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# Polymerase Chain Reaction Testing: Selected Indications

[Clinical Policy Bulletins](#) | [Medical Clinical Policy Bulletins](#)

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Table Of Contents

[Policy](#)

[Applicable CPT / HCPCS / ICD-10 Codes](#)

[Background](#)

[References](#)

Policy History

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[Review History](#)

[Definitions](#)

Policy

Scope of Policy

This Clinical Policy Bulletin addresses qualitative and quantitative polymerase chain reaction (PCR) testing.

Additional Information

[Clinical Policy Bulletin Notes](#)

## I. Medical Necessity

### A. Qualitative Polymerase Chain Reaction (PCR) Testing

Aetna considers the following qualitative polymerase chain reaction (PCR) testing medically necessary (not an all-inclusive list):

1. Acanthamoeba in corneal ulceration
2. Actinomyces, for identification of actinomyces species in tissue specimens
3. Adenovirus, to diagnose adenovirus myocarditis, and to diagnose adenovirus infection in immunocompromised hosts, including transplant recipients
4. Anaplastic lymphoma kinase (ALK) testing as an alternative to FISH for selecting individuals for ALK inhibitor therapy
5. Avian influenza A virus, for diagnosis of avian influenza A (H5N1) in persons with both:
  - a. Symptoms consistent with Avian influenza A virus (see background); and
  - b. A history of travel to or contact with persons or birds from a country with documented H5N1 avian influenza infections within 10 days of symptom onset. Ongoing listings of countries affected by avian influenza are available from the World Organization for Animal Health, available at [OIE - World Organization for Animal Health](#) (<https://www.oie.int/en/disease/avian-influenza/#ui-id-5>)
6. Babesiosis (*Babesia* spp.), diagnosis

7. Bacterial vaginosis (*Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus mulieris*, *M. curtisi*, *megasphaera* type 1 and type 2) for the diagnosis in symptomatic vaginitis (see [CPB 0643 - Diagnosis of Vaginitis](#) ([https://www.aetna.com/cpb/medical/data/600\\_699/0643.html](https://www.aetna.com/cpb/medical/data/600_699/0643.html)))
8. Bartonella species (*B. henselae*, *B. quintana*, *B. clarridgeiae*, *B. elizabethae* ), to confirm diagnosis in acutely or severely ill members with systemic symptoms of Cat-Scratch Disease, particularly persons with hepatosplenomegaly or persons with large painful adenopathy and immunocompromised hosts; and to distinguish *B. henselae* from *B. quintana* infection in HIV-infected members and other immunocompromised members with signs and symptoms of bacillary angiomatosis or peliosis hepatitis (see also [CPB 0215 - Lyme Disease and Other Tick-Borne Diseases](#) ([./200\\_299/0215.html](#)))
9. Borrelia miyamotoi infection, diagnosis in acute phase of *B. miyamotoi* infection in persons from endemic areas with signs of symptoms suggesting these infections
10. Beta-tyrosinase, to detect hematogenous spread of melanoma cells in members with melanoma
11. BK polyomavirus in transplant recipients receiving immunosuppressive therapies and persons with immunosuppressive diseases (e.g., immune complex glomerulonephritis associated with HIV/AIDS)
12. Bordetella holmesii, *B. pertussis* and *B. parapertussis* , for diagnosis of whooping cough in individuals with 1 of the following that has occurred in less than 21 days (or 3 weeks):
  - a. Coughing with documented exposure to pertussis; or
  - b. Paroxysms of coughing, or
  - c. Inspiratory whoop, or
  - d. Post-tussive vomiting, or
  - e. Apnea (with or without cyanosis) (for infants less than 1 year of age only)
13. BRAF mutation analysis for hairy cell leukemia
14. Breast and ovarian cancer (BRCA1, BRCA2), for persons who meet criteria for BRCA testing (see [CPB 0227 - Breast and Ovarian Cancer Susceptibility Gene Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy](#) ([./200\\_299/0227.html](#)))
15. Brucella spp., for members with signs and symptoms of Brucellosis, and history of direct contact with infected animals and their carcasses or secretions or by ingesting unpasteurized milk or milk products
16. Burkholderia infections (including *B. cepacia*, *B. gladioli*), diagnosis
17. Candidiasis (*Candida albicans*, *glabrata*, *krusei*, *parapsilosis* and *tropicalis*) for diagnosis of symptomatic vaginitis (see [CPB 0643 - Diagnosis of Vaginitis](#) ([https://www.aetna.com/cpb/medical/data/600\\_699/0643.html](https://www.aetna.com/cpb/medical/data/600_699/0643.html)))
18. Chancroid (*Haemophilus ducreyi*), for diagnosis of persons with genital ulcer disease
19. Chikungunya virus infection, diagnosis
20. Chlamydophila (Chlamydia) pneumoniae, in members with signs or symptoms suggestive of Chlamydial pneumonia
21. Chlamydia trachomatis, for screening\* and diagnosis for persons who meet criteria in [CPB 0433 - Chlamydia Trachomatis - Screening and Diagnosis](#) ([./400\\_499/0433.html](#)).
22. Chromosome 18q assay for persons with colorectal cancer
23. Clostridium difficile, diagnosis (e.g., persons with diarrhea, not for asymptomatic or "test of cure")
24. Colorado tick fever virus, for diagnosis of Colorado tick fever within the first 14 days, before serologic tests become positive
25. Colorectal cancer, hereditary non-polyposis colorectal cancer (MLH1, MSH2, MSH6 and microsatellite instability) and familial adenomatous polyposis (FAP, APC), for persons who meet criteria in [CPB 0140 - Genetic Testing](#) ([./100\\_199/0140.html](#))

26. Coronavirus COVID-19, for indications consistent with CDC Guidance related to COVID-19. Note: Standard benefit plans exclude coverage of testing required for work, school, or recreational activities. Please check benefit plan descriptions. In addition, testing required for employment, school or recreational activities is not considered medically necessary treatment of disease.
27. Coxiella burnetii, for confirmation of acute Q fever
28. Cytomegalovirus (CMV), for persons with signs or symptoms suggestive of CMV infection
29. Dengue, for laboratory confirmation of clinical diagnosis
30. Diphtheria toxin (*Corynebacterium diphtheriae*), detection
31. Ebola, diagnosis
32. Ehrlichiosis (*Ehrlichia spp.*), for diagnosis in acute phase in persons from endemic areas with signs or symptoms suggesting this diagnosis
33. Entamoeba histolytica, to distinguish *E. histolytica* from *E dispar* and *E moshkovskii*
34. Enterovirus infections (Group A and B coxsackieviruses, polioviruses, and echoviruses), for detecting viral RNA in cerebrospinal fluid (CSF), for immunocompromised persons suspected of having persistent central nervous system (CNS) infection (aseptic meningitis)
35. Epidemic typhus (*Rickettsia prowazekii*), diagnosis
36. Epstein Barr Virus (EBV): for detection of EBV in post-transplant lymphoproliferative disorder; or for testing for EBV in persons with lymphoma; or for those who are immunocompromised for other reasons.
37. Factor II (prothrombin) G20210A mutation, for persons who meet criteria in [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)
38. Factor V Leiden mutation, for persons who meet criteria in [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)
39. Fragile X syndrome, for persons who meet medical necessity criteria for Fragile X genetic testing (see [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)), FMR1 gene analysis by PCR is considered medically necessary to confirm diagnosis of fragile X syndrome and to rule out FRAXE and FRAXF.
40. Francisella tularensis, for presumptive diagnosis of tularemia
41. Genetic testing in Canavan disease, Niemann Pick disease, cystic fibrosis, Gaucher disease, Tay Sachs, connexin 26, Rett syndrome, fetal sickle cell anemia, Huntington's disease, and Angelman and Prader Willi syndromes, for persons who meet criteria in [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)
42. Gonorrhea (*Neisseria gonorrhoeae*), for screening\* and diagnosis
43. Group B streptococcal (GBS) infection screening, for the following: (i) intrapartum testing of women with unknown GBS colonization status and no intrapartum risk factors (temperature of greater than or equal to 100.4° F [greater than or equal to 38.0°C] or rupture of amniotic membranes greater than or equal to 18 hours) at the time of testing and who are delivering at term; and (ii) antepartum testing with broth enrichment for pregnant women at 36 to 37 weeks gestation.
44. Haemophilus influenzae, for capsular typing of invasive disease
45. Hantavirus, diagnosis
46. Hemochromatosis mutation in persons who meet criteria for genetic testing for hereditary hemochromatosis in [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)
47. Hemorrhagic fevers and related syndromes caused by viruses of the family Bunyaviridae (Rift Valley fever, Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndromes), for diagnosis in acute phase in persons with clinical presentation suggestive of these conditions
48. Hepatitis B virus, for selection of candidates to receive antiviral therapy and to monitor the response to therapy
49. Hepatitis C virus genotyping, for determining the risks/benefits and duration of treatment
50. Hepatitis C virus, for diagnosis of infection, and for monitoring members receiving anti-viral therapy
51. Hepatitis D virus, for confirmation of active infection in persons with anti-HDV

antibodies

52. Hepatitis E virus, for definitive diagnosis in persons with anti-HEV antibodies
53. Herpes simplex virus (HSV), for diagnosis of HSV infection for active lesions or symptoms of active disease
54. Human granulocytic anaplasmosis (*Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilum*))
55. Human herpesvirus type 6 (HHV-6), for diagnosis of infection in immunocompromised persons such as persons with AIDS or transplant recipients, and to diagnose HHV-6 infection in members with mononucleosis-like syndrome in members without heterophile antibodies or antibodies specific to Epstein-Barr virus (EBV)
56. Human immunodeficiency virus (HIV), to diagnose HIV infection in infants and young children less than 18 months of age
57. Human leukocyte antigen (HLA) typing for:
  - a. Assessing histocompatibility in tissue grafts and organ transplants;
  - b. Diagnosis of ankylosing spondylitis or Reiters syndrome (HLA B27);
  - c. Persons suspected of having celiac disease who meet criteria in [CPB 0561 - Celiac Disease Laboratory Testing \(./500\\_599/0561.html\)](#)
58. Human metapneumovirus, diagnosis
59. Human papilloma virus (HPV), for indications listed in [CPB 0443 - Cervical Cancer Screening and Diagnosis \(./400\\_499/0443.html\)](#)
60. Human T Lymphotropic Virus type 1 and type 2 (HTLV-I and HTLV-II), to confirm the presence of HTLV-I and HTLV-II in the cerebrospinal fluid of persons with signs or symptoms of HTLV-I/HTLV-II myeloradiculopathy who have traveled to areas where HTLV-I/HTLV-II infection is endemic (Japan, the Caribbean, and parts of South America)
61. Influenza virus (including influenza A and B), for hospitalized persons with suspected influenza and for other persons with suspected influenza for whom a diagnosis of influenza will inform decisions regarding clinical care, infection control, or management of close contacts
62. Janus Kinase 2 gene mutation, for initial diagnostic assessment of BCR-ABL negative adults presenting with symptoms of myeloproliferative disorders, and for diagnostic assessment of polycythemia vera in adults
63. JC polyomavirus, in transplant recipients receiving immunosuppressive therapies, in persons with immunosuppressive diseases (e.g., AIDS), and for diagnosing progressive multifocal leukoencephalopathy (PML) in persons with multiple sclerosis or Crohn's disease receiving natalizumab (Tysabri)
64. JC polyomavirus for PML for members before starting ocrelizumab or rituximab therapy
65. JC polyomavirus for members receiving vedolizumab (Entyvio)
66. Lactobacillus vaginitis
67. Leishmaniasis, diagnosis
68. Leukemias, acute myelogenous (FLT3 mutation), acute myelocytic (AM1/ETO t(8;21) translocation), acute myelomonocytic (CBFB/MYH11 inv(16) inversion), acute promyelocytic (PML/RARA t(15;17) translocation), acute lymphocytic (bcr-abl gene rearrangement), chronic myelogenous (bcr-abl gene rearrangement), and chronic lymphocytic (IgVh mutation analysis)
69. Lymphogranuloma venereum (Chlamydia trachomatis)
70. Lymphomas, B cell (bcl-2 gene translocation t(14;18)), mantle cell (bcl-1 gene translocation t(11;14)(q13;q32)) and T cell (gene rearrangements)
71. Lymphomas, B cell or T cell, to determine clonality
72. Malaria, for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or a rapid diagnostic test
73. Measles virus (Morbilliviruses), for diagnosis of measles (rubeola)
74. Methicillin resistant *Staphylococcus aureus* (MRSA), to distinguish MRSA from non-resistant forms of *S. aureus* (not for detection of the mere presence of *S. aureus*)

75. Microsporidia, diagnosis
76. Monkeypox virus (Orthopoxvirus) using an FDA cleared test when either of the following criteria are met:
- a. Member with any of the following epidemiological risk factors for infection and presents with a rash or lesion suspicious of monkeypox:
    - i. Reports having contact with a person or people with a similar appearing rash or who received a diagnosis of confirmed or probable monkeypox; or
    - ii. Had close or intimate in-person contact with individuals in a social network experiencing monkeypox activity (this includes men who have sex with men (MSM) who meet partners through an online website, digital application, or social event [e.g., a bar or party]); or
  - b. Member presents with genital ulcer disease or proctitis that does not respond to empiric treatment for typical sexually transmitted infections
77. Mucosa-associated lymphoid tissue (MALT) lymphomas and marginal zone lymphomas, for evaluating persons who have non-diagnostic atypical lymphoid infiltrates that are positive for *H. pylori* infection, to assess likelihood of response to antibiotic therapy
78. Mumps, in persons with acute parotitis of 2 or more days' duration
79. *Mycobacterium tuberculosis*, for rapid diagnostic testing of acid fast stain positive respiratory tract specimens
80. *Mycoplasma genitalium*, diagnosis in persons with urethritis or cervicitis
81. *Mycoplasma hominis*, diagnosis
82. *Mycoplasma pneumoniae*, for diagnosis
83. *Neisseria meningitidis*, to establish diagnosis where antibiotics have been started before cultures have been obtained
84. Oncotype Dx test for breast cancer, when criteria in [CPB 0352 - Tumor Markers \(./300\\_399/0352.html\)](#) are met
85. Parvovirus, for detecting chronic infection in immunocompromised persons (e.g., pregnant women with known exposure to parvovirus B19 infection and serologic testing is negative)
86. *Pneumocystis jirovecii*, diagnosis
87. Psittacosis, for diagnosis of *Chlamydophila (Chlamydia) psittaci* infection and distinguishing *C. psittaci* from other *Chlamydophila* infections
88. Respiratory syncytial virus (RSV)
89. RET proto-oncogene mutations, for diagnosis of multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid carcinoma (FMTC) in persons who meet criteria in [CPB 0319 - RET Proto-Oncogene Testing \(./300\\_399/0319.html\)](#)
90. Rocky Mountain Spotted Fever (*Rickettsia rickettsii*), for diagnosis in acute phase (first 2 weeks of infection) in persons from endemic areas with signs or symptoms suggestive of this diagnosis
91. Rubella, diagnosis
92. Severe acute respiratory syndrome (SARS), for detection of SARS coronavirus RNA in persons with signs or symptoms of SARS who have traveled to endemic areas or have been exposed to persons with SARS
93. Shiga toxin (from *E. coli* and *Shigella*), detection
94. Streptococcal pharyngitis (group A *Streptococcus*), diagnosis
95. Syphilis (*Treponema pallidum*), for diagnosis of oral or other lesions where contamination with commensal treponemes is likely
96. Thiopurine methyltransferase (TPMT) genotyping (see [CPB 0249 - Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolic](#)

[Assessment of Thiopurine Therapy \(./200\\_299/0249.html\)](#)

97. Toxoplasma gondii, for detection of T. gondii infection in immunocompromised persons with signs and symptoms of toxoplasmosis, and for detection of congenital Toxoplasma gondii infection (including testing of amniotic fluid for toxoplasma infection)
98. Trichomoniasis (*Trichomonas vaginalis*), diagnosis of trichomonas in symptomatic men and women, and screening of women at high risk of infection (i.e., women who have new or multiple partners, have a history of STDs, exchange sex for payment, or use injection drugs)
99. Ureaplasma parvum, diagnosis
100. Ureaplasma urealyticum, diagnosis
101. Varicella-Zoster infections, for diagnosis and also to distinguish wild-type virus from vaccination in previously immunized persons with signs or symptoms of Varicella zoster infection
102. West Nile Virus, to confirm diagnosis in acute-phase serum, CSF or tissue specimens, and to screen bone marrow transplant donors in endemic areas.
103. Whipple's disease (*T. whippeli*), biopsy tissue from small bowel, abdominal or peripheral lymph nodes, or other organs of persons with signs and symptoms
104. Zika virus infection:
  - a. To establish diagnosis in symptomatic or asymptomatic pregnant women who have traveled to endemic areas; or
  - b. Testing of infants with microcephaly or intracranial calcifications born to women who traveled to or resided in an area with Zika virus transmission while pregnant; or
  - c. Infants born to mothers with positive or inconclusive test results for Zika virus infection; or
  - d. Persons with symptoms consistent with Zika virus infection who have traveled to or resided in an area with Zika virus transmission; or
  - e. Persons who may have been exposed to Zika virus infection through sex.

\* Note: Screening tests are covered only for members with preventive services benefits. Please check benefit plan descriptions for details.

B. Qualitative Multiple PCR Tests

Aetna considers the following qualitative multiplex PCR tests medically necessary:

1. Cerebrospinal fluid (CSF) pathogen panel when the following criteria are met:
  - a. Member has clinical findings consistent with a central nervous system (CNS) infection (e.g., meningitis, encephalitis); and
  - b. Viral etiology is suspected or the CSF culture is inconclusive for pathogen etiology (i.e., viral, bacterial, fungal);
2. Coronavirus COVID-19 (SARS-CoV-2) respiratory panel (up to 5 respiratory pathogens) when member has signs and symptoms of COVID-19 and test will be used to guide therapy
3. Gastrointestinal pathogen panel (e.g., Thermo Fisher Scientific TaqPath Enteric Bacterial Select Panel) when any of the following criteria is met:
  - a. Member has community-acquired diarrhea of 7 days or more duration; or
  - b. Member has travel-related diarrhea; or
  - c. Member has diarrhea with signs or risk factors for severe disease (fever, bloody diarrhea, dysentery, dehydration, severe abdominal pain, hospitalization and/or immunocompromised state);
4. Gastrointestinal pathogen panels of up to 11 targets are considered medically necessary for immune competent persons who meet the above-listed criteria;

more than 11 targets are considered medically necessary for persons who meet criteria and are critically ill or immunocompromised;

5. Herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), and varicella zoster virus (VZV) assay (e.g., Abbott Alinity m HSV 1 & 2 / VZV assay) to confirm diagnosis of HSV-1, HSV-2 and/or VZV infections in members with acute onset of symptoms suggestive of a varicella or unusual HSV infection (e.g., suspicious skin lesions or rash in members with a fever or who are immunocompromised and/or considered high-risk for complications, orchitis, encephalitis);
6. Pneumonia panel (e.g., BioFire FilmArray Pneumonia Panel) or Respiratory virus and/or bacteria panel (e.g., BioFire FilmArray Respiratory Panel (EZ, RP, or RP2), ePlex RP Panel, Respiratory Pathogen with ABR (RPX) Panel) when all of the following criteria are met:

- a. Member has signs and symptoms of a respiratory infection, and
- b. Member is immunocompromised and/or considered high-risk for complications (e.g., infants up to 1 to 2 months of age, receiving cancer treatment, organ transplant recipient); and
- c. Test will be used to guide therapy;

7. Sepsis pathogen panel (e.g., BioFire BCID panel, ePlex BCID-GP, GN, or FP) when all of the following criteria are met:

- a. Member has signs and symptoms consistent with sepsis; and
- b. Molecular testing is performed directly on a positive culture obtained from body fluid (e.g., blood); and
- c. Test will be used to guide therapy;

#### C. Quantitative PCR Testing

Aetna considers the following quantitative PCR tests medically necessary:

1. Acute myeloid leukemia: AM1/ETO t(8, 21) translocation, to monitor disease progression and response to therapy
2. Acute myelomonocytic leukemia: CBF $\beta$ /MYH11 inv(16), to monitor disease progression and response to therapy
3. Acute promyelocytic leukemia: PML/RARA t(15;17), to monitor disease progression and response to therapy
4. Adenovirus viral load, to monitor response to antiviral therapy in infected immunocompromised hosts, including transplant recipients
5. B cell lymphomas: bcl-2 gene translocation, to monitor disease progression and response to therapy
6. BK polyomavirus viral load for diagnosis and monitoring response to therapy in transplant recipients
7. Chronic myelogenous leukemia and acute lymphocytic leukemia: bcr/abl gene rearrangement, to monitor disease progression and response to therapy
8. Cytomegalovirus (CMV) viral load, to monitor response to therapy
9. EBV viral load, to monitor for EBV viral replication in hematopoietic stem cell and solid organ transplant recipients (Note: For high-risk allogeneic stem cell transplant recipients, screening should begin the day of transplantation and continue at least weekly for the first 3 months and even longer if the member
  - a. Is being treated for graft-versus-host disease,
  - b. Has a haploidentical graft, or
  - c. Has already experienced EBV viremia.

More frequent testing is worth considering if the EBV load is rising. For standard-risk hematopoietic stem cell and solid organ transplant recipients, testing should be done only when they are suspected to have EBV infection.)

10. *Gardnerella vaginalis* and vaginitis (including *Atopobium vaginae*, *Lactobacillus* species, and *Megasphaera* species) for the diagnosis in symptomatic vaginitis
11. Hepatitis B viral load, to monitor response to therapy
12. Hepatitis C viral load, to monitor response to therapy
13. HIV RNA viral load testing, to monitor disease progression and response to therapy
14. Human herpesvirus type 6, to monitor response to therapy in immunocompromised hosts, including transplant recipients
15. Mantle cell lymphoma: *bcl-1/JH t(11;14)* gene rearrangement, to monitor disease progression and response to therapy
16. T cell lymphomas: gene rearrangements, to monitor disease progression and response to therapy
17. Varicella-zoster infection diagnosis in previously immunized persons, to distinguish wild-type virus from vaccination.

## II. Experimental, Investigational, or Unproven

### A. Qualitative PCR Testing

Aetna considers the following qualitative PCR testing experimental, investigational, or unproven because of insufficient evidence in the peer-reviewed literature:

- *Acinetobacter baumannii*
- Aspergillosis (e.g., MycoDART Dual Amplification Real-Time PCR Aspergillus diagnostic panel)
- *Astrovirus*
- Autoimmune lymphoproliferative syndrome
- *Bacteroides* spp. (*B. fragilis*, *B. ureolyticus*)
- Blastomycosis
- Caliciviruses (noroviruses and sapoviruses)
- Campylobacteriosis (Campylobacter infection)
- Castleman's disease
- Cervical intraepithelial neoplasia (CIN) metastasis
- Chlamydia pneumoniae, for assessment of atherosclerotic cardiovascular disease, asthma, Alzheimer disease, multiple sclerosis, or Kawasaki disease
- Coagulase-negative staphylococcus (including *Staphylococcus saprophyticus*, and *Staphylococcus lugdunensis*)
- Coccidiomycosis (*Coccidioides* species)
- *Cochliobolus lunatus*
- *Cochliobolus spicifer*
- Colorectal cancer screening (PreGen Plus) (see [CPB 0516 - Colonoscopy and Colorectal Cancer Screening \(../500\\_599/0516.html\)](#))
- Coronavirus (other than SARS-coronavirus and COVID-19)
- Creutzfeldt-Jakob disease
- *Cryptococcus* (*Cryptococcus neoformans*)
- Cryptosporidiosis (cryptosporidium infection)
- *Cutibacterium acnes* (formerly *propionibacterium acnes*) for diagnosis of corneal ulcers
- Cyclosporiasis (Cyclospora infection)
- Cytochrome P450 genotyping (see [CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing \(../700\\_799/0715.html\)](#))
- Differential diagnosis of onychauxis versus onychogryphosis
- Donovanosis, or granuloma inguinale (*Klebsiella granulomatis*)
- Drainage from otitis externa
- Eastern equine encephalitis
- *Eggerthella* (screening)
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Enterococcus faecalis*

- Enterococcus faecium
- Escherichia coli (except for detection of Shiga toxin)
- Evaluation of suspected candida auris
- Giardia lamblia
- Hepatitis A virus
- Hepatitis G virus (HGV)/GB virus type C
- Histoplasma capsulatum histoplasmosis
- Human bocavirus
- Human herpesvirus type 7 (HHV-7)
- Human herpesvirus type 8 (HHV-8)
- Joint effusion
- Kawasaki disease
- Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria
- LaCrosse encephalitis
- Legionella pneumophila
- Leptospirosis (Leptospira organisms)
- Listeria
- Lyme disease (Borrelia burgdorferi)
- Malaise and fatigue (including chronic fatigue syndrome)
- Management of pruritis ani
- Melanoma (p16, Melaris) (see [CPB 0140 - Genetic Testing \(../100\\_199/0140.html\)](#))  
and melanoma micrometastases
- MTHFR mutation in persons with hyper-homocysteinemia
- Molluscum contagiosum
- Moraxella catarrhalis
- Mycobacterium species (other than M. tuberculosis), including Mycobacterium-avium intracellulare (MIA)
- Mycoplasma fermentans
- Mycoplasma hominis screening in asymptomatic pregnant women
- Mycoplasma penetrans
- Nanobacteria
- Non-albicans Candida;
- Onychomycosis (tinea unguium)
- Oral swab for mycoplasma hominis and ureaplasma urealyticum for screening of oral infection
- Parainfluenza virus
- Parechovirus for recurrent fever
- Parvovirus for mast cell activation syndrome
- PCR panels in podiatry for detecting multiple bacteria and fungi and antibiotic resistance testing
- Peptic ulcer disease (*Helicobacter pylori*) (other than in persons with MALT lymphomas and marginal zone lymphomas)
- Peripheral neuropathy
- Pharyngitis panel for diagnosis stomatitis and otitis media
- Pharyngitis panel for persons in the community presenting with symptoms of pharyngitis
- Plesiomonas shigelloides
- Pneumococcal infections (*S. pneumoniae*)
- Pneumocystis pneumonia (Pneumocystis jiroveci (formerly *P. carinii*))
- Prevotella spp. (except for suspected bacterial vaginosis)
- Prostate cancer micrometastasis
- Prostatitis (e.g., Pathnostics Comprehensive Guidance)
- Proteus mirabilis
- Pseudomonas (*P. aeruginosa*)
- Pleuropulmonary coccidioidomycosis
- Rectal swab testing for identification of antibiotic resistant bacteria before prostate biopsy
- Repeat monkeypox testing to confirm resolution of the lesion
- Respiratory virus for children with febrile seizure

- Rhinovirus
- Rotavirus
- Routine screening of trichomonas in asymptomatic women
- Saccharomyces cerevisiae
- Salmonella
- Screening newborns for congenital cytomegalovirus infection
- Serratia spp. (including *S. marcescens*)
- Sporotrichosis (*Sporothrix schenckii*)
- St. Louis encephalitis
- *Staphylococcus epidermidis*
- *Staphylococcus aureus*
- *Stenotrophomonas maltophilia*
- Stool *H. pylori* detection for evaluation of dysphagia (e.g., the PylorDx and the PyloriAR/Stool *H. pylori* Antibiotic Resistance Panel (NGS))
- *Streptococcus* group C
- *Streptococcus* group G
- Toxoplasmosis in uveitis
- Trichosporonosis (*Trichosporon* spp.)
- Urinary tract infection panels (e.g., Lab Genomics Urogenital Pathogen Test [UPX], Qlear UTI [Lifescan Labs, Thermo Fisher Scientific])
- Vancomycin-resistant enterococcus
- *Vibrio cholerae*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- Western equine encephalitis
- Wound infection panels (e.g., Lesion Infection (Wound) [Lab Genomics, Thermo Fisher Scientific])
- Xenotropic murine leukemia
- *Yersinia enterocolitica*.

#### B. Quantitative PCR Tests

Aetna considers the following quantitative PCR tests experimental, investigational, or unproven because their role in clinical management has not been established:

- *Bartonella* (*B. henselae*, *B. quintana*)
- Candidiasis (*Candida albicans* and other *Candida* species)
- Chlamydia species (*Chlamydia (Chlamydophila) pneumoniae*, *C. trachomatis*)
- EoGenius (a 96-gene quantitative PCR array and an associated dual-algorithm) for eosinophilic esophagitis
- Evaluation of JC polyomavirus for progressive multifocal leukoencephalopathy before starting ocrelizumab or rituximab therapy
- Evaluation of suspected candida auris
- Hepatitis G virus (HGV)/GB virus type C
- Herpes simplex virus (HSV)
- Influenza
- Karius Test (microbial cell-free DNA)
- Kawasaki disease (e.g., KawasakiDx)
- Legionella pneumophila
- Lyme disease (*Borrelia burgdorferi*)
- Management of pruritis ani
- *Mycobacterium* species (including *Mycobacterium avium-intracellulare* and *Mycobacterium tuberculosis*)
- Mycoplasma
- *Neisseria gonorrhoeae*
- Oral swab for mycoplasma hominis and ureaplasma urealyticum for screening of oral infection
- Parvovirus (including chronic fatigue immune dysfunction syndrome secondary to parvovirus B19 infection)

- Pharyngitis panel for diagnosis stomatitis and otitis media
- Pharyngitis panel for persons in the community presenting with symptoms of pharyngitis
- Rectal swab testing for identification of antibiotic resistant bacteria before prostate biopsy
- Repeat monkeypox testing to confirm resolution of the lesion
- Respiratory virus for children with febrile seizure
- uBiome (e.g., SmartGut, SmartJane, SmartFlu, and Explorer).

## I. Related Policies

- [CPB 0140 - Genetic Testing \(./100\\_199/0140.html\)](#)
- [CPB 0177 - Helicobacter Pylori Infection Testing \(./100\\_199/0177.html\)](#)
- [CPB 0215 - Lyme Disease and Other Tick-Borne Diseases \(./200\\_299/0215.html\)](#)
- [CPB 0227 - Breast and Ovarian Cancer Susceptibility Gene Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy \(./200\\_299/0227.html\)](#)
- [CPB 0249 - Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolitic Assessment of Thiopurine Therapy \(./200\\_299/0249.html\)](#)
- [CPB 0319 - RET Proto-Oncogene Testing \(./300\\_399/0319.html\)](#)
- [CPB 0352 - Tumor Markers \(./300\\_399/0352.html\)](#)
- [CPB 0433 - Chlamydia Trachomatis - Screening and Diagnosis \(./400\\_499/0433.html\)](#)
- [CPB 0443 - Cervical Cancer Screening and Diagnosis \(./400\\_499/0443.html\)](#)
- [CPB 0516 - Colonoscopy and Colorectal Cancer Screening \(./500\\_599/0516.html\)](#)
- [CPB 0561 - Celiac Disease Laboratory Testing \(./500\\_599/0561.html\)](#)
- [CPB 0643 - Diagnosis of Vaginitis  
\(\[https://www.aetna.com/cpb/medical/data/600\\\_699/0643.html\]\(https://www.aetna.com/cpb/medical/data/600\_699/0643.html\)\)](#)
- [CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing \(./700\\_799/0715.html\)](#)

## CPT Codes /HCPCS Codes/ICD-10 codes

PCR testing for genetic or inherited disorder:

<b>Code</b>	<b>Code Description</b>
CPT codes covered if selection criteria are met:	
0017U	Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected
0040U	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis, major breakpoint, quantitative
0353U	Infectious agent detection by nucleic acid (DNA), Chlamydia trachomatis and Neisseria gonorrhoeae, multiplex amplified probe technique, urine, vaginal, pharyngeal, or rectal, each pathogen reported as detected or not detected
81206 - 81208	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis
81210	BRAF (b-raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81220 - 81224	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis
81225	CYP2C19 (cytochrome P450, family 2, subfamily C, polypeptide 19) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *8, *17)
81226	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg,

	drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, *4XN)
81227	CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *5, *6)
81240	F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
81242	FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A>T)
81243 - 81244	FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis
81251	GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G>A)
81252 - 81253	GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence or known familiar variants
81255	HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G>C, G269S)
81256	HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D)
81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)
81261	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B- cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)
81264	IGK@ (Immunoglobulin kappa light chain locus) (eg, leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)
81270	JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
81292 - 81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81295 - 81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81298 - 81300	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81315 - 81316	PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis
81317 - 81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis
81330 - 81331	SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)
81340 - 81342	TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s)
81370 - 81377	HLA Class I and II typing, low resolution (eg, antigen equivalents)

81378 - 81383	HLA Class I typing, high resolution (ie, alleles or allele groups)
81400 - 81408	Molecular pathology
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
86711	Antibody; JC (John Cunningham) virus
86828 - 86835	Antibody to human leukocyte antigens (HLA), solid phase assays (eg, microspheres or beads, ELISA, flow cytometry)
CPT codes not covered for indications listed in the CPB:	
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
Other HCPCS codes related to the CPB:	
J2323	Injection, natalizumab, 1 mg
Q5134	Injection, natalizumab-sztn (tyruko), biosimilar, 1 mg
ICD-10 codes covered if selection criteria are met:	
C18.0 - C20	Malignant neoplasm of colon, rectosigmoid junction, and rectum
C43.0 - C43.9	Malignant melanoma of skin
C81.00 - C81.99	Hodgkin lymphoma
C82.00 - C82.99	Follicular lymphoma
C83.10 - C83.19	Mantle cell lymphoma
C83.30 - C83.3A	Diffuse large B-cell lymphoma
C83.70 - C83.79	Burkitt lymphoma
C84.00 - C84.09	Mycosis fungoides
C84.40 - C84.49	Peripheral T-cell lymphoma, not classified
C84.60 - C84.79	Anaplastic large cell lymphoma
C88.40 - C88.41	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT-lymphoma) [Diffuse large B cell lymphoma (DLBCL)]
C91.00 - C91.02	Acute lymphoblastic leukemia [ALL]
C91.10 - C91.12	Chronic lymphoid leukemia
C91.40 - C91.42	Hairy-cell leukemia
C92.00 - C92.02	Acute myeloblastic leukemia
C92.10 - C92.12	Chronic myeloid leukemia, BCR/ABL-positive
D12.0 - D12.6	Benign neoplasm of colon
D57.00 - D57.819	Sickle-cell disorders
D68.51 - D68.59	Primary thrombophilia
E75.02	Tay-sachs disease
E75.21	Fabry (-Anderson) disease
E75.22	Gaucher disease
E75.240 - E75.249	Niemann-pick disease
E83.110 - E83.119	Hemochromatosis
E84.0 - E84.9	Cystic fibrosis
F84.2	Rett's syndrome
G10	Huntington's disease
K90.0	Celiac disease
M45.0 - M45.AB	Ankylosing spondylitis
Q87.10 - Q87.19	Congenital malformation syndromes predominantly associated with short stature [Prader-Willi syndrome]
Q93.51	Angelman syndrome

Q99.2	Fragile X chromosome
Z13.0	Encounter for screening for disease of the blood and blood-forming organs and certain disorders involving the immune mechanism [sickle-cell disease or trait]
Z13.228	Encounter for screening for other metabolic disorders [cystic fibrosis]
Z52.000 - Z52.9	Donors of organs and tissues
ICD-10 codes not covered for indications listed in the CPB:	
C94.80 - C94.82	Other specified leukemia [Xenotropic murine leukemia]
G60.0 - G60.9	Hereditary and idiopathic neuropathy
C61	Malignant neoplasm of prostate
D06.0 - D06.9	Carcinoma in situ of cervix uteri
D47.Z2	Castleman disease
E72.10 - E72.19	Disorders of sulphur-bearing amino-acid metabolism
N87.0 - N87.9	Dysplasia of cervix uteri
Z12.10	Encounter for screening for malignant neoplasm of intestinal tract, unspecified
Z12.5	Encounter for screening for malignant neoplasm of prostate
Z12.83	Encounter for screening for malignant neoplasm of skin
Z85.41	Personal history of malignant neoplasm of cervix uteri
Z85.46	Personal history of malignant neoplasm of prostate
PCR testing for microbial identification - amplified probe:	
CPT codes covered if selection criteria are met:	
0301U	Infectious agent detection by nucleic acid (DNA or RNA), <i>Bartonella henselae</i> and <i>Bartonella quintana</i> , droplet digital PCR (ddPCR);
0302U	following liquid enrichment
0455U	Infectious agents (sexually transmitted infection), <i>Chlamydia trachomatis</i> , <i>Neisseria gonorrhoeae</i> , and <i>Trichomonas vaginalis</i> , multiplex amplified probe technique, vaginal, endocervical, gynecological specimens, oropharyngeal swabs, rectal swabs, female or male urine, each pathogen reported as detected or not detected
86328	Immunoassay for infectious agent antibody(ies), qualitative or semiquantitative, single step method (eg, reagent strip); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
86769	Antibody; severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19])
87150	Culture, typing; identification by nucleic acid (DNA or RNA) probe, amplified probe technique, per culture or isolate, each organism probed
87154	Culture, typing; identification of blood pathogen and resistance typing, when performed, by nucleic acid (DNA or RNA) probe, multiplexed amplified probe technique including multiplex reverse transcription, when performed, per culture or isolate, 6 or more targets
87468	Infectious agent detection by nucleic acid (DNA or RNA); <i>Anaplasma phagocytophilum</i> , amplified probe technique
87469	<i>Babesia microti</i> , amplified probe technique
87471	<i>Bartonella henselae</i> and <i>Bartonella quintana</i> , amplified probe technique
87478	<i>Borrelia miyamotoi</i> , amplified probe technique
87484	<i>Ehrlichia chaffeensis</i> , amplified probe technique
87486	<i>Chlamydia pneumoniae</i> , amplified probe technique

87491	Chlamydia trachomatis, amplified probe technique
87493	clostridium difficile, toxin gene(s), amplified probe technique [not covered for asymptomatic persons or for "test of cure"]
87496	cytomegalovirus, amplified probe technique
87498	enterovirus, amplified probe technique
87501	influenza virus, includes reverse transcription, when performed, and amplified probe technique, each type or subtype
87502	influenza virus, for multiple types or sub-types, includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, first 2 types or sub-types
87503	each additional influenza virus type or sub-type
87516	hepatitis B virus, amplified probe technique
87521	hepatitis C virus, amplified probe technique
87523	hepatitis D (delta), quantification, including reverse transcription, when performed
87529	Herpes simplex virus, amplified probe technique
87532	Herpes virus-6, amplified probe technique
87535	HIV-1, amplified probe technique
87538	HIV-2, amplified probe technique
87556	Mycobacterium tuberculosis, amplified probe technique
87581	Mycoplasma pneumoniae, amplified probe technique
87591	Neisseria gonorrhoeae, amplified probe technique
87634	respiratory syncytial virus, amplified probe technique
87635	severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique
87636	severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) and influenza virus types A and B, multiplex amplified probe technique
87637	severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), influenza virus types A and B, and respiratory syncytial virus, multiplex amplified probe technique
87641	Staphylococcus aureus, methicillin resistant, amplified probe technique
87651	streptococcus, group A, amplified probe technique
87653	Streptococcus, group B, amplified probe technique
87661	Trichomonas vaginalis, amplified probe technique
87662	Zika virus, amplified probe technique
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique
87910	Infectious agent genotype analysis by nucleic acid (DNA or RNA); cytomegalovirus
87912	Hepatitis B virus
CPT codes not covered for indications listed in the CPB:	
Central nervous system pathogen panel, rectal swab testing for identification of antibiotic resistant bacteria - no specific code	
0068U	Candida species panel (C. albicans, C. glabrata, C. parapsilosis, C. krusei, C tropicalis, and C. auris), amplified probe technique with qualitative report of the presence or absence of each species
0109U	Infectious disease (Aspergillus species), real-time PCR for detection of

	DNA from 4 species (A. tumigatus, A. terreus, A. niger, and A. flavus), blood, lavage fluid, or tissue, qualitative reporting of presence or absence of each species
0140U	Infectious disease (fungi), fungal pathogen identification, DNA (15 fungal targets), blood culture, amplified probe technique, each target reported as detected or not detected
0141U	Infectious disease (bacteria and fungi), gram-positive organism identification and drug resistance element detection, DNA (20 gram-positive bacterial targets, 4 resistance genes, 1 pan gram-negative bacterial target, 1 pan Candida target), blood culture, amplified probe technique, each target reported as detected or not detected
0142U	Infectious disease (bacteria and fungi), gram-negative bacterial identification and drug resistance element detection, DNA (21 gram-negative bacterial targets, 6 resistance genes, 1 pan gram-positive bacterial target, 1 pan Candida target), amplified probe technique, each target reported as detected or not detected
0152U	Infectious disease (bacteria, fungi, parasites, and DNA viruses), DNA, PCR and next-generation sequencing, plasma, detection of >1,000 potential microbial organisms for significant positive pathogens
0321U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 20 bacterial and fungal organisms and identification of 16 associated antibiotic-resistance genes, multiplex amplified probe technique.
0339U	Oncology (prostate), mRNA expression profiling of HOXC6 and DLX1, reverse transcription polymerase chain reaction (RT-PCR), first-void urine following digital rectal examination, algorithm reported as probability of high-grade cancer
0370U	Infectious agent detection by nucleic acid (DNA and RNA), surgical wound pathogens, 34 microorganisms and identification of 21 associated antibiotic- resistance genes, multiplex amplified probe technique, wound swab
0371U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogen, semiquantitative identification, DNA from 16 bacterial organisms and 1 fungal organism, multiplex amplified probe technique via quantitative polymerase chain reaction (qPCR), urine
0372U	Infectious disease (genitourinary detection, multiplex amplified probe technique, urine, reported as an antimicrobial stewardship risk score
0374U	Infectious agent detection by nucleic acid (DNA or RNA), genitourinary pathogens, identification of 21 bacterial and fungal organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique, urine
0389U	Pediatric febrile illness (Kawasaki disease [KD]), interferon alpha-inducible protein 27 (IFI27) and mast cell-expressed membrane protein 1 (MCEMP1), RNA, using quantitative reverse transcription polymerase chain reaction (RT-qPCR), blood, reported as a risk score for KD
0504U	Infectious disease (urinary tract infection), identification of 17 pathologic organisms, urine, real-time PCR, reported as positive or negative for each organism
87476	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, amplified probe technique
87500	Infectious agent detection by nucleic acid (DNA or RNA); vancomycin resistance (eg, enterococcus species van A, van B), amplified probe technique

	Technique
87526	hepatitis G virus, amplified probe technique
87541	Legionella pneumophilia, amplified probe technique
87551	Mycobacteria species, amplified probe technique
87561	Mycobacteria avium-intracellulare, amplified probe technique
87640	Staphylococcus aureus, amplified probe technique
87913	Infectious agent genotype analysis by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]), mutation identification in targeted region(s)
HCPCS codes covered if selection criteria are met:	
D0606	Molecular testing for a public health related pathogen, including coronavirus
U0001	CDC 2019 Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel
U0002	2019-nCoV Coronavirus, SARS-CoV-2/2019-nCoV (COVID-19), any technique, multiple types or subtypes (includes all targets), non-CDC
U0003	Infectious agent detection by nucleic acid (DNA or RNA); Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), amplified probe technique, making use of high throughput technologies as described by CMS-2020-01-R
U0004	2019-nCoV Coronavirus, SARS-CoV-2/2019-nCoV (COVID-19), any technique, multiple types or subtypes (includes all targets), non-CDC, making use of high throughput technologies as described by CMS-2020-01-R
U0005	Infectious agent detection by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]), amplified probe technique, CDC or non-CDC, making use of high throughput technologies, completed within 2 calendar days from date of specimen collection (list separately in addition to either HCPCS code U0003 or U0004) as described by CMS-2020-01-R2
Other HCPCS codes related to the CPB:	
J2350	Injection, ocrelizumab, 1 mg
J3380	Injection, vedolizumab, 1 mg
J9312	Injection, rituximab, 10 mg
Q5115	Injection, rituximab-abbs, biosimilar, (Truxima), 10 mg
Q5119	Injection, rituximab-pvvr, biosimilar, (Ruxience), 10 mg
Q5123	Injection, rituximab-arrx, biosimilar, (Riabni), 10 mg
ICD-10 codes covered if selection criteria are met:	
A03.0 - A03.9	Shigellosis [not covered for <i>Plesiomonas shigelloides</i> ]
A04.71 - A04.72	Enterocolitis due to <i>Clostridium difficile</i>
A06.0 - A06.9	Amebiasis
A15.0 - A19.9	Tuberculosis
A21.0 - A21.9	Tularemia
A23.0 - A23.9	Brucellosis
A24.1 - A24.9	Melioidosis [when caused by <i>Burkholderia</i> infection]
A28.1	Cat-scratch disease
A36.0 - A36.9	Diphtheria
A37.00 - A37.91	Whooping cough
A39.0	Meningococcal meningitis [ <i>Neisseria meningitidis</i> ]
A41.01 - A41.02	Sepsis due to <i>Staphylococcus aureus</i>

A42.0 - A42.2,	Actinomycosis
A42.81 - A42.89	
A44.0 - A44.9	Bartonellosis [not covered for <i>Bartonella baciliformis</i> ]
A49.01 - A49.02	Methicillin susceptible and resistant <i>Staphylococcus aureus</i> infection, unspecified site
A49.2	Hemophilus influenzae infection, unspecified site
A49.3	Mycoplasma infection, unspecified site [not covered for oral swab for <i>hominis</i> and urea plasma <i>urealyticum</i> ]
A50.01 - A53.9	Syphilis
A54.00 - A54.9	Gonococcal infections
A55	Chlamydial lymphogranuloma ( <i>venereum</i> )
A57	Chancroid
A59.00 - A59.9	Trichomoniasis
A69.8	Other specified spirochetal infections [ <i>Borrelia miyamotoi</i> , acute phase]
A70	Chlamydia psittaci infections
A71.0 - A71.9	Trachoma
A74.0	Chlamydial conjunctivitis
A74.81 - A74.89	Other chlamydial diseases
A74.9	Chlamydial infection, unspecified
A75.0	Epidemic louse-borne typhus fever due to <i>Rickettsia prowazekii</i>
A75.2	Typhus fever due to <i>rickettsia typhi</i>
A77.0 - A77.3	Spotted fever [tick-borne rickettsioses]
A77.9	
A77.40 - A77.49	Ehrlichiosis
A78	Q Fever
A80.0 - A80.9	Acute poliomyelitis
A81.2	Progressive multifocal leukoencephalopathy
A87.0	Enteroviral meningitis
A90	Dengue fever [classical dengue]
A91	Dengue hemorrhagic fever
A92.30 - A92.39	West Nile virus infection
A92.4	Rift Valley fever
A92.5	Zika virus disease
A92.8	Other specified mosquito-borne viral fevers
A93.2	Colorado tick fever
A95.0 - A95.9	Yellow fever
A98.0	Crimean-Congo hemorrhagic fever
A98.4	Ebola virus disease
B00.0 - B00.9	Herpesviral [herpes simplex] infections
B01.0 - B01.9	Varicella [chickenpox]
B02.0 - B02.9	Zoster [herpes zoster]
B05.0 - B05.9	Measles
B06.00 - B06.9	Rubella [German measles]
B08.21	Exanthema subitum [sixth disease] due to human herpesvirus 6
B10.01	Human herpesvirus 6 encephalitis
B10.81	Human herpesvirus 6 infection
B16.0 - B16.9	Acute hepatitis B

B17.10 - B17.11	Acute hepatitis C
B18.0 - B18.1	Chronic viral hepatitis B
B18.2	Chronic viral hepatitis C
B19.10 - B19.11	Unspecified viral hepatitis B
B19.20 - B19.21	Unspecified viral hepatitis C
B20	Human immunodeficiency virus [HIV] disease
B25.0 - B25.9	Cytomegaloviral disease
B26.0 - B26.9	Mumps
B34.0, B97.0	Adenovirus infection, unspecified and as the cause of diseases classified elsewhere
B34.1	Enterovirus infection, unspecified [Group A and B]
B34.3	Parvovirus infection, unspecified [not covered for persons with autoimmune neutropenia]
B37.0 - B37.9	Candidiasis [not covered for suspected candida auris]
B47.1	Actinomycetoma
B47.9	Mycetoma, unspecified
B50.0 - B54	Malaria
B55.0 - B55.9	Leishmaniasis
B58.00 – B58.0, 1	Toxoplasmosis
B58.1 – B58.9	
B60.0	Babesiosis
B60.19	Other acanthamebic disease
B95.1	Streptococcus group B, as the cause of diseases classified elsewhere
B95.61 - B95.62	Methicillin susceptible or resistant Staphylococcus aureus infection as the cause of diseases classified elsewhere
B96.00 - B96.09	Mycoplasma pneumoniae [M. pneumoniae] as the cause of diseases classified elsewhere
B96.3	Hemophilus influenzae [H. influenzae] as the cause of diseases classified elsewhere
B97.11	Coxackievirus as the cause of diseases classified elsewhere
B97.12	Echovirus as the cause of diseases classified elsewhere [not covered for parechovirus for recurrent fever]
B97.21	SARS-associated coronavirus as the cause of diseases classified elsewhere
B97.30 - B97.39	Retrovirus as the cause of diseases classified elsewhere
D45	Polycythemia vera
D47.z1	Post-transplant lymphoproliferative disorder (PTLD)
D89.810 - D89.813	Graft-versus-host disease
G93.89	Other specified disorders of brain [intracranial calcification in infants born to women who traveled to or resided in an area with Zika virus transmission while pregnant]
H16.001 – H16.079	Corneal ulcer [not covered Cutibacterium acnes (formerly proionibacterium acnes)]
J02.0	Streptococcal pharyngitis
J09.x1 - J09.x9	Influenza due to identified novel influenza A virus
J10.00 - J10.89	Influenza due to other identified influenza virus
J11.00 - J11.89	Influenza due to unidentified influenza virus
J15.212	Pneumonia due to methicillin resistant Staphylococcus aureus
J15.7	Pneumonia due to Mycoplasma pneumoniae

J16.0	Chlamydial pneumonia
J20.0	Acute bronchitis due to Mycoplasma pneumoniae
J20.1	Acute bronchitis due to Hemophilus influenzae
J20.3	Acute bronchitis due to coxsackievirus
J20.7	Acute bronchitis due to echovirus
J21.0	Acute bronchiolitis due to respiratory syncytial virus
K90.81	Whipple's disease
L29.2	Pruritus vulvae
L29.3	Anogenital pruritus, unspecified
M02.30 - M02.39	Reiter's disease
N34.1 - N34.2	Nonspecific and other urethritis
N72	Inflammatory disease of cervix uteri
N76.89	Other specified inflammation of vagina and vulva [vaginal burning, irritation and erythema]
N89.8	Other specified noninflammatory disorders of vagina [Vaginal discharge]
N94.10 – N94.19	Dyspareunia
O09.00 - O09.93	Supervision of high risk pregnancy [antenatal screening with broth enrichment for group B streptococcal infection in pregnant women at 36 to 37 weeks gestation]
O20.0 - O29.93	Other maternal disorders predominantly related to pregnancy [antenatal screening with broth enrichment for group B streptococcal infection in pregnant women at 36 to 37 weeks gestation]
O98.511 - O98.53	Other viral diseases complicating pregnancy, childbirth and the puerperium
P28.40, P28.41, P28.42, P28.43, P28.49	Other apnea of newborn [apnea with/without cyanosis]
P35.0	Congenital rubella syndrome
P35.4	Congenital Zika virus disease
Q02	Microcephaly [infants born to women who traveled to or resided in an area with Zika virus transmission while pregnant]
R05.1 - R05.9	Cough
R11.10	Vomiting, unspecified [posttussive vomiting]
R19.7	Diarrhea, unspecified [for Clostridium difficile diagnosis]
R30.0	Dysuria
R75	Inconclusive laboratory evidence of human immunodeficiency virus [HIV]
R87.610 - R87.613, R87.619	Abnormal cytological findings in specimens from cervix uteri
R87.810	Cervical high risk human papillomavirus [HIV] DNA test positive
S10.16XA - S10.16XS, S10.86XA - S10.86XS, S10.96XA - S10.96XS, S20.161A - S20.169S, S20.361A - S20.369S, S20.461A -	Insect bite (nonvenomous)

S20.469S,  
S20.96XA -  
S20.96XS,  
S30.860A -  
S30.867S,  
S40.261A -  
S40.269S,  
S40.861A -  
S40.869S,  
S50.361A -  
S50.369S,  
S50.861A -  
S50.869S,  
S60.361A -  
S60.369S,  
S60.460A -  
S60.469S,  
S60.561A -  
S60.569S,  
S60.861A -  
S60.869S,  
S70.261A -  
S70.269S,  
S70.361A -  
S70.369S,  
S80.261A -  
S80.269S,  
S80.861A -  
S80.869S,  
S90.461A -  
S90.466S,  
S90.561A -  
S90.569S,  
S90.861A -  
S90.869S

W57.XXXA -	Bitten or stung by nonvenomous insect and other nonvenomous arthropods
W57.XXXS	
Z01.42	Encounter for cervical smear to confirm findings of recent normal smear following initial abnormal smear
Z11.1	Encounter for screening respiratory tuberculosis
Z11.2	Encounter for screening for other bacterial diseases
Z11.3	Encounter for screening for infections with a predominantly sexual mode of transmission [not covered for routine screening of trichomonas in asymptomatic men and women]
Z11.4	Encounter for screening for human immunodeficiency virus [HIV]
Z11.59	Encounter for screening for other viral diseases
Z11.8	Encounter for screening for other infectious and parasitic diseases [not covered for routine screening of trichomonas in asymptomatic women]
Z16.11	Resistance to penicillins
Z20.4	Contact with and (suspected) exposure to rubella
Z20.5	Contact with and (suspected) exposure to viral hepatitis
Z20.6	Contact with and (suspected) exposure to human immunodeficiency virus [HIV]
Z20.820	Contact with and (suspected) exposure to varicella
Z20.821	Contact with and (suspected) exposure to Zika virus

Z20.828	Contact with and (suspected) exposure to other viral communicable diseases [includes Zika virus] [symptoms of or exposure to Zika virus]
Z20.89	Contact with and (suspected) exposure to other communicable diseases
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z22.4	Carrier of infections with a predominantly sexual mode of transmission
Z22.51	Carrier of viral hepatitis B
Z22.52	Carrier of viral hepatitis C
Z34.00 - Z34.93	Encounter for supervision of normal pregnancy [antenatal screening with broth enrichment for group B streptococcal infection in pregnant women at 36 to 37 weeks gestation] [not covered for routine screening of trichomonas in asymptomatic men and women; not covered for mycoplasma hominis screening in asymptomatic pregnant women]
Z36.85	Encounter for antenatal screening for Streptococcus B [antenatal screening with broth enrichment for group B streptococcal infection in pregnant women at 36 to 37 weeks gestation]
Z72.51 - Z72.53	High risk sexual behavior
Z77.21	Contact with and (suspected) exposure to potentially hazardous body fluids [women at high risk for infection, who have new or multiple partners, a history of STDs, exchange sex for payment] [symptoms of or exposure to Zika virus]
Z94.0 - Z94.84	Transplanted organ and tissue status
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):	
A02.0 - A02.9	Other salmonella infections
A04.5	Campylobacter enteritis
A04.6	Enteritis due to Yersinia enterocolitica
A04.8	Other specified bacterial intestinal infections [Enterobacter aerogenes]
A05.3	Foodborne Vibrio parahaemolyticus intoxication
A07.1	Giardiasis [lambliasis]
A07.2	Cryptosporidiosis
A07.4	Cyclosporiasis
A08.32	Astrovirus enteritis
A27.0 - A27.9	Leptospirosis
A32.0 - A32.9	Listeriosis
A40.3	Sepsis due to Streptococcus, pneumoniae
A41.51	Sepsis due to Escherichia coli [E. coli]
A41.52	Sepsis due to Pseudomonas
A41.53	Sepsis due to serratia
A41.81	Sepsis due to Enterococcus
A49.01	Methicillin susceptible Staphylococcus aureus infection, unspecified site [Staphylococcus saprophyticus]
A49.1	Streptococcus infection [other than group B]
A58	Granuloma inguinale
A69.20 - A69.29	Lyme disease
A81.00 - A81.09	Creutzfeldt-Jakob disease
B08.1	Molluscum contagiosum
B08.20	Exanthema subitum [sixth disease], unspecified
B08.22	Exanthema subitum [sixth disease] due to human herpesvirus 7
B09	Unspecified viral infection characterized by skin and mucous membrane lesion

B10.09	Other human herpesvirus encephalitis
B10.82	Human herpesvirus 7 infection
B10.89	Other human herpesvirus infection
B17.8	Other specified acute viral hepatitis
B35.1	Tinea unguium
B35.3	Tinea pedis
B36.2	White piedra
B38.0 - B38.9	coccidioidomycosis
B39.0 - B39.5	Histoplasmosis
B40.0 - B40.9	Blastomycosis
B42.0 - B42.9	Sporotrichosis
B44.0 - B44.7, B44.89 - B44.9	Aspergillosis
B45.0 - B45.9	Cryptococcosis
B48.8	Other specified mycoses [Cochliobolus spicifer, Cochliobolus lunatus]
B58.09	Other toxoplasma oculopathy [uveitis]
B59	Pneumocystosis
B95.0, B95.2, B95.3 - B95.5	Streptococcus as the cause of diseases classified elsewhere [other than group B]
B95.7 - B95.8	Other and unspecified staphylococcus as the cause of diseases classified elsewhere [Staphylococcus saprophyticus] [Staphylococcus lugdunensis]
B96.20 - B96.29	Escherichia coli [E.coli] as the cause of diseases classified elsewhere
B96.4	Proteus (mirabilis) (morganii) as the cause of diseases classified elsewhere
B96.5	Pseudomonas (aeruginosa) (mallei) (pseudomallei) as the cause of diseases classified elsewhere
B96.6	Bacteroides fragilis [B. fragilis] as the cause of diseases classified elsewhere
B96.81	Helicobacter pylori [H. pylori] as the cause of diseases classified elsewhere
B96.89	Other specified bacterial agents as the cause of diseases classified elsewhere [Acinetobacter baumannii, Enterobacter cloacae, Stenotrophomonas maltophilia, Vibrio vulnificus, Vibrio cholerae, Eggerthella, Prevotella bivia]
B97.29	Other coronavirus as the cause of diseases classified elsewhere
B97.6	Parvovirus as the cause of diseases classified elsewhere, unspecified
B97.7	Papillomavirus as the cause of diseases classified elsewhere
C46.0 - C46.9	Kaposi's sarcoma
D06.0 - D06.9	Carcinoma in situ of cervix uteri
D89.40 - D89.49	Mast cell activation syndrome and related disorders
D89.82	Autoimmune lymphoproliferative syndrome [ALPS]
G30.0 - G30.9	Alzheimer's disease
H60.00 - H60.93	Otitis externa
I25.10 - I25.119	Atherosclerotic heart disease of native coronary artery
I25.700 - I25.812	Atherosclerosis of coronary artery bypass graft(s)
I70.0 - I70.92	Atherosclerosis
J13	Pneumonia due to Streptococcus pneumoniae
J15.0	Pneumonia due to Klebsiella pneumoniae
J15.1	Pneumonia due to Pseudomonas

J15.4	Pneumonia due to other streptococci
J45.20 - J45.998	Asthma, mild intermittent, mild persistent, moderate persistent, or severe persistent, and uncomplicated, exacerbation, or status asthmaticus
K25.0 - K28.9	Gastric, duodenal, peptic or gastrojejunal ulcer
L29.0	Pruritus ani
L60.2	Onychogryphosis
L70.8	Other acne [Cutibacterium acnes (formerly propionibacterium acnes)]
M00.10 - M00.19	Pneumococcal arthritis and polyarthritis
M25.40 - M25.48	Effusion of joint
M30.3	Mucocutaneous lymph node syndrome [Kawasaki]
N39.0 - N39.9	Urinary tract infection
N41.0 - N41.9	Prostatitis
N87.0 - N87.9	Dysplasia of cervix uteri
O98.611 - O98.619	Protozoal diseases complicating pregnancy
P35.1	Congenital cytomegalovirus infection
Q84.5	Enlarged and hypertrophic nails
R13.10 – R13.19	Dysphagia
R53.0 - R53.83	Malaise and fatigue
R87.810	Cervical high risk human papillomavirus (HPV) DNA test positive
Z00.00 - Z00.01	Encounter for general adult medical examination [not covered for routine screening of trichomonas in asymptomatic women]
Z01.411 - Z01.419	Encounter for gynecological examination (general) (routine) [not covered for routine screening of trichomonas in asymptomatic women]
Z11.51	Encounter for screening for human papillomavirus (HPV)
Z11.6	Encounter for screening for other protozoal diseases and helminthiases [malaria]
Z13.6	Encounter for screening for cardiovascular disorders
Z13.89	Encounter for screening for other disorder [genitourinary]
Z16.21 - Z16.22	Resistance to vancomycin and vancomycin related antibiotics
Z30.40 - Z30.9	Encounter for surveillance of contraceptives [not covered for routine screening of trichomonas in asymptomatic men and women]
Z34.00 - Z34.93	Encounter for supervision of normal pregnancy [not covered for routine screening of trichomonas in asymptomatic men and women]
Z85.41	Personal history of malignant neoplasm of cervix uteri
Z87.11	Personal history of peptic ulcer disease
Monkeypox virus (Orthopoxvirus):	
CPT codes covered if selection criteria are met:	
87593	Infectious agent detection by nucleic acid (DNA or RNA); orthopoxvirus (e.g., monkeypox virus, cowpox virus, vaccinia virus), amplified probe technique, each [not covered for repeat testing]
ICD-10 codes covered if selection criteria are met:	
K51.20 - K51.219	Ulcerative (chronic) proctitis
K62.6	Ulcer of anus and rectum
L08.0	Pyoderma
L98.411 - L98.499	Non-pressure chronic ulcer of skin
L98.8	Other specified disorders of the skin and subcutaneous tissue
L98.9	Disorder of the skin and subcutaneous tissue, unspecified
N76.5 - N76.6	Ulceration of vagina and vulva

R21	Rash and other nonspecific skin eruption
Z20.2	Contact with and (suspected) exposure to infections with a predominantly sexual mode of transmission
Z20.828	Contact with and (suspected) exposure to other viral communicable diseases
Z20.89	Contact with and (suspected) exposure to other communicable diseases
Z72.51	High risk heterosexual behavior
Z72.52	High risk homosexual behavior
Z72.53	High risk bisexual behavior
Z77.128	Contact with and (suspected) exposure to other hazards in the physical environment

Cerebrospinal fluid (CSF) pathogen panel:

CPT codes covered if selection criteria are met:

87483	Infectious agent detection by nucleic acid (DNA or RNA); central nervous system pathogen (eg, Neisseria meningitidis, Streptococcus pneumoniae, Listeria, Haemophilus influenzae, E. coli, Streptococcus agalactiae, enterovirus, human parechovirus, herpes simplex virus type 1 and 2, human herpesvirus 6, cytomegalovirus, varicella zoster virus, Cryptococcus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
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ICD-10 codes covered if selection criteria are met:

G00.0 - G03.9	Meningitis
G04.00 - G05.4	Encephalitis, myelitis, and encephalomyelitis
M43.6	Torticollis
R11.2	Nausea and vomiting, unspecified
R40.20 - R40.2444	Coma
R41.82	Altered mental status, unspecified
R41.0	Disorientation, unspecified [confusion]
R44.0 - R44.3	Hallucinations
R45.1	Restlessness and agitation
R50.9	Fever, unspecified
R51.0 - R51.9	Headache
R55	Syncope and collapse [loss of consciousness]
R56.9	Unspecified convulsions [seizures]

PCR testing for Coronavirus COVID-19 more than 5 respiratory pathogens:

CPT codes covered if selection criteria are met:

0202U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected
0223U	Infectious disease (bacterial or viral respiratory tract infection), pathogen-specific nucleic acid (DNA or RNA), 22 targets including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), qualitative RT-PCR, nasopharyngeal swab, each pathogen reported as detected or not detected
0225U	Infectious disease (bacterial or viral respiratory tract infection) pathogen-specific DNA and RNA, 21 targets, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected

87632	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets
87633	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
CPT codes not covered for indications listed in the CPB:	
87913	Infectious agent genotype analysis by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]), mutation identification in targeted region(s)
ICD-10 codes covered if selection criteria are met:	
B20	Human immunodeficiency virus [HIV] disease
B59	Pneumocystosis
B97.35	Human immunodeficiency virus, type 2 [HIV 2] as the cause of diseases classified elsewhere
C00.0 - C96.9	Malignant neoplasm
D70.0 - D77	Other disorders of blood and blood-forming organs
D80.0 - D89.9	Certain disorders of the immune mechanism
E08.00 - E13.9	Diabetes mellitus
E40 - E46	Malnutrition
G35	Multiple sclerosis
I12.0	Unstable angina
I13.11	Hypertensive heart and chronic kidney disease without heart failure, with stage 5 chronic kidney disease, or end stage renal disease
I13.2	Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease
J43.0 - J43.9, J98.2, J98.3	Emphysema
J44.0 - J44.9	Other chronic obstructive pulmonary disease
J45.20 - J45.998	Asthma, mild intermittent, mild persistent, moderate persistent, or severe persistent, and uncomplicated, exacerbation, or status asthmaticus
J60 - J70.9	Lung disease due to external agents
J80	Acute respiratory distress syndrome
J81.0 - J81.1	Pulmonary edema
J82.1 - J82.89	Pulmonary eosinophilia, not elsewhere classified
J84.01 - J84.9	Other interstitial pulmonary diseases
J85.0 - J86.9	Suppurative and necrotic conditions in the lower respiratory tract
J90 - J94.9	Other diseases of the pleura
J95.00 - J95.89	Intraoperative and postprocedural complications and disorders of respiratory system, not elsewhere classified
J96.00 - J96.92	Respiratory failure
J98.01	Acute bronchospasm
J98.11 - J98.19	Pulmonary collapse
K91.2	Postsurgical malabsorption, not elsewhere classified
M32.0 - M32.9	Systemic lupus erythematosus (SLE)
M34.0 - M34.9	Systemic sclerosis [scleroderma]

M35.00 - M35.9	Other systemic involvement of connective tissue
N18.5	Chronic kidney disease, stage 5
N18.6	End stage renal disease
T86.00 - T86.99	Complications of transplanted organs and tissue
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z48.21 - Z48.298	Encounter for aftercare following heart organ transplant
Z49.01 - Z49.32	Encounter for care involving renal dialysis
Z51.11	Encounter for antineoplastic chemotherapy
Z51.12	Encounter for antineoplastic immunotherapy
Z79.52	Long term (current) use of systemic steroids
Z94.0 - Z94.84	Transplanted organ and tissue status
Z99.2	Dependence on renal dialysis
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):	
R56.00 - R56.01	Febrile convulsions
PCR testing for Coronavirus COVID-19 up to 5 respiratory pathogens:	
CPT codes covered if selection criteria are met:	
0240U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 3 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B), upper respiratory specimen, each pathogen reported as detected or not detected
0241U	Infectious disease (viral respiratory tract infection), pathogen-specific RNA, 4 targets (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], influenza A, influenza B, respiratory syncytial virus [RSV]), upper respiratory specimen, each pathogen reported as detected or not detected
87631	Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
CPT codes not covered for indications listed in the CPB:	
87913	Infectious agent genotype analysis by nucleic acid (DNA or RNA); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (coronavirus disease [COVID-19]), mutation identification in targeted region(s)
Other HCPCS codes related to the CPB:	
C9803	Hospital outpatient clinic visit specimen collection for severe acute respiratory syndrome coronavirus 2 (sars-cov-2) (coronavirus disease [covid-19])
G2023	Specimen collection for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]), any specimen source
G2024	Specimen collection for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Coronavirus disease [COVID-19]) from an individual in a SNF or by a laboratory on behalf of a HHA, any specimen source
G2025	Distant site telehealth services provided by Rural Health Clinics (RHCs) and Federally Qualified Health Centers (FQHCs)
ICD-10 codes covered if selection criteria are met (not all-inclusive):	
B20	Human immunodeficiency virus [HIV] disease
B59	Pneumocystosis
B97.29	Other coronavirus as the cause of diseases classified elsewhere

B97.35	Human immunodeficiency virus, type 2 [HIV 2] as the cause of diseases classified elsewhere
C00.0 - C96.9	Malignant neoplasm
D70.0 - D77	Other disorders of blood and blood-forming organs
D80.0 - D89.9	Certain disorders of the immune mechanism
E08.00 - E13.9	Diabetes mellitus
E40 - E46	Malnutrition
G35	Multiple sclerosis
I12.0	Unstable angina
I13.11	Hypertensive heart and chronic kidney disease without heart failure, with stage 5 chronic kidney disease, or end stage renal disease
I13.2	Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease
J12.82	Pneumonia due to coronavirus disease 2019
J12.89	Other viral pneumonia
J20.8	Acute bronchitis due to other specified organisms
J22	Unspecified acute lower respiratory infection
J40	Bronchitis, not specified as acute or chronic
J43.0 - J43.9, J98.2, J98.3	Emphysema
J44.0 - J44.9	Other chronic obstructive pulmonary disease
J45.20 - J45.998	Asthma, mild intermittent, mild persistent, moderate persistent, or severe persistent, and uncomplicated, exacerbation, or status asthmaticus
J60 - J70.9	Lung disease due to external agents
J80	Acute respiratory distress syndrome
J81.0 - J81.1	Pulmonary edema
J82.1 - J82.89	Pulmonary eosinophilia, not elsewhere classified
J84.01 - J84.9	Other interstitial pulmonary diseases
J85.0 - J86.9	Suppurative and necrotic conditions in the lower respiratory tract
J90 - J94.9	Other diseases of the pleura
J95.00 - J95.89	Intraoperative and postprocedural complications and disorders of respiratory system, not elsewhere classified
J96.00 - J96.92	Respiratory failure
J98.01	Acute bronchospasm
J98.11 - J98.19	Pulmonary collapse
J98.8	Other specified respiratory disorders
K91.2	Postsurgical malabsorption, not elsewhere classified
M32.0 - M32.9	Systemic lupus erythematosus (SLE)
M34.0 - M34.9	Systemic sclerosis [scleroderma]
M35.00 - M35.9	Other systemic involvement of connective tissue
N18.5	Chronic kidney disease, stage 5
N18.6	End stage renal disease
R05.1 - R05.9	Cough
R06.02	Shortness of breath
R50.9	Fever, unspecified
T86.00 - T86.99	Complications of transplanted organs and tissue
U07.1	COVID-19

Z03.818	Encounter for observation for suspected exposure to other biological agents ruled out
Z20.828	Contact with and (suspected) exposure to other viral communicable diseases
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z48.21 - Z48.298	Encounter for aftercare following heart organ transplant
Z49.01 - Z49.32	Encounter for care involving renal dialysis
Z51.11	Encounter for antineoplastic chemotherapy
Z51.12	Encounter for antineoplastic immunotherapy
Z79.52	Long term (current) use of systemic steroids
Z94.0 - Z94.84	Transplanted organ and tissue status
Z99.2	Dependence on renal dialysis

ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):

R56.00 - R56.01	Febrile convulsions
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PCR testing for Gastrointestinal pathogen panel up to 11 targets:

CPT codes covered if selection criteria are met:

87505	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 3-5 targets
87506	gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 6-11 targets

ICD-10 codes covered if selection criteria are met:

A04.71 - A04.72	Enterocolitis due to Clostridium difficile
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A04.8	Other specified bacterial intestinal infections
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R19.7	Diarrhea, unspecified
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PCR testing for Gastrointestinal pathogen panel more than 11 targets:

CPT codes covered if selection criteria are met:

0369U	Infectious agent detection by nucleic acid (DNA and RNA), gastrointestinal pathogens, 31 bacterial, viral, and parasitic organisms and identification of 21 associated antibiotic-resistance genes, multiplex amplified probe technique
87507	Infectious agent detection by nucleic acid (DNA or RNA); gastrointestinal pathogen (eg, Clostridium difficile, E. coli, Salmonella, Shigella, norovirus, Giardia), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets

ICD-10 codes covered if selection criteria are met (R19.7 also required):

B20	Human immunodeficiency virus [HIV] disease
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B59	Pneumocystosis
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B97.35	Human immunodeficiency virus, type 2 [HIV 2] as the cause of diseases classified elsewhere
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C00.0 - C96.9	Malignant neoplasm
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D70.0 - D77	Other disorders of blood and blood-forming organs
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D80.0 - D89.9	Certain disorders of the immune mechanism
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E08.00 - E13.9	Diabetes mellitus
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E40 - E46	Malnutrition
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G35	Multiple sclerosis
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I12.0	Unstable angina
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I13.11	Hypertensive heart and chronic kidney disease without heart failure, with stage 5 chronic kidney disease, or end stage renal disease
I13.2	Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease
J43.0 - J43.9, J98.2, J98.3	Emphysema
J44.0 - J44.9	Other chronic obstructive pulmonary disease
J45.20 - J45.998	Asthma, mild intermittent, mild persistent, moderate persistent, or severe persistent, and uncomplicated, exacerbation, or status asthmaticus
J60 - J70.9	Lung disease due to external agents
J80	Acute respiratory distress syndrome
J81.0 - J81.1	Pulmonary edema
J82.1 - J82.89	Pulmonary eosinophilia, not elsewhere classified
J84.01 - J84.9	Other interstitial pulmonary diseases
J85.0 - J86.9	Suppurative and necrotic conditions in the lower respiratory tract
J90 - J94.9	Other diseases of the pleura
J95.00 - J95.89	Intraoperative and postprocedural complications and disorders of respiratory system, not elsewhere classified
J96.00 - J96.92	Respiratory failure
J98.01	Acute bronchospasm
J98.11 - J98.19	Pulmonary collapse
K91.2	Postsurgical malabsorption, not elsewhere classified
M32.0 - M32.9	Systemic lupus erythematosus (SLE)
M34.0 - M34.9	Systemic sclerosis [scleroderma]
M35.00 - M35.9	Other systemic involvement of connective tissue
N18.5	Chronic kidney disease, stage 5
N18.6	End stage renal disease
R19.7	Diarrhea, unspecified [required for all claims]
T86.00 - T86.99	Complications of transplanted organs and tissue
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z48.21 - Z48.298	Encounter for aftercare following heart organ transplant
Z49.01 - Z49.32	Encounter for care involving renal dialysis
Z51.11	Encounter for antineoplastic chemotherapy
Z51.12	Encounter for antineoplastic immunotherapy
Z79.52	Long term (current) use of systemic steroids
Z94.0 - Z94.84	Transplanted organ and tissue status
Z99.2	Dependence on renal dialysis
HSV-1, HSV- 2, and varicella zoster virus (VZV) assay:	
CPT codes covered if selection criteria are met:	
0527U	Herpes simplex virus (HSV) types 1 and 2 and Varicella zoster virus (VZV), amplified probe technique, each pathogen reported as detected or not detected
ICD-10 codes covered if selection criteria are met:	
A60.00 - A60.9	Anogenital herpesviral [herpes simplex] infections
B00.0 - B00.9	Herpes viral [herpes simplex] infections
B01.0 - B01.9	Varicella [chickenpox]
B02.0 - B02.9	Zoster [herpes zoster]
D84.81	Immunodeficiency due to conditions classified elsewhere
D84.822	Immunodeficiency due to external causes

D84.89	Other immunodeficiencies
G04.00 - G04.91	Encephalitis, myelitis and encephalomyelitis
L98.8	Other specified disorders of the skin and subcutaneous tissue [skin lesions]
L98.9	Disorder of the skin and subcutaneous tissue, unspecified [skin lesions]
N45.2	Orchitis
N45.3	Epididymo-orchitis
P35.2	Congenital herpesviral [herpes simplex] infection
R21	Rash and other nonspecific skin eruption [skin lesions]

PCR testing for Respiratory Viral Panels - amplified probe:

CPT codes covered if selection criteria are met:

0115U	Respiratory infectious agent detection by nucleic acid (DNA and RNA), 18 viral types and subtypes and 2 bacterial targets, amplified probe technique, including multiplex reverse transcription for RNA targets, each analyte reported as detected or not detected
0373U	Infectious agent detection by nucleic acid (DNA and RNA), respiratory tract infection, 17 bacteria, 8 fungus, 13 virus, and 16 antibiotic-resistance genes, multiplex amplified probe technique, upper or lower respiratory specimen
0528U	Lower respiratory tract infectious agent detection, 18 bacteria, 8 viruses, and 7 antimicrobial-resistance genes, amplified probe technique, including reverse transcription for RNA targets, each analyte reported as detected or not detected with semiquantitative results for 15 bacteria

ICD-10 codes covered if selection criteria are met:

B20	Human immunodeficiency virus [HIV] disease
B59	Pneumocystosis
B97.35	Human immunodeficiency virus, type 2 [HIV 2] as the cause of diseases classified elsewhere
C00.0 - C96.9	Malignant neoplasm
D70.0 - D77	Other disorders of blood and blood-forming organs
D80.0 - D89.9	Certain disorders of the immune mechanism
E08.00 - E13.9	Diabetes mellitus
E40 - E46	Malnutrition
G35	Multiple sclerosis
I12.0	Unstable angina
I13.11	Hypertensive heart and chronic kidney disease without heart failure, with stage 5 chronic kidney disease, or end stage renal disease
I13.2	Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end stage renal disease
J43.0 - J43.9, J98.2, J98.3	Emphysema
J44.0 - J44.9	Other chronic obstructive pulmonary disease
J45.20 - J45.998	Asthma, mild intermittent, mild persistent, moderate persistent, or severe persistent, and uncomplicated, exacerbation, or status asthmaticus
J60 - J70.9	Lung disease due to external agents
J80	Acute respiratory distress syndrome
J81.0 - J81.1	Pulmonary edema
J82.0 - J82.89	Pulmonary eosinophilia, not elsewhere classified
J84.01 - J84.9	Other interstitial pulmonary diseases

J85.0 - J86.9	Suppurative and necrotic conditions in the lower respiratory tract
J90 - J94.9	Other diseases of the pleura
J95.00 - J95.89	Intraoperative and postprocedural complications and disorders of respiratory system, not elsewhere classified
J96.00 - J96.92	Respiratory failure
J98.01	Acute bronchospasm
J98.11 - J98.19	Pulmonary collapse
K91.2	Postsurgical malabsorption, not elsewhere classified
M32.0 - M32.9	Systemic lupus erythematosus (SLE)
M34.0 - M34.9	Systemic sclerosis [scleroderma]
M35.00 - M35.9	Other systemic involvement of connective tissue
N18.5	Chronic kidney disease, stage 5
N18.6	End stage renal disease
T86.00 - T86.99	Complications of transplanted organs and tissue
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z48.21 - Z48.298	Encounter for aftercare following heart organ transplant
Z49.01 - Z49.32	Encounter for care involving renal dialysis
Z51.11	Encounter for antineoplastic chemotherapy
Z51.12	Encounter for antineoplastic immunotherapy
Z79.52	Long term (current) use of systemic steroids
Z94.0 - Z94.84	Transplanted organ and tissue status
Z99.2	Dependence on renal dialysis
ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):	
R56.00 - R56.01	Febrile convulsions
PCR testing for Symptomatic Vaginitis:	
CPT codes covered if selection criteria are met:	
0330U	Infectious agent detection by nucleic acid (DNA or RNA), vaginal pathogen panel, identification of 27 organisms, amplified probe technique, vaginal swab
0352U	Infectious disease (bacterial vaginosis and vaginitis), multiplex amplified probe technique, for detection of bacterial vaginosis-associated bacteria (BVAB-2, Atopobium vaginae, and Megasphaera type 1), algorithm reported as detected or not detected and separate detection of Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata/Candida krusei, and trichomonas vaginalis, vaginal-fluid specimen, each result reported as detected or not detected
81513	Infectious disease, bacterial vaginosis, quantitative real-time amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis
81514	Infectious disease, bacterial vaginosis and vaginitis, quantitative real-time amplification of DNA markers for Gardnerella vaginalis, Atopobium vaginae, Megasphaera type 1, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), and Lactobacillus species (L. crispatus and L. jensenii), utilizing vaginal-fluid specimens, algorithm reported as a positive or negative for high likelihood of bacterial vaginosis, includes separate detection of Trichomonas vaginalis and/or Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, when reported
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida

	species, amplified probe technique
87511	Gardnerella vaginalis, amplified probe technique
87512	Gardnerella vaginalis, quantification
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
ICD-10 codes covered if selection criteria are met:	
B37.31, B37.32	Candidiasis of vulva and vagina
L29.2	Pruritus vulvae
L29.3	Anogenital pruritus, unspecified
N76.0	Acute vaginitis
N76.1	Subacute and chronic vaginitis
N76.2	Acute vulvitis
N76.3	Subacute and chronic vulvitis
N77.1	Vaginitis, vulvitis and vulvovaginitis in diseases classified elsewhere
N89.8	Other specified noninflammatory disorders of vagina
Sepsis pathogen panel:	
CPT codes covered if selection criteria are met:	
Sepsis pathogen panel - no specific code:	
Other CPT codes related to the CPB:	
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates (includes anaerobic culture, if appropriate)
ICD-10 codes covered if selection criteria are met:	
A40.0 - A41.9	Sepsis
R00.0	Tachycardia, unspecified
R11.2	Nausea and vomiting, unspecified
R19.7	Diarrhea, unspecified
R23.1	Pallor
R34	Anuria and oliguria
R50.9	Fever, unspecified
R53.83	Other fatigue
R53.1	Weakness
R61	Generalized hyperhidrosis
R68.0	Hypothermia, not associated with low environmental temperature
Pharyngitis panel (qualitative and quantitative) PCR testing:	
CPT codes not covered for indications listed in the CPB:	
Pharyngitis panel (qualitative and quantitative) PCR testing -no specific code	
ICD-10 codes not covered for indications listed in the CPB:	
H65.00 - H65.93	Acute serous otitis media
H66.001 - H66.93	Suppurative and unspecified otitis media
J02.8	Acute pharyngitis due to other specified organisms
J02.9	Acute pharyngitis, unspecified
J31.2	Chronic pharyngitis
K12.0	Recurrent oral aphthae
K12.1	Other forms of stomatitis
PCR testing for microbial identification - quantification:	
CPT codes covered if selection criteria are met:	
87497	Infectious agent detection by nucleic acid (DNA or RNA); cytomegalovirus, quantification
87517	hepatitis B virus, quantification
87522	hepatitis C virus, quantification
87533	Herpes virus-6 quantification

87536	HIV-1, quantification
87539	HIV-2, quantification
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism

CPT codes not covered for indications listed in the CPB:

EoGenius (a 96-gene quantitative PCR array and an associated dual-algorithm), uBiome  
SmartGut, SmartJane, SmartFlu and Explorer, rectal swab testing for identification of antibiotic  
resistant bacteria - no specific code

87472	Infectious agent detection by nucleic acid (DNA or RNA); Bartonella (B. henselae, B Quintana), quantification
87477	Borrelia burgdorferi, quantification
87482	Candida species, quantification
87487	Chlamydia pneumoniae, quantification
87527	hepatitis G, quantification
87530	Herpes simplex virus, quantification
87533	Herpes virus-6, quantification
87542	Legionella pneumophilia, quantification
87562	Mycobacteria avium-intracellulare, quantification
87582	Mycoplasma pneumoniae, quantification
87592	Neisseria gonorrhoeae, quantification
87652	Streptococcus, group A, quantification

Other HCPCS codes related to the CPB:

J2350	Injection, ocrelizumab, 1 mg
J9312	Injection, rituximab, 10 mg
Q5115	Injection, rituximab-abbs, biosimilar, (Truxima), 10 mg
Q5119	Injection, rituximab-pvvr, biosimilar, (ruxience), 10 mg
Q5123	Injection, rituximab-arrx, biosimilar, (riabni), 10 mg

ICD-10 codes covered if selection criteria are met:

B01.0 - B01.9	Varicella [chickpox] [for diagnosis and also to distinguish wild-type virus from vaccination in previously immunized persons with signs or symptoms of varicella-zoster infection]
B08.21	Exanthema subitum [sixth disease] due to human herpesvirus 6
B10.01	Human herpesvirus 6 encephalitis
B10.81	Human herpesvirus 6 infection
B16.0 - B16.9	Acute hepatitis B
B17.10 - B17.11	Acute hepatitis C
B18.0 - B18.1	Chronic viral hepatitis B with or without delta-agent
B18.2	Chronic viral hepatitis C
B19.10 - B19.11	Unspecified viral hepatitis B with or without hepatic coma
B19.20 - B19.21	Unspecified viral hepatitis C with or without hepatic coma
B20	Human immunodeficiency virus [HIV] disease
B25.0 - B25.9	Cytomegaloviral disease
B34.0, B97.0	Adenovirus infection, unspecified and as the cause of diseases classified elsewhere
P35.1	Congenital cytomegalovirus infection
T86.10 - T86.19	Complications of kidney transplant
Z21	Asymptomatic human immunodeficiency virus [HIV] infection status
Z94.0 - Z94.84	Transplanted organ and tissue status

ICD-10 codes not covered for indications listed in the CPB:

A31.0 - A31.9	Diseases due to other mycobacterium
A44.0 - A44.9	Bartonellosis
A48.1	Legionnaires' disease
A48.8	Other specified bacterial disease [gardnerella vaginalis]
A49.3	Mycoplasma infection, unspecified site
A54.00 - A54.9	Gonococcal infections
A69.20 - A69.29	Lyme disease
A74.89	Other chlamydial diseases
A74.9	Chlamydial infection, unspecified
A81.2	Progressive multifocal leukoencephalopathy
A98.5	Hemorrhagic fever with renal syndrome
B00.0 - B00.9	Herpesviral [herpes simplex] infections
B02.0 - B02.9	Zoster [herpes zoster]
B08.21	Exanthema subitum [sixth disease]due to human herpesvirus 6
B08.22	Exanthema subitum [sixth disease]due to human herpesvirus 7
B10.01	Human herpesvirus 6 infection
B10.09	Other human herpesvirus encephalitis
B10.81	Human herpesvirus 6 infection
B10.82	Human herpesvirus 7 infection
B10.89	Other human herpesvirus infection
B17.8, B18.8 -	Other and unspecified viral hepatitis [GB virus type C]
B18.9	
B33.4	Hantavirus (cardio)-pulmonary syndrome [HPS] [HCPS]
B34.3	Parvovirus infection, unspecified
B37.0 - B37.9	Candidiasis
J09.X1 - J11.89	Influenza due to certain or other identified or unidentified influenza viruses
J16.0	Chlamydial pneumonia
K20.0	Eosinophilic esophagitis
L29.0	Pruritus ani
M30.3	Mucocutaneous lymph node syndrome [Kawasaki]
N76.0 - N76.3	Acute, subacute, chronic vaginitis and vulvitis
N77.1	Vaginitis, vulvitis and vulvovaginitis in diseases classified elsewhere
R53.82	Chronic fatigue, unspecified
R56.00 – R56.01	Febrile convulsions

## Background

The development of the polymerase chain reaction (PCR) has greatly simplified DNA analysis and shortened laboratory time (ACOG, 2002). Polymerase chain reaction allows the exponential amplification of the targeted gene or DNA sequence. Only minute quantities of DNA, typically 0.1 to 1.0 mg, are necessary for PCR. DNA can be amplified from a single cell. One important prerequisite of PCR is that the sequence of the gene, or at least the borders of the region of DNA to be amplified, must be known.

The PCR procedure has 3 steps: (i) DNA is denatured by heating to render it single

stranded, (ii) the PCR primers, which are short pieces of DNA (oligonucleotides) 20 to 30 base pairs in length exactly complementary to the ends of each piece of the double-stranded DNA to be amplified, anneal to their complementary regions of the DNA, and (iii) synthesis of the complementary strand of DNA occurs in the presence of the enzyme Taq polymerase and nucleotides triphosphates (dATP, dCTP, dGTP, and dTTP). The reaction cycle of denaturation, annealing, and extension is repeated 25 to 30 times to produce millions of copies of DNA. Typically, fragments several kilobases (kb) in size can be amplified, but sequences up to 10 kb have been successfully amplified. The exact cycling parameters and conditions for PCR must be determined empirically for each set of primers.

Polymerase chain reaction is very sensitive; therefore, extreme care must be taken to avoid amplification of contaminant DNA from aerosolized secretions or sloughed skin cells. These precautions are particularly important when DNA from a single cell is being amplified.

PCR amplification techniques raise considerable concerns regarding contamination from one specimen to another, creating the potential for false-positive results. The clinical interpretation of PCR results may also be challenging. Amplification of organisms representing latent infection or colonization can not be distinguished from active, clinically significant infections. Additionally, PCR may amplify fragments of nucleic acids, representing dead microorganisms, thus further clouding the clinical interpretation. Finally, specificities, sensitivities, and positive and negative predictive values of PCR have not been reported in large groups of patients for many of the microorganisms.

Polymerase chain reaction may be useful when culture is difficult due to the low numbers of the organisms, for fastidious or lengthy culture requirements, or when there is difficulty in collecting an appropriate sample. Quantification of viral load via PCR may be useful when the viral load can be used as a prognostic indicator, or when necessary follow the patient's response to therapy.

Several manufacturers have produced PCR Assays designed to detect multiple pathogens. The INFINITI® Bacterial Vaginosis QUAD Assay is designed to detect the following pathogens: *Bacteroides fragilis*, *Gardnerella vaginalis*, *Mobiluncus mulieris*, *Mobiluncus curtisi*, *Atopobium vaginae*, and *Prevotella bivia* (AutoGenomics, Bacterial Vaginosis, 2010). The INFINITI® Candida Vaginitis QUAD Assay is designed to detect 5 fungal species: *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* (AutoGenomics Candida Vaginitis 2010). Quest Diagnostics has developed the Quest SureSwab, which includes tests for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* as well as tests for bacterial vaginosis and *Candida* spp. LabCorp has developed the NuSwab test, which includes *Atopobium vaginae*, BVAB-2, megasphaera-1), *C. albicans*, *C. glabrata*, *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis*. GenPath has developed the GenPap, which includes bacterial vaginosis, *Candida* spp., *T. vaginalis*, *N. gonorrhoeae*, *T. pallidum*, *C. trachomatis*, and herpes simplex virus. The BD Max Vaginal Panel detects bacterial vaginosis, *Candida* spp. and *T. vaginalis*. Medical Diagnostics Laboratories has developed the OneSwab, which tests for dozens of pathogens. The uBiome SmartJane tests for 14 high-risk HPV strains, 5 low-risk HPV strains, *C. trachomatis*, *N. gonorrhoea*, *T. pallidum*, *M. genitalium* and 23 other vaginal flora.

#### Actinomycosis

Actinomycosis is a subacute or chronic infection due to *Actinomyces israelii* or one of several other *Actinomyces* species. The body's reaction tends to be granulomatous or suppurative. Hematogenous spread can occur to distant sites but lymphatic spread is uncommon. The 3 major anatomic types of actinomycosis are cervicofacial, thoracic, and abdominal (AAP, 2006). *Actinomyces* species are slow-growing, microaerophilic or facultative anaerobic gram-positive, filamentous,

branching bacilli that can be part of the normal oral, gastrointestinal, or vaginal flora. Diagnosis is suspected clinically and confirmed by x-rays and identification of *Actinomyces* spp. in sputum, pus, or biopsy specimen. In pus or tissue, the microorganism appears as the distinctive sulfur granules or as tangled masses of branched and unbranched wavy bacterial filaments, pus cells, and debris, surrounded by an outer zone of radiating, club-shaped, hyaline, and refractive filaments that take hematoxylin-eosin stain in tissue but are positive on Gram stain. Current serologic tests have no role in diagnosis. Nucleic acid probes and PCR methods are being developed for more rapid and more accurate identification. *Actinomyces* species can be identified in tissue specimens using the 16s rRNA sequencing and PCR assay (AAP, 2009). Initial therapy should include intravenous penicillin G or ampicillin for 4 to 6 weeks followed by high-doses of oral penicillin, amoxicillin, erythromycin, clindamycin, doxycycline, or tetracycline for a total of 6 to 12 months.

#### Acute Myelogenous Leukemia: FLT3 Mutation

Mutations in FLT3 are common in acute myelogenous leukemia (AML) and have been associated with poorer survival in children and in younger adults with normal cytogenetics receiving intensive chemotherapy (Chin et al, 2006). FLT3 mutation analysis is used to predict survival in AML patients.

#### Acute Myeloid Leukemia: AM1/ETO t(8, 21) Translocation

The translocation t(8;21)(q22;q22) is one of the most common structural chromosomal aberrations in patients with AML, occurring in about 15 % of adult AML cases and 40 % of AML cases with differentiation (AML-M2) (Chin et al, 2006). AML with t(8;21) has a mean age onset of about 30 years and is most common in children and younger adults; it is relatively rare in elderly persons. The presence of t(8;21) is associated with the highest complete remission rate (90 %) and the highest probability (50 % to 70 %) of remaining in complete remission at 5 years. However, the disease may become resistant to therapy upon relapse.

The t(8;21)(q22;q22) translocation results in constant expression of AM1/ETO fusion mRNA, which can be detected by reverse transcription-polymerase chain reaction (RT-PCR). The quantitative t(8;21) assay can be used not only to diagnose AML, but also to serially monitor patients, evaluate the effectiveness of treatment, and predict early relapse.

#### Acute Myelomonocytic Leukemia: CBFB/MYH11 inv(16)

The pericentric inversion of chromosome 16(p13;q22), and less frequently the t(16;16) (p13;q22) translocation, accounts for 16 % of the chromosomal aberrations associated with acute myelomonocytic leukemia (Chin et al, 2006). This inversion results in fusion of the core binding factor β (CBFβ) gene on 16q22 with the smooth muscle myosin heavy chain gene (MYH11) on 16p13, leading to the formation of a chimeric CBFβ/MYH11 fusion protein. Clinically, the inv(16) or t(16;16) is associated with AML with abnormal eosinophils (French-American-British classification M4EO subtype), with abnormal eosinophils being part of the malignant clone. Patients with inv(16) or t(16;16) generally have relatively good response and long-term disease-free survival rates.

#### Acute Promyelocytic Leukemia: PML/RARA t(15;17)

Acute promyelocytic leukemia (APL or AML-M3) is a subtype of acute myeloblastic leukemia characterized by distinct clinical and histopathologic features as well as a unique cytogenetic abnormality, t(15;17)(q22;q12-21) (Chin et al, 2006). Historically one of the most lethal forms of acute myeloid leukemia, APL leads to disseminated intravascular coagulation and death when not diagnosed and treated. Treatment with all-trans-retinoic acid substantially improves survival in patients

who have failed anthracycline chemotherapy or for whom anthracycline is contraindicated. Sequential treatment with all-trans-retinoic acid and chemotherapy results in remission in the majority of cases. If chemotherapy is given initially, retinoic acid should be given upon confirmation of the molecular diagnosis of APL.

The PML/RAR-alpha/ t(15;17) translocation results in fusion of the retinoic acid receptor alpha (RARA) gene on chromosome 17 with the PML gene on chromosome 15. Detection of the PML/RARA fusion transcript by RT-PCR is more sensitive than conventional cytogenetic detection of the t(15;17) translocation and best predicts therapeutic response to all-trans-retinoic acid.

More than 99 % of APL patients harbor this translocation. A positive RT-PCR test is diagnostic for APL; thus the test can also be used for initial diagnosis as well as detection of minimal residual disease or recurrence.

#### Adenovirus

Adenoviruses are DNA viruses. The most common site of adenovirus infection is the upper respiratory tract. Manifestations include symptoms of the common cold, pharyngitis, pharyngoconjunctival fever, tonsillitis, otitis media, and keratoconjunctivitis, often associated with fever. Life-threatening disseminated infection, severe pneumonia, meningitis, and encephalitis occasionally occur, especially among young infants and immunocompromised hosts. Adenoviruses are infrequent causes of acute hemorrhagic conjunctivitis, a pertussis-like syndrome, croup, bronchiolitis, hemorrhagic cystitis, and genitourinary tract disease. A few adenovirus serotypes can cause gastroenteritis.

Infection in infants and children may occur at any age. Adenoviruses causing respiratory tract infection usually are transmitted by respiratory tract secretions through person-to-person contact, fomites, and aerosols. Because adenoviruses are stable in the environment, fomites may be important in their transmission. Other routes of transmission have not been defined clearly and may vary with age, type of infection, and environmental or other factors.

According to AAP guidelines, although PCR testing has been used to detect adenovirus DNA, detection of adenovirus infection by culture or antigen is the preferred diagnostic method. Adenoviruses associated with respiratory tract disease can be isolated from pharyngeal secretions, eye swabs, and feces by inoculation of specimens into a variety of cell cultures. Adenovirus antigens can be detected in body fluids of infected persons by immunoassay techniques, which are especially useful for diagnosis of diarrheal disease, because enteric adenovirus types 40 and 41 usually can not be isolated in standard cell cultures. Enteric adenoviruses also can be identified by electron microscopy of stool specimens. Multiple methods to detect group-reactive hexon antigens in body secretions and tissue have been developed. Also, detection of viral DNA can be accomplished with genomic probes, synthetic oligonucleotide probes, or gene amplification by polymerase chain reaction. Serodiagnosis is based on detecting a 4-fold or greater rise in antibodies to a common adenovirus antigen (e.g., hexon). According to the AAP (2006), serodiagnosis is used primarily for epidemiologic studies.

Polymerase chain reaction has been used to diagnose adenovirus myocarditis (Martin et al, 1994; Towbin et al, 1994; Shirali et al, 2001). Routine viral cultures and histopathology are rarely positive in cases of presumed viral myocarditis (AAP, 2006).

Re-activation of adenovirus infection occurs in greater than 80 % of autologous and allogeneic hematopoietic stem cell transplant recipients but causes severe disease in fewer than 2 % (Anaissie, 2008). There are four clinically significant adenoviral

syndromes: pneumonitis, nephritis, diarrhea and hemorrhagic colitis, and hemorrhagic cystitis. Disseminated disease with multi-organ failure can also occur. Quantitative PCR assays have been developed to detect viremia in hematopoietic stem cell transplant recipients. In several studies, rising blood viral loads were associated with invasive adenovirus disease. Adenovirus viral loads also can be utilized to help monitor responses to therapy (Flomenberg and Munoz, 2008).

Treatment of adenovirus infection in immunocompetent persons is supportive.

#### Anaplastic Lymphoma Kinase (ALK) Testing

Leigh and colleagues (2014) noted that the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) guideline on molecular testing for the selection of patients with lung cancer for epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors was considered for endorsement. The American Society of Clinical Oncology (ASCO) staff reviewed the CAP/IASLC/AMP guideline for developmental rigor; an ASCO ad hoc review panel of experts reviewed the guideline content. The ASCO panel concurred that the recommendations are clear, thorough, and based on the most relevant scientific evidence in this content area and presented options that will be acceptable to patients. The CAP/IASLC/AMP guideline comprises 37 recommendations (evidence grade A or B), expert consensus opinions, or suggestions that address the following 5 principal questions: (i) When should molecular testing be performed? (ii) How should EGFR testing be performed? (iii) How should ALK testing be performed? (iv) Should other genes be routinely tested in lung adenocarcinoma? and (v) How should molecular testing be implemented and operationalized? The authors concluded that the ASCO review panel endorsed the CAP/IASLC/AMP guideline. This guideline represents an important advance toward standardization of EGFR and ALK testing practices and is of major clinical relevance in advancing the care of patients with lung cancer. The ASCO review panel highlighted 3 evolving areas: (i) advances in ALK testing methodology, (ii) considerations for selecting appropriate populations for molecular testing, and (iii) emergence of other targetable molecular alterations. Specifically, the guideline does not recommend ALK real-time PCR as an alternative to FISH for selecting patients for ALK inhibitor therapy.

#### Aspergillosis

Aspergillosis comprises a variety of manifestations of infection. Allergic bronchopulmonary aspergillosis manifests as episodic wheezing, expectoration of brown mucus plugs, low-grade fever, eosinophilia, and transient pulmonary infiltrates (AAP, 2009). This form of aspergillosis occurs most frequently in immunocompetent children with chronic asthma or cystic fibrosis. Allergic sinusitis is a far less common allergic response to colonization by Aspergillus species than allergic bronchopulmonary syndrome. It occurs in children with nasal polyps or previous episodes of sinusitis or who have undergone sinus surgery and is characterized by symptoms of chronic sinusitis with dark plugs of nasal discharge.

Aspergillomas and otomycosis are 2 syndromes of non-allergic colonization by Aspergillus species in immunocompetent children (AAP, 2009). Aspergillomas grow in pre-existing cavities or bronchogenic cysts without invading pulmonary tissue; almost all patients have underlying lung disease, typically cystic fibrosis. Patients with otomycosis have underlying chronic otitis media with colonization of the external auditory canal by a fungal mat that produces a dark discharge.

Invasive aspergillosis occurs almost exclusively in immunocompromised patients with neutropenia or an underlying disease (e.g., chronic granulomatous disease) or medication use (e.g., corticosteroids) that causes neutrophil dysfunction or after cytotoxic chemotherapy or immunosuppressive therapy (e.g., organ

transplantation) (AAP, 2009). Invasive infection usually involves pulmonary, sinus, cerebral, or cutaneous sites, and the hallmark is angioinvasion with resulting thrombosis, dissemination to other organs, and, occasionally, erosion of the blood vessel wall and catastrophic hemorrhage. Rarely, endocarditis, osteomyelitis, meningitis, infection of the eye or orbit, and esophagitis occur.

Dichotomously branched and septate hyphae, identified by microscopic examination of wet preparations, tissue specimens or bronchoalveolar lavage, are suggestive of the diagnosis. Isolation of an Aspergillus species in culture is required for definitive diagnosis. The organism usually is not recoverable from blood but is isolated readily from lung, sinus, and skin biopsy specimens cultured on special media.

Biopsy of a lesion usually is required to confirm the diagnosis. According to the AAP (2009), a serologic assay for detection of galactomannan, a molecule found in the cell wall of Aspergillus species, is available commercially but has not been evaluated widely in infants and children. A positive test result in adults supports a diagnosis of invasive aspergillosis, and monitoring of serum antigen concentrations may be useful to assess response to therapy.

In allergic aspergillosis, diagnosis is suggested by a typical clinical syndrome and elevated concentrations of total and Aspergillus-specific serum immunoglobulin E, eosinophilia, and a positive skin test to Aspergillus antigens. In persons with cystic fibrosis, the diagnosis is more difficult because wheezing, eosinophilia, and a positive skin test unassociated with allergic bronchopulmonary aspergillosis often are present.

According to the Infectious Diseases Society of America (2000), although PCR assays for Aspergillus RNA and DNA have been developed, these PCR assays must be tested with body fluids in prospective trials of invasive aspergillosis, and reproducibility must be verified before a role for these tests are established.

An UpToDate review on "Diagnosis of invasive aspergillosis" (Marr, 2012) states that "Investigational DNA detection assays (e.g., by polymerase chain reaction [PCR]) have shown mixed results, with some studies suggesting superior performance compared to antigen based assays, and others reporting the opposite. Results of multiple assays that use different technologies and microbial targets have been reported. A systemic review and meta-analysis suggested that sensitivity and specificity of PCR to detect invasive aspergillosis was 88 and 75 percent. However, this review emphasized that results cannot be generalized with non-homogeneity of methods and patients evaluated".

The development of rapid diagnostic tests may allow for the early detection of invasive fungal infections in immune-compromised patients, such as those undergoing transplants, or those with cancer and AIDS. MycoDART, Inc., affiliated with RealTime Laboratories, Inc, offers MycoDART-PCR DNA tests which utilizes qualitative dual amplification real-time PCR to rapidly test the DNA in whole blood, plasma, and bronchial alveolar lavage specimens within in 4-6 hours. The company developed the MycoDART-PCR Aspergillus probe Diagnostic Panel to detect *A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*.

MycoDART states that their rapid PCR tests provide accuracy as high as 95% compared to blood cultures which are only be 50% accurate and can take days to get results. A clinical study is underway comparing MycoDART-PCR and blood cultures, the current gold standard, in order to demonstrate efficacy in persons exposed to Candida. In addition, the company is teaming up with Baylor Health System to lead a clinical study of the Aspergillus assay (BioSpace, 2019).

According the Infectious Disease Society of America (2016), committee members were not in agreement for the utility of blood-based PCR in diagnosing invasive Aspergillus (IA). "One group favored recommendations for PCR testing, based on publications validating its role when used in conjunction with other tests such as antigen detection assays to diagnose IA and/or reduce preemptive antifungal usage. The other group thought that PCR assays are promising but could not be recommended for routine use in clinical practice at present due to the lack of conclusive validation for commercially available assays, the variety of methodologies in the literature, and questions about the extent to which results assisted diagnosis". As research in the area continues, the panel recommend that clinicians who choose to use PCR assays employ them carefully in the management of patients on a case-by-case basis. When PCR assays are used, results should be considered in conjunction with other diagnostic tests and the clinical context (strong recommendation; moderate-quality evidence).

Kauffman (2019) states that "investigational DNA detection assays (eg, by PCR) have shown mixed results, with some studies suggesting superior performance compared with antigen-based assays and others reporting the opposite. Results of multiple assays that use different technologies and microbial targets have been reported. In a meta-analysis of 25 studies, sensitivity and specificity of PCR to detect invasive aspergillosis was 84 and 76 percent, respectively. When at least two PCR results were positive, the sensitivity was 64 percent and the specificity was 95 percent. Another meta-analysis had similar findings. These results suggest that two positive PCR results are highly suggestive of invasive aspergillosis".

#### Astrovirus

Commercial tests for diagnosis of astrovirus are not available in the United States, although enzyme immunoassays are available in many other countries (AAP, 2009). The following tests are available in some research and reference laboratories: electron microscopy for detection of viral particles in stool, enzyme immunoassay for detection of viral antigen in stool or antibody in serum, latex agglutination in stool, and RT-PCR assay for detection of viral RNA in stool. Of these tests, RT-PCR assay is the most sensitive. Rehydration with oral or intravenous fluid and electrolyte solutions is recommended for treatment of astrovirus infection (AAP, 2009). No specific control measures are available.

#### Babesiosis

Babesiosis is a tick-borne disease caused by hemoplasma parasites of the genus Babesia. While more than 100 species have been reported, only a few have been identified as causing human infections. *Babesia microti* and *Babesia divergens* have been identified in most human cases, but variants (considered different species) have been recently identified. Little is known about the occurrence of Babesia species in malaria-endemic areas where Babesia can easily be misdiagnosed as Plasmodium.

Worldwide, but little is known about the prevalence of Babesia in malaria-endemic countries, where misidentification as Plasmodium probably occurs. In the United States, *B. microti* is the agent most frequently identified (Northeast and Midwest).

Infectious Disease Society of America Lyme disease guidelines (Wormser, 2000) stated that the diagnosis of Babesiosis should be suspected in patients from areas where babesiosis is endemic who develop fever (especially if fever is very high (greater than 37 degrees)) in the absence of erythema migrans after an *Ixodes* tick bite. Infection may also be suspected in patients who have residual symptoms after treatment for early Lyme disease.

According to the CDC Guidelines on Identification and Diagnosis of Parasites of Public Health Concern (2001), PCR testing has a limited role in the diagnosis of Babesia infection. Diagnosis of Babesia infection should be made by microscopy detection of parasites in patients' blood smears. However, indirect fluorescent antibody (IFA) tests are useful for detecting infected individuals with very low levels of parasitemia (such as asymptomatic blood donors in transfusion-associated cases), for diagnosis after infection is cleared by therapy, and for discrimination between *Plasmodium falciparum* and Babesia infection in patients whose blood smear examinations are inconclusive and whose travel histories cannot exclude either parasite.

According to the CDC, molecular techniques, such as PCR, are necessary only in limited situations, specifically:

- Where the morphologic characteristics observed on microscopic examination of blood smears do not allow an unambiguous differentiation between Babesia and Plasmodium.
- In research investigations of new Babesia variants (or species) observed in recent human infections in the U.S. and in Europe.

#### Bacterial Vaginosis

Bacterial vaginosis (BV) is a condition in which the natural balance of organisms found in the vagina is changed from a predominance of *Lactobacillus* to an overgrowth of other bacteria including *Gardnerella vaginalis*, *Mobiluncus* and other anaerobes. *Atopobium vaginae* is a recently recognized bacterium that has been found in bacterial vaginosis (Ferris et al, 2004). Its clinical significance is unknown. Bacterial vaginosis can be diagnosed by the use of clinical or Gram-stain criteria (CDC, 2002; AGM-MSSVD, 2002). The most widely accepted method for diagnosis of bacterial vaginosis is the presence of 3 of the following four Amsel criteria: (i) a homogenous vaginal discharge; (ii) a vaginal pH greater than 4.5; (iii) the presence of clue cells; and (iv) a fishy odor after addition of potassium hydroxide to the vaginal secretions (the amine test). Diagnosis on the basis of Amsel criteria has a reported sensitivity of 81 % and a specificity of 94 % (WHO, 1999). Gram stain of a vaginal smear has a sensitivity of 89 % and a specificity of 93 %. According to the CDC, culture of *G. vaginalis* is not recommended as a diagnostic tool because it is not specific.

A DNA probe based test for high concentrations of *G. vaginalis* (Affirm™ VP III, manufactured by Becton Dickinson, Sparks, MD) does have clinical utility (CDC, 2002). This test has sensitivity for *G. vaginalis* of 94 % and a specificity of 81 % (WHO, 1999). Data from the Affirm VPIII labeling indicates a reconciled sensitivity/specificity of the Affirm VPIII test as compared to clinically significant culture levels and Gram stain morphology for patients with clinical BV by 3 of 4 Amstel Criteria was 98%/100% and 95%/100% respectively (BD, 2010). Sheiness, et al. (1992) reported that the combination of a positive DNA probe and vaginal pH > 4.5 had a sensitivity and specificity of 95 and 99 percent, respectively, for diagnosis of BV when clinical criteria were used as the diagnostic standard.

Other commercially available tests that may be useful for the diagnosis of BV include a card test for the detection of elevated pH and trimethylamine (FemExam® test card, manufactured by Cooper Surgical, Shelton, CT) and prolineaminopeptidase (Pip Activity TestCard™, manufactured by Litmus Concepts, Inc., Santa Clara, CA). The FemExam is a rapid test that measures vaginal pH and volatile amines, corresponding to 2 of the 4 Amsel criteria. The proline aminopeptidase test is an indirect test for a chemical produced by the organisms associated with bacterial vaginosis. Prolineaminopeptidase assay has a reported sensitivity of 93 % and a specificity of 93 % for BV (WHO, 1999). The BVBlue system (Gryphus Diagnostics, L.L.C.) is a point of care chromogenic diagnostic test for BV based on the presence of elevated sialidase enzyme in vaginal fluid samples.

Sensitivity ranging from 88 to 94 percent and specificity ranging from 91 to 98 percent have been reported when compared with Amsel and Nugent criteria (Myziuk, et al., 2003; Sumeksri, et al., 2005; Bradshaw, et al., 2005).

Current guidelines do not recommend PCR testing for bacterial vaginosis (CDC, 2010; SOGC, 2008; AAP, 2012; NYSDOH, 2009; BASHH, 2012; AHMAC, 2012; Queensland Health, 2013; Alberta Health Services, 2014). Guidelines on BV from the CDC (Workowski et al, 2010) stated that "PCR also has been used in research settings for the detection of a variety of organisms associated with BV, but evaluation of its clinical utility is uncertain." Sobel (2015) noted that PCR-based tests are being investigated for molecular diagnosis of BV, mostly based upon molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae*. Citing studies by Cartwright, et al. (2012), Menard, et al. (2008) and Menard, et al. (2010), Sobel found that these quantitative PCR tests have good sensitivity and specificity compared to standard tests, but "these tests are expensive and of questionable advantage."

The CDC's 2015 Sexually Transmitted Diseases Treatment Guidelines on bacterial vaginitis (BV) stated that "PCR has been used in research settings for the detection of a variety of organisms associated with BV, but evaluation of its clinical utility is still underway. Detection of specific organisms might be predictive of BV by PCR. Additional validation is needed before these tests can be recommended to diagnose BV."

Also, an UpToDate review on "Bacterial vaginosis" (Sobel, 2020) states that "Molecular tests that assay the vaginal microbiome for evidence of BV, vaginal candidiasis, and trichomonas have shown promise in clinical studies, and two assays have been approved by the FDA for use.".

Cox et al (2015) stated that *Gardnerella vaginalis* is a Gram-variable anaerobic bacterium present in 100 % of women with BV, which is a complex polymicrobial condition with no single causative agent. The current laboratory detection method for BV relies on a Gram-stain Nugent score to estimate the quantity of different bacterial morphotypes in the vaginal micro flora. While the Nugent score can distinguish between women with and without BV, a significant proportion are categorized as intermediate, which fails to differentiate a normal from an abnormal vaginal micro flora. A singleplex *G. vaginalis* TaqMan real-time quantitative PCR (qPCR) assay was developed and compared with the "gold standard" Nugent score. Detection and quantification of *G. vaginalis* was performed on vaginal specimens with positive, negative and intermediate Nugent scores. The *G. vaginalis* qPCR assay demonstrated high analytical specificity against a broad microbial panel and analytical sensitivity down to  $3.1 \times 10(4)$  copies ml(-1). There was a significantly higher *G. vaginalis* load in women with BV compared with intermediate and non-BV women ( $p$  value =  $5.1 \times 10(-14)$ ). All Nugent scores in keeping with BV had qPCR loads of greater than or equal to  $10(7)$  copies ml(-1). Among the 24 undefined women (11.8 %) in the study with an intermediate flora, 14 (58.3 %) had a *G. vaginalis* load of greater than or equal to  $10(7)$  copies ml(-1). The authors concluded that a threshold of  $107$  copies ml(-1) had positive and negative predictive values of 57.1 and 100 % for BV; the high qPCR loads among the intermediate Nugent scores suggested the need for a new approach in classifying BV and the potential for qPCR to play a role.

Jespers et al (2016) stated that a next-generation diagnostic tool for BV, consisting of quantitative and/or qualitative molecular criteria, has not yet been identified. The optimal diagnostic tool should not only diagnose BV in diverse populations, but should also detect early signs of transition to dysbiosis. These researchers evaluated a tool based on log<sub>10</sub>-transformed qPCR data for *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, *Lactobacillus vaginalis*, *Lactobacillus* genus, *Atopobium vaginae* and *Gardnerella vaginalis* in

vaginal specimens of 426 African women to detect dysbiosis and predict transition to dysbiosis. *G. vaginalis* ( $p = 0.204$ ) and *A. vaginae* ( $p = 0.001$ ) were more commonly present in women who evolved to an intermediate (Nugent 4 to 6) or BV score (Nugent 7 to 10) compared to women who continued to have a normal Nugent score. The combination of *G. vaginalis*, *A. vaginae* and *Lactobacillus* genus counts performed best for diagnostic accuracy for BV -- sensitivity 93.4 % and specificity 83.6 %; and for predictive accuracy for BV -- sensitivity 79 % and specificity 52 %. *L. crispatus* combinations did not perform well. The authors concluded that a triple -- *G. vaginalis*-*A. vaginae*-*Lactobacillus* genus -- qPCR tool holds promise for research in sub-Saharan Africa or when developed as a next-generation clinical diagnostic modality for BV, ideally engineered as a rapid assay.

Schwebke et al (2018) stated that vaginitis is a common complaint, diagnosed either empirically or using Amsel's criteria and wet mount microscopy. These researchers determined characteristics of an investigational test (a molecular test for vaginitis), compared to reference, for detection of bacterial vaginosis, *Candida* spp., and *Trichomonas vaginalis*. Vaginal specimens from a cross-sectional study were obtained from 1,740 women (greater than or equal to 18 years old), with vaginitis symptoms, during routine clinic visits (across 10 sites in the United States). Specimens were analyzed using a commercial PCR/fluorogenic probe-based investigational test that detects bacterial vaginosis, *Candida* spp., and *Trichomonas vaginalis*. Clinician diagnosis and in-clinic testing (Amsel's test, potassium hydroxide preparation, and wet mount) were also employed to detect the 3 vaginitis causes. All testing methods were compared to the respective reference methods (Nugent Gram stain for bacterial vaginosis, detection of the *Candida* gene *its2*, and *Trichomonas vaginalis* culture). The investigational test, clinician diagnosis, and in-clinic testing were compared to reference methods for bacterial vaginosis, *Candida* spp., and *Trichomonas vaginalis*. The investigational test resulted in significantly higher sensitivity and negative predictive value (NPV) than clinician diagnosis or in-clinic testing. In addition, the investigational test showed a statistically higher overall percent agreement with each of the t3 reference methods than did clinician diagnosis or in-clinic testing. The investigational test showed significantly higher sensitivity for detecting vaginitis, involving more than 1 cause, than did clinician diagnosis. The authors concluded that the results from the current study support the potential utility of the investigational test in the differential diagnosis of vaginitis. While some laboratory tests take 2 to 7 days to provide results, the investigational test results are generally available within 24 hours. These researchers stated that although more work is needed to establish the cost/benefit ratio regarding the application of this investigational test in a practical setting, its high sensitivity, specificity, and accuracy (across a large spectrum of disease prevalence) should impart benefits and decrease the chance of needless treatment of patients that are negative for the disease. This may prove especially important with cases of vaginitis that involve multiple causes, where the sensitivity of clinician diagnosis may be limited.

The authors stated that this study had limitations that prevent an exact interpretation of the findings. Several analyses involved observations for each type of infection that were excluded due to non-compliance or inability to report. It is possible, for example, that listing these types of observations as "not compliant" or "not reportable" for the investigational test, in lieu of "failure to correctly diagnose", may have artificially improved its operating characteristics. Other limitations included the fact that the investigational assay may have resulted in an over-diagnosis of vaginitis, as it cannot distinguish non-pathogenic colonization from pathogenic growth; this would be considered for clinician diagnosis. However, the clinical cut-off for the investigational test was set by the current reference standard for diagnosing *Candida* spp. (positive fungal culture report), and thus, the results were consistent with everyday practice. Moreover, bacterial vaginosis may be detected by the Nugent score (7 to 10) but also be asymptomatic. The investigational test showed the best agreement with the Nugent score, which is the

gold standard, but may have included asymptomatic bacterial vaginosis. The bacterial vaginosis algorithm for the investigational test was set by the composite reference method of concordant positive and negative Nugent and Amsel's criteria. Therefore, only unambiguous specimens for bacterial vaginosis status were used to develop the algorithm. Additionally, this study employed a cross-sectional design that did not evaluate clinical outcomes for patients with discordant reference method results and clinician diagnosis. Only clinics with expertise and resource availability for detection of the 4 Amsel's criteria and wet mount procedures were chosen as study sites. Therefore, clinician diagnosis benefited from reliability of in-clinic results in a way that might not occur under real-life conditions. Thus, the actual difference in clinician diagnosis versus the investigational test may likely be greater than that observed in this study. Finally, in this study these investigators omitted the intermediate values for Nugent scoring (4 to 6), whereas Gaydos et al (2017) used the composite reference method of Nugent score combined with the modified Amsel 2/3 criteria without discharge to discriminate intermediate Nugent scoring (4 to 6). These researchers may have missed some cases of bacterial vaginosis, the exclusion of which could have led to either an over- or under-estimation of performance in the investigational test. However, the prevalence of bacterial vaginosis in this study (58 %) was very close to that reported by Gaydos et al (55.8 %).

Workowski et al (2021) state that bacterial vaginosis (BV) is a "vaginal dysbiosis resulting from replacement of normal hydrogen peroxide and lactic-acid-producing *Lactobacillus* species in the vagina with high concentrations of anaerobic bacteria, including *G. vaginalis*, *Prevotella* species, *Mobiluncus* species, *A. vaginae*, and other BV-associated bacteria". "The infections most frequently associated with vaginal symptoms are BV (i.e., replacement of the vaginal flora by an overgrowth of anaerobic bacteria including *G. vaginalis*, *Prevotella bivia*, *A. vaginae*, *Megasphaera* type 1, and numerous other fastidious or uncultivated anaerobes), trichomoniasis, and vulvovaginal candidiasis (VVC). Women with BV are at increased risk for STI acquisition. Multiple BV NAATs are available for BV diagnosis among symptomatic women. These tests are based on detection of specific bacterial nucleic acids and have high sensitivity and specificity for BV." In addition, PCR tests are available. "Three laboratory-developed tests (NuSwab VG, OneSwab BV Panel PCR with Lactobacillus Profiling by qPCR, and SureSwab BV) have to be internally validated before use for patient care yet have good sensitivity and specificity, similar to FDA-cleared assays. BV NAATs should be used among symptomatic women only (e.g., women with vaginal discharge, odor, or itch) because their accuracy is not well defined for asymptomatic women. Despite the availability of BV NAATs, traditional methods of BV diagnosis, including the Amsel criteria, Nugent score, and the Affirm VP III assay, remain useful for diagnosing symptomatic BV because of their lower cost and ability to provide a rapid diagnosis."

#### Bacteroides and Prevotella Infections

*Bacteroides* species (including *B. fragilis*, *B. ureolyticus*) and *Prevotella* species are anaerobic bacteria that are predominant components of the bacterial flora of mucous membranes and are therefore a common cause of endogenous infections. Bacteroides and Prevotella infections can develop in all body sites, including the central nervous system, the head, the neck, the chest, the abdomen, the pelvis, the skin, and the soft tissues. Anaerobic culture media are necessary for recovery of *Bacteroides* and *Prevotella* species (AAP, 2009). Because infections usually are polymicrobial, aerobic cultures also should be obtained. Nucleic acid probes and PCR methods, available in research laboratories, are being developed for rapid identification.

#### Bartonella

The predominant sign of *Bartonella henselae* (cat-scratch disease, CSD) is regional lymphadenopathy in an immunocompetent person. Fever and mild systemic symptoms occur in one-third of patients (AAP, 2009; CPS, 2002).

*Bartonella henselae* is the causative organism for most cases of CSD. Other less common causes of CSD are *B. clarridgeiae* and *B. elizabethae*. *B. henselae* are fastidious, slow-growing, gram-negative bacilli that also have been identified as the causative agent of bacillary angiomatosis and peliosis hepatitis, 2 infections that have been reported primarily in patients infected with the human immunodeficiency virus.

Infection with *B. henselae* results in disease syndromes of variable severity, ranging from lymphadenopathy only (CSD) to systemic disease. The severity and presentation of disease are related to immune status. In general, immunocompetent patients who are otherwise healthy tend to present with classic CSD when infected with *B. henselae*. Patients who are immunocompromised by having AIDS, chronic alcoholism, immunosuppression, or other serious health problems tend to have systemic disease. However, there have been rare reports of systemic disease, including bacillary angiomatosis, in immunocompetent persons.

Cat-scratch disease is believed to be a relatively common infection, although the true incidence is unknown. Most cases occur in patients younger than 20 years of age. Cats are the common reservoir for human disease, and bacteremia in cats associated with patients with CSD is common. More than 90 % of patients have a history of recent contact with cats, often kittens, which usually are healthy. No evidence of person-to-person transmission exists.

The IFA test for detection of serum antibody to antigens of *Bartonella* species is sensitive and specific and useful for the diagnosis of CSD (AAP, 2009; CPS, 2002). Enzyme immunoassays for detection of antibody to *B. henselae* have been developed; however, they have not been demonstrated to be more sensitive or specific than the IFA test. If involved tissue is available, the putative agent of the disease may be visualized by the Warthin-Starry silver impregnation stain; however, this test is not specific for *B. henselae*. Pathologic and microbiologic examinations also are useful to exclude other diseases. Histologic findings in lymph node sections are characteristic but not pathognomonic for CSD. A cat-scratch antigen skin test, which was used formerly to confirm the clinical diagnosis, was prepared from aspirated pus from suppurative lymph nodes of patients with apparent CSD. The AAP (2006) stated that this test should not be used.

Polymerase chain reaction assays are available in some commercial laboratories, and from reference laboratories and the CDC (AAP, 2009). According to the Canadian Paediatric Society (2002), PCR assays can differentiate between *B. henselae* and *B. Quintana*, the cause of trench fever and of bacillary angiomatosis and bacillary peliosis hepatitis in HIV-infected patients.

Management is primarily symptomatic since the disease usually is self-limited, resolving spontaneously in 2 to 4 months. Painful suppurative nodes can be treated with needle aspiration for relief of symptoms; surgical excision generally is unnecessary.

Antibiotic therapy may be considered for acutely or severely ill patients with systemic symptoms, particularly persons with hepatosplenomegaly or persons with large painful adenopathy and immunocompromised hosts. No well-controlled randomized clinical trials have been performed that clearly demonstrate a clinically significant benefit of antimicrobial therapy for CSD. Reports suggest that several oral antibiotics (rifampin, trimethoprim-sulfamethoxazole, azithromycin, and ciprofloxacin) and parenteral gentamicin may be effective in CSD. Doxycycline,

erythromycin, and azithromycin are effective for treatment of signs and symptoms associated with bacillary angiomatosis if administered for prolonged periods to immunocompromised persons.

#### Bartonellosis

Bartonellosis, or Carrion's disease, is a biphasic disease caused by *Bartonella bacilliformis* and transmitted by sandflies. The disease is characterized by an initial life-threatening febrile phase known as Oroya fever followed by an eruptive phase known as verruga peruana. *Bartonella bacilliformis*, is a small, gram-negative, intra-cellular bacteria. Bartonellosis is endemic to certain areas of the Andean regions of Peru, Columbia, and Ecuador. The diagnosis of Oroya fever is made by blood culture or by identifying *B. bacilliformis* organisms on Giemsa-stained blood smears. The diagnosis of Bartonellosis in patients with verruga peruana is generally based on the characteristic clinical features with or without a skin biopsy specimen that shows compatible findings on a Giemsa-stained sample viewed under light microscopy. A more definitive diagnosis can be made by visualizing inclusions with light microscopy or by visualizing individual microorganisms with electron microscopy. Antibody tests for *B. bacilliformis* infection have been developed. Although these tests have been useful in epidemiologic studies, their sensitivity and specificity for clinical practice have not been determined. There is a lack of evidence on the performance characteristics and clinical utility of PCR tests for *B. bacilliformis*. Anti-microbial therapy is essential in patients suspected of having Oroya fever. Rifampin has been recommended as the preferred therapy for patients with verruga peruana.

#### B-Cell Lymphomas: bcl-2 Gene Translocation

The bcl-2 gene translocation, t(14;18), is the rearrangement of the bcl-2 proto-oncogene on chromosome 18 with the immunoglobulin heavy chain region on chromosome 14 (Chin et al, 2006). The majority of translocations occur in the major breakpoint cluster region (mbr) of the bcl-2 gene and result in over-expression of the bcl-2 protein. Over-expression, in turn, results in resistance to apoptosis (natural cell death), which leads to abnormally high levels of B-cell lymphocytes in the lymph nodes, spleen and peripheral blood.

The bcl-2 translocation is a characteristic of B-cell lymphomas. It is observed in 70 to 90 % of follicular non-Hodgkin B-cell lymphomas, 20 to 30 % of large diffuse B-cell lymphomas, and 50 % of undifferentiated B-cell lymphomas, but not in other lymphomas. Thus, the bcl-2 translocation is useful in the differential diagnosis of B-cell neoplasms. In addition, presence of the bcl-2 translocation is an indicator of poor prognosis in large cell diffuse lymphoma. Testing during and after treatment may assist in monitoring therapeutic response and detection of minimal residual disease or recurrent lymphoma.

#### B-Cell / T-Cell Clonality for Lymphomas

According to Kim et al (2013) a clonality test for immunoglobulin (IG) and T-cell receptor (TCR) is a useful adjunctive method for the diagnosis of lymphoproliferative diseases (LPDs). Recently, the BIOMED-2 multiplex PCR assay has been established as a standard method for assessing the clonality of LPDs. Clonality in LPDs was tested in Korean patients using the BIOMED-2 multiplex PCR and compared the results with those obtained in European, Taiwanese, and Thai participants. The usefulness of the test as an ancillary method for diagnosing LPDs was also evaluated. A total of 219 specimens embedded in paraffin, including 78 B-cell lymphomas, 80 T-cell lymphomas and 61 cases of reactive lymphadenitis, were used for the clonality test. Mature B-cell malignancies showed 95.7 % clonality for IG, 2.9 % co-existing clonality, and 4.3 % polyclonality. Mature T-cell malignancies exhibited 83.8 % clonality for TCR, 8.1 % co-existing clonality, and 16.2 % polyclonality. Reactive lymphadenitis showed 93.4 % polyclonality for IG and TCR.

The majority of these results were similar to those obtained in Europeans. However, the clonality for IGK of B-cell malignancies and TCRG of T-cell malignancies was lower in Koreans than Europeans. The authors concluded that the BIOMED-2 multiplex PCR assay was a useful adjunctive method for diagnosing LPDs.

Shin et al (2013) stated that the evaluation of bone marrow (BM) involvement is important for diagnosis and staging in patients with lymphoid neoplasia. These researchers evaluated Ig and/or TCR gene re-arrangements in the BM for diagnosis and staging in patients with lymphoid neoplasia, using the standardized BIOMED-2 multiplex PCR clonality assays. The results were compared with microscopic findings such as histology and CD10, CD20, CD79a, CD3 and CD5 immunohistochemistry. A total of 151 samples were enrolled; 119 B-cell neoplasia, 29 T-cell neoplasia, and 3 Hodgkin's lymphoma. The molecular clonality assay and microscopic diagnosis were concordant in 66.9 % (n = 101) and discordant in 33.1 % (n = 50); Ig/TCR gene clonality assay detected 43 cases of BM involvement which was not presented in the morphology. Two cases among them turned into microscopic BM involvement during a close follow-up. Clonal TCR gene rearrangements were detected in 12.6 % of B-cell neoplasia and Ig gene rearrangement were found in 3.4 % of T-cell neoplasia. The authors concluded that this molecular clonality assay is valuable particularly in diagnosing BM involvement of lymphoid neoplasia if it is morphologically uncertain. But it should be carefully interpreted because molecular clonality may be present in the reactive lympho-proliferation. Therefore, comprehensive analysis with morphologic analysis should be important to reach a final diagnosis.

Shaw et al (2014) stated that re-biopsy rates as high as 12 % have been reported in previous studies of primary central nervous system lymphoma (PCNSL). This can lead to secondary operations, increasing risks of morbidity to the patient and costs for the NHS. Polymerase chain reaction testing for clonality in hematological malignancies has been applied to cases of lymphoma outside the CNS, but is less commonly used in the diagnosis of CNS lymphomas. Clonality in B- and T-cell populations may indicate the presence of malignancy. The present study aimed to identify factors to reduce the re-biopsy rate in PCNSL. A cohort of 102 suspected cerebral lymphoma cases biopsied at Frenchay Hospital, Bristol over a 10-year period (2000 to 2010) was examined. Clinical data, including age, sex, location, pre-biopsy steroid use, the need for re-biopsy and histological diagnosis, were collected. Re-biopsied cases were retrospectively reviewed and they subsequently underwent PCR testing for clonality. Overall, 96/102 (94 %) cases achieved a histological diagnosis after 1 or more biopsies; 81/96 (84 %) of these were lymphomas involving the brain and 15/96 (16 %) were spinal lymphomas. The majority of these were B-cell lymphomas (95/96 (99 %)), with 1 case of peripheral T-cell lymphoma (1/96 (1 %)). Due to insufficient histological evidence of PCNSL after the 1st biopsy, 9/102 (9 %) of cases had required re-biopsy. In 7/9 (78 %) of these cases, these investigators undertook PCR testing for clonality on tissue from the 1st biopsy; 3/7 (43 %) cases were monoclonal for B or T populations, raising the possibility of PCNSL. Based on these results, the authors recommended that all CNS lympho-proliferative lesions be assessed by hematopathologists, with the inclusion of PCR testing particularly in equivocal cases. This would reduce the number of patients going for re-biopsy and reduce the patient morbidity and costs for the NHS.

Ribera et al (2014) noted that in up to 5 to 15 % of studies of LPDs, flow cytometry (FCM) or immunomorphologic methods cannot discriminate malignant from reactive processes. These researchers determined the usefulness of PCR for solving these diagnostic uncertainties. Immunoglobulin heavy-chain gene (IGH) and TCRy genes were analyzed by PCR in 106 samples with inconclusive FCM results. A clonal result was registered in 36/106 studies, with a LPD being confirmed in 27 (75 %) of these cases. Specifically, 9/9 IGH clonal and 16/25 TCRy clonal results were finally diagnosed with LPD. Additionally, 2 clonal TCRy samples with suspicion of

undefined LPD were finally diagnosed with T LPD. Although polyclonal results were obtained in 47 of the cases studied (38 IGH and 9 TCRy), hematologic neoplasms were diagnosed in 4/38 IGH polyclonal and in 1/9 TCRy polyclonal studies. There were also 14 PCR polyclonal results (4 IGH, 10 TCRy), albeit non-conclusive. Of these, 2/4 were eventually diagnosed with B-cell lymphoma and 3/10 with T-cell LPD. In 8 IGH samples, the results of PCR techniques were non-informative but in 3/8 cases a B-lymphoma was finally confirmed. The authors concluded that PCR is a useful technique to identify LPD when FCM is inconclusive. A PCR clonal B result is indicative of malignancy but IGH polyclonal and non-conclusive results do not exclude lymphoid neoplasms. Interpretation of T-cell clonality should be based on all the available clinical and analytical data.

Furthermore, the NCCN' clinical practice guideline on "Non-Hodgkin's lymphomas" (Version 2.2014) recommends determination of T-cell clonality in T-cell lymphomas, including mycosis fungoides/Sezary syndrome. The guideline also recommends determining B-cell clonality for the B-cell lymphomas.

#### BK and JC Polyomaviruses

BK virus (BKV) and JC virus (JCV) are double-stranded DNA, human polyomaviruses (Quest Diagnostics, 2005). More than 70 % of the adult population has antibodies to BKV and JCV, with primary infections typically occurring in childhood. In immunocompetent individuals, primary BKV infections usually cause a mild respiratory illness and, rarely, cystitis, whereas primary JCV infections are typically asymptomatic. After initial infection, polyomaviruses establish latency in various tissues. The primary sites of latency are uroepithelial cells for BK virus and B-lymphocytes and renal tissue for JCV. Re-activation of latent as well as primary BKV and JCV infections may occur in immunocompromised individuals. BKV infections can lead to interstitial nephritis, hemorrhagic cystitis, and kidney allograft rejection. BKV nephropathy is associated with BK viremia of greater than 5,000 copies/mL (plasma) and BK viruria greater than 107 copies/mL and is seen in approximately 8 % of kidney transplant recipients. Though latency is typically associated with the absence of viremia, low levels (less than 2,000 copies/mL plasma) are seen in some asymptomatic individuals. JCV is responsible for progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system seen in up to 70 % of AIDS patients. Additionally, BKV and JCV viruria are found in approximately 40 % of bone marrow transplant patients.

Polymerase chain reaction testing detects the presence of the virus, not antibodies to the virus; thus, the detection of viral DNA may be indicative of an active infection (Quest Diagnostics, 2005; Randhawa et al, 2005). The identification of viral DNA may warrant the institution of antiviral therapies and/or a decrease of immunosuppressive therapies. Determination of viral DNA presence or concentration is also useful in establishing the cause of allograft rejection.

JC virus has been identified as the cause of PML in persons receiving natalizumab (Tysabri), which is indicated for certain persons with Crohn's disease and multiple sclerosis. Natalizumab increases the risk of PML, which is a rare and frequently fatal demyelinating disease of the CNS. Use of natalizumab requires enrollment of the prescriber, patient, and infusion center and pharmacy in a risk-minimization program, called the TOUCH Prescribing Program, in an attempt to identify cases of PML as early as possible. Prior to initiating the therapy a MRI scan must be obtained for each patient with multiple sclerosis to help differentiate potential, future symptoms of multiple sclerosis from PML. A baseline brain MRI may also be helpful in patients with Crohn's disease, although baseline lesions are uncommon. The FDA-approved labeling recommends that clinicians monitor patients for any new sign or symptom that may be suggestive of PML. Natalizumab should immediately be withheld at the first sign or symptom suggestive of PML. For

diagnosis of PML, the labeling recommends an evaluation that includes a gadolinium-enhanced MRI scan of the brain and, when indicated, cerebrospinal fluid analysis for JC viral DNA.

Furmaga et al (2021) state that the BK polyomavirus (BKPyV) is activated in cases of immune deficiency, both pharmacological and pathological, and BKPyV infection is of particular importance in recipients of kidney transplants or HSC transplantation. BKPyV infection can lead to the loss of the transplanted kidney or to hemorrhagic cystitis. The authors state that "The development of molecular methods has enabled significant improvement not only in BKPyV diagnostics, but in monitoring the effectiveness of treatment as well. Amplification of viral DNA from urine by PCR (Polymerase Chain Reaction) and qPCR Quantitative Polymerase Chain Reaction) is a non-invasive method that can be used to confirm the presence of the genetic material of the virus and to determine the viral load". The authors state that the "presence of BKPyV in organ transplant recipients most often induces urinary tract infections. In the case of kidney transplants, the disease occurs in the transplanted kidney, while in the case of transplants of other organs, such as a heart, liver or lung, BKPyV infection is observed in the recipient's native kidneys, and occasionally in other organs".

Dube et al (2023) states that "BK virus DNAemia (BKPyV) and nephropathy are common after kidney transplant; however, there are limited data on BK infections in nonrenal solid organ transplant recipients". The authors "examined the frequency, clinical and pathologic features, and kidney and lung outcomes of BKPyV and BK virus native kidney nephropathy (BKVN) in lung transplant recipients" at a single center. The authors found that "among 878 recipients transplanted from 2003 to 2019, 56 (6%) developed BKPyV at a median of 30.1 months after transplant (range, 0.6-213) and 11 (1.3%) developed BKVN at a median of 46 months after transplant (range, 9-213)". The incidence of end-stage kidney disease was significantly higher in those patients with peak viral load  $\geq 10\ 000$  copies/mL ( $p < .001$ ). "All cases of BKVN were in patients with peak viral load of  $\geq 10\ 000$  copies/mL, and 55% of these patients developed end-stage kidney disease. Despite the reduction of immunosuppression to treat BKVN, only 1 patient developed acute rejection, and lung function was stable  $> 1$  year". Per their review, BKPyV and nephropathy were found to be more common after lung transplantation than previously reported. Thus, the authors concluded that routine screening for BKPyV should be considered in all lung transplant recipients.

#### Blastomycosis

An UpToDate review on "Clinical manifestations and diagnosis of blastomycosis" (Bradsher, 2012) states that "Molecular identification of *B. dermatitidis* has been accomplished by a variety of techniques, including PCR assays for ribosomal genes, the ITS-region, repetitive sequences, and species- or genus-specific genes. PCR techniques, although promising, are labor-intensive, are not routinely available, and have not been examined in large prospective studies".

#### Brucella

Brucella species are small, non-motile, gram-negative coccobacilli. The species that infect humans are *Brucella abortus*, *B. melitensis*, *Brucella suis*, and, rarely, *Brucella canis* (AAP, 2006).

Brucellosis in children frequently is a mild self-limited disease compared with the more chronic disease observed among adults. However, in areas where *Brucella melitensis* is the endemic species, disease can be severe. Onset of illness can be acute or insidious. Manifestations are nonspecific and include fever, night sweats, weakness, malaise, anorexia, weight loss, arthralgia, myalgia, abdominal pain, and

headache. Physical findings include lymphadenopathy, hepatosplenomegaly, and, occasionally, arthritis. Serious complications include meningitis, endocarditis, and osteomyelitis.

Brucellosis is a zoonotic disease of wild and domestic animals. Humans are accidental hosts, contracting the disease by direct contact with infected animals and their carcasses or secretions or by ingesting unpasteurized milk or milk products. Persons in occupations such as farming, ranching, and veterinary medicine, as well as abattoir workers, meat inspectors, and laboratory personnel, are at increased risk. Approximately 100 cases of brucellosis occur annually in the United States.

A definitive diagnosis is established by recovery of *Brucella* organisms from blood, bone marrow, or other tissues. A presumptive diagnosis can be made by serologic testing. The serum agglutination test (SAT), which is the most commonly used test, will detect antibodies against *B. abortus*, *B. suis*, and *B. melitensis*, but not *B. canis*. Detection of antibodies against *B. canis* requires use of *B. canis*-specific antigen. Enzyme immunoassay is a sensitive method for determining IgG, IgA, and IgM anti-*Brucella* antibodies, but until better standardization is established, EIA should be used for suspected cases with negative SAT titers or for evaluation of patients with suspected relapse or re-infection. The PCR test has been developed but is not available in most clinical laboratories (AAP, 2009).

Prolonged therapy is imperative for achieving a cure. Oral doxycycline or tetracycline given orally should be administered for 4 to 6 weeks. Oral trimethoprim-sulfamethoxazole is appropriate therapy for younger patients. For life-threatening complications of brucellosis, such as meningitis or endocarditis, the duration of therapy often is extended for several months.

#### Burkholderia Infections

Culture is the appropriate method to diagnose *B. cepacia* complex infection. In cystic fibrosis lung infection, culture of sputum on selective agar is recommended to decrease the potential for over-growth by mucoid *P. aeruginosa*. *B. cepacia* and *B. gladioli* can be identified by PCR assay, but this assay is not available routinely (AAP, 2009).

#### Caliciviruses

Caliciviruses are RNA viruses. The 2 recognized genera that cause disease in humans are noroviruses (formerly Norwalk-like viruses) and sapoviruses. Symptoms of infection include diarrhea and vomiting, commonly accompanied by fever, headache, malaise, myalgia, and abdominal pain. Symptoms last from 1 day to 2 weeks. According to the AAP (2009), commercial assays for diagnosis are not available in the United States. Tests available in some research and reference laboratories include electron microscopy, PCR, and serologic testing. There is no specific treatment for calicivirus infection; supportive therapy includes oral rehydration solution to replace fluids and electrolytes. No specific control measures are available. Given the self-limited nature of infection and the lack of specific treatment, there is no indication for testing other than by public health laboratories in investigating outbreaks.

#### Campylobacteriosis (Campylobacter Infection)

Campylobacter infection is diagnosed when a culture of a stool specimen yields the organism (CDC, 2009; CDC, 2010). Visualization of motile and curved, spiral or S-shaped rods by stool phase-contrast or darkfield microscopy can provide rapid presumptive evidence for *Campylobacter enteritis*. *Campylobacter* species can be detected directly in stool specimens by commercially available enzyme immunoassay or in research laboratories by PCR assay (AAP, 2009).

## Candidiasis

A diagnosis of *Candida vaginitis* is suggested clinically by pruritus and erythema in the vulvovaginal area; a white discharge may be present (CDC, 2002). The diagnosis can be made in a woman who has signs and symptoms of vaginitis when either a) a wet preparation (saline, 10 % KOH) or Gram stain of vaginal discharge demonstrates yeasts or pseudohyphae, or b) a culture or other test yields a positive result for a yeast species (CDC, 2002; AGM-MSSVD, 2002). *Candida vaginitis* is associated with a normal vaginal pH (less than 4.5). According to the CDC (2002), identifying Candida by culture in the absence of symptoms is not an indication for treatment, because approximately 10 % to 20 % of women harbor Candida species and other yeasts in the vagina. Culture may be indicated in women with recurrent vulvovaginal candidiasis (defined as more than four episodes of vulvovaginal Candidiasis per year) to confirm the clinical diagnosis and to identify unusual species, including non-albicans species, including *C. glabrata*. A rapid antigen detection assay for Trichomonas and Candidiasis has a sensitivity of 61 to 81 % and a specificity of 97 % for *C. albicans* (WHO, 1999). A commercially available, rapid, automated hybridization assay is available that uses DNA probes to directly detect Candida, Trichomonas and Gardnerella in vaginal swab samples (WHO, 1999). This assay has a reported sensitivity 80 % and specificity of 98 % for *Candida vaginalis*.

Current guidelines do not include a recommendation for PCR testing for candidiasis (ACOG; 2006; BASHH, 2006; CDC, 2006; AAP, 2012; Workowski et al, 2010).

Guidelines on diagnostic procedures for candidiasis from the European Society of Clinical Microbiology and Infections Disease (Cuenca-Estrella, et al., 2012) state that "PCR-based procedures have not been validated, and no recommendations can be made."

According to guidelines from the CDC (2006), *C. glabrata* may be suspected in persons with recurrent vulvovaginal candidiasis. The CDC explains that *C. glabrata* and other nonalbicans Candida species are observed in 10 to 20 % of patients with recurrent vulvovaginal candidiasis, and that conventional anti-myotic therapies are not as effective against these species as against *C. albicans*. The CDC guidelines state that *C. glabrata* does not form pseudohyphae or hyphae and is not easily recognized on microscopy. The CDC states that the clinical diagnosis of *C. glabrata* can be confirmed by vaginal cultures, and that such cultures should be obtained from patients with RVVC to confirm the clinical diagnosis and to identify unusual species.

*Candida albicans*, *C. dubliniensis*, and *C. stellatoidea* can be identified morphologically via germ-tube formation (hyphae are produced from yeast cells after 2 to 3 hrs of incubation) on microscopy, or with biochemical assays.

CHROMagar Candida is a specialized media for Candida isolation, which allows for the presumptive identification of several Candida species by using color reactions in specialized media that demonstrate different colony colors depending on the species of Candida. CHROMagear distinguishes *C. albicans*, *C. tropicalis*, and *C. krusei* based on the species' distinctive pigments.

Also, fluorescent in-situ hybridization (FISH) has been used to distinguish *C. albicans* from non-albicans species. The peptide nucleic acid FISH (PNA-FISH) is used to distinguish *C. albicans* from non-albicans Candida species. The *C. albicans* PNA-FISH test can be used to identify *C. albicans* in 24 to 48 hrs when the probe is added to smears that are made directly from the blood culture bottle and followed by hybridization. A newer version of this test now allows for the simultaneous identification of either *C. albicans* or *C. glabrata*.

Culture of Candida species allows for susceptibility testing. According to current guidelines, susceptibility testing may be considered in situations where there is a failure to respond to initial antifungal therapy.

Updated guidelines on the management of candidiasis from the Infectious Diseases Society of America (Pappas et al, 2009) concluded: "Real-time PCR is a non-validated but intriguing methodology that holds promise as an early diagnostic aid for candidemia. These encouraging data offer new perspectives for early diagnosis of Candida infections, but continued evolution of these assays will be required before they can be used routinely." These recommendations are in line with previous guidelines from other authorities on the management of candida infectious that indicated no role for PCR testing (CDC, 2006; White et al, 2006; Pickering et al, 2006). According to guidelines from the British Association for Sexual Health and HIV (White et al, 2006), PCR testing for Candida species "is currently of use only as a research tool." More recently, Sobel (2020) stated that PCR tests are now available commercially, "but are costly and offer no proven benefit over culture in symptomatic women."

#### Cerebrospinal Fluid (CSF) Pathogen Testing

Pfefferle et al. (2020) state that infectious meningitis is a serious disease in which patient outcomes relies on fast and reliable diagnostic testing such as the FilmArray ME multiplex PCR test. The authors analyzed clinical performance of the assay in a real life setting at a tertiary university hospital using a pragmatic and simple sample selection strategy to reduce the overall cost burden. FilmArray ME analysis was restricted to CSF-samples (n=171) that had high pretest probability of infectious meningitis, e.g. positive Gram-stain, samples in which leukocytes and/or bacteria were evident or urgent suspicion of infection was communicated by clinicians. Those samples were also analyzed by reference methods: culture only (n = 45), PCR only (n = 20) or both methods (n = 106). The authors found that 56/171 (32.75%) were FilmArray ME positive. Bacterial pathogens were detected in 30/56 (53.57%), viral pathogens were detected in 27/56 (48.21%) and yeast DNA was detected in 1/56 (1.79%) of positive samples. Double detection occurred in 2/56 samples. In 52/56 (92.86%) FilmArray ME positive samples, results could be confirmed by the reference assays (sensitivity = 96.30%, specificity = 96.58%). The authors concluded that the "FilmArray ME assay is a fast and reliable diagnostic tool for the management of infectious meningitis and can easily be implemented in routine diagnostic workflows. However, correlation of test results and underlying clinical symptoms requires experienced users and the awareness of potentially false negative or false positive results. Moreover, considering the need for antimicrobial susceptibility testing, the use of molecular tests as a stand-alone diagnostic cannot be recommended".

Multiplex or panel-based nucleic acid amplification tests are available for testing multiple bacterial and viral pathogens simultaneously in a single CSF sample (eg, FilmArray meningitis/encephalitis panel [BioFire]). These tests have been found to be highly sensitive and specific, though false-positive and false-negative results can occur. If a multiplex panel is performed, it should be used in conjunction with standard microbiologic tests (e.g., cultures of CSF and blood). Multiplex panels do not detect all causes of CNS infection, nor do they provide any information on antimicrobial susceptibility (Kaplan, 2018).

Domingues et al. (2019) state that the FilmArray meningitis/encephalitis (ME) panel is a PCR multiplex for simultaneous and rapid identification of 14 pathogens, including 6 bacteria, 7 viruses, and Cryptococcus. The authors evaluated 436 CSF samples using the FilmArray ME Panel. Among them, 25 cases were positive for bacteria, being Streptococcus pneumonia the most frequent (48 %). Among positive cases for bacteria, 60 % were positive only with FilmArray. All the bacterial meningitis cases in which the only positive test was FilmArray had CSF findings

suggestive of bacterial meningitis, including neutrophilic pleocytosis, increased CSF protein and lactate, and decreased CSF glucose. The authors concluded that these findings suggest that FilmArray may increase the diagnostic sensitivity for bacterial meningitis.

Eichinger et al. (2019) conducted a retrospective analysis of CSF multiplex PCRs (Biofire FilmArray) in children with clinical suspicion of meningitis, encephalitis or sepsis-like illness. During the 1-year study period, the multiplex PCR panel was performed on 187 individual CSF samples that met the inclusion criteria. About half of the patients (92/187) were less than 1 year of age. In 27 cases (14.4%), the PCR yielded a positive result with the majority (12/27) being indicative of an enteroviral infection. In the age group of 8-84 days of life, 36.4% of the patients had a positive result. When the patients with a PCR positive for a viral agent were compared to an age-matched group of patients, no differences were observed regarding symptoms and laboratory parameters. However, the duration of antimicrobial therapy could be significantly reduced through the use of multiplex PCR. The authors concluded that the use of on-site diagnostic multiplex PCR was able to reduce the use of antimicrobials in selected cases, and that this test can guide clinical decisions earlier during the course of medical care compared to standard diagnostics.

The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based in vitro diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The FilmArray ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing (FDA, 2020).

The FDA approved BioFire FilmArray meningitis/encephalitis (ME) panel targets 14 different pathogens simultaneously in 1 test with results in one hour. This test uses 0.2 mL of cerebrospinal fluid (CSF), and is able to detect bacteria (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*), viruses (Cytomegalovirus (CMV), Enterovirus, Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Human herpesvirus 6 (HHV-6), Human parechovirus, and Varicella zoster virus), as well as, yeast (*Cryptococcus neoformans/gattii*). The panel is reported to have an overall sensitivity of 94.2% and a specificity of 99.8% (BioFire, 2020b).

The Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (Miller et al, 2018) state that "FDA-cleared multiplex PCR targeting 14 organisms is available for diagnosing meningitis and encephalitis"; however, "it should not be considered a replacement for culture since clinical experience with the assay is limited and specificity issues have been reported". The IDSA state that although molecular testing has replaced viral culture for the diagnosis of enteroviral meningitis, it is not routinely relied on for the detection of bacteria in CSF where Gram stain and bacterial culture should be ordered. "NAAT of CSF is more sensitive than viral culture for the diagnosis of enteroviral CNS infection".

An UpToDate review on "Clinical features and diagnosis of acute bacterial meningitis in adults" (Hasbun, 2020) state that nucleic acid amplification tests, such as the PCR assay, has shown high sensitivity and specificity in patients with bacterial meningitis; however, the author does not routinely recommend for in those with suspected bacterial meningitis. The author states that multiplex PCR assays may be useful in selected patients whose CSF findings are consistent with the diagnosis, but who have negative-CSF Gram stain or culture, or have received prior antimicrobial therapy. Use of PCR for the two most common meningeal pathogens (*S. pneumoniae* and *N. meningitidis*) is routinely recommended in the

United Kingdom guidelines for patients presenting with meningitis. Studies evaluating the multiplex PCR assay for detection of *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type b have shown sensitivities and specificities of 100 percent. Problems with false-positive results have been reported with PCR but false negatives are very uncommon. Similar to bacterial meningitis, viral meningitis presents acutely with classic signs and symptoms of meningitis. Unlike bacterial meningitis, the CSF normally has a lymphocytic pleocytosis, normal glucose, moderate elevation of protein, and negative-CSF Gram stain and culture. Definitive diagnosis of viral meningitis is made by CSF polymerase chain reaction (PCR).

#### Cervical Intraepithelial Neoplasia (CIN) Metastases

Current evidence-based guidelines from the National Comprehensive Cancer Network (NCCN) (2003) and information from the National Cancer Institute (NCI) (2002) mentioned no role for PCR testing in the detection of metastases from cervical cancer.

#### Chancroid

Chancroid is a genital ulcer disease caused by the bacterium *Haemophilus ducreyi*. In the United States, chancroid usually occurs in discrete outbreaks, although the disease is endemic in some areas. The accuracy of clinical diagnosis varies due to the atypical presentation of the ulcer. According to the AAP (2006), the diagnosis of chancroid usually is made on the basis of clinical findings and the exclusion of other infections associated with genital ulcer disease, such as syphilis or HSV, or adenopathies, such as lymphogranuloma venereum. Direct examination of clinical material by Gram stain may strongly suggest the diagnosis if large numbers of gram-negative coccobacilli, sometimes in "school of fish" patterns, are seen.

Confirmation by recovery of *H. ducreyi* from a genital ulcer or lymph node aspirate is the more available alternative diagnostic test. The AAP (2009) notes that fluorescent monoclonal antibody stains and PCR assays can provide a specific diagnosis but are not available in most laboratories.

According to the Association for Genitourinary Medicine (AGUM) of the Medical Society for the Study of Venereal Disease (MSSVD) (2002), in addition to culture or direct examination of gram stain, *H. ducreyi* may be identified by detection of nucleic acid (DNA) by amplification techniques such as PCR techniques, using nested techniques.

According to the CDC, a culture for *H. ducreyi* should be performed in patients with genital ulcer disease from regions where *H. ducreyi* is prevalent (CDC, 2002). According to the WHO (1999), the sensitivity and specificity of culture is 56 to 90 % and 100 %, respectively; the WHO notes that the sensitivity of culture can only be estimated because there is no gold standard on which to base the diagnosis of chancroid. The resolved sensitivity of PCR using *H. ducreyi* compared to culture is 77 to 98 %, and the specificity is 98 to 100 % (WHO, 1999). Conversely, culture may be only 75 % sensitive relative to PCR. Yet, PCR may be negative in a number of culture-proven chancroid cases, owing to the presence of Taq polymerase inhibitors in the DNA preparations extracted from genital ulcer specimens.

To circumvent the many problems of positive diagnosis of chancroid, the CDC proposes that a "probable diagnosis", for both clinical and surveillance purposes, be made if the patient has one or more painful genital ulcers, and (i) no evidence of T. pallidum infection by dark field examination of ulcer exudates or by a serologic test for syphilis, and (ii) the clinical presentation, appearance of the genital ulcers and regional lymphadenopathy, if present, is typical for chancroid and a test for HSV is negative (CDC, 2002).

The Centers for Disease Control and Prevention (CDC) states that preliminary diagnosis of chikungunya virus infection is based on the patient's clinical features, places and dates of travel, and activities (Staples et al, 2014). Laboratory diagnosis is generally accomplished by testing serum to detect virus, viral nucleic acid, or virus-specific IgM and neutralizing antibodies.

During the first week after onset of symptoms, Chikungunya virus infection can often be diagnosed by using viral culture or RT-PCR on serum (Staples et al, 2014). Chikungunyavirus-specific IgM and neutralizing antibodies normally develop toward the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative.

Testing for Chikungunyavirus IgM and IgG is commercially available (Staples et al, 2014). However, confirmatory neutralizing antibody testing is only available through CDC and a few state health laboratories.

#### Chlamydia Trachomatis

*Chlamydia trachomatis* is an important cause of urethritis and cervicitis (WHO, 1999; AGM-MSSVD, 2002). Laboratory detection of *C. trachomatis* is necessary because as many as 70 % to 80 % of women and up to 50 % of men who are infected do not experience any symptoms. The U.S. Preventive Services Task Force (USPSTF) strongly recommends that clinicians routinely screen all sexually active women 25 years and younger, and other asymptomatic women at increased risk of infection, for chlamydial infection (USPSTF, 2002). The conventional method for the laboratory diagnosis of *C. trachomatis* has been inoculation of a cell culture with a genital specimen. According to the WHO (1999), however, this method is expensive, labor-intensive, time-consuming, and requires considerable expertise. For these reasons, culture tests are now used less frequently and antigen and nucleic acid detection techniques have become common methods for detection of *C. trachomatis* infection.

The leukocyte esterase assay is a rapid urine dipstick test for the presence of an enzyme found in the urine when leukocytes are present due to inflammation. The LE test can diagnose urethritis but can not identify the specific cause of the infection. The sensitivity and specificity of LE for the detection of chlamydial and gonococcal infection are 54 % to 97 % and 36 % to 95 %, respectively (WHO, 1999). Microscopy detection of *C. trachomatis* has a reported sensitivity and specificity of 74 % to 90 % and a specificity of 9 8% to 99 %. According to the AAP's Committee on Infectious Diseases, nucleic acid amplification methods, such as PCR, transcription-mediated amplification (TMA), and strand displacement amplification (SDA) are more sensitive than cell culture and more specific and sensitive than DNA probe, direct fluorescent antibody (DFA) tests, or enzyme immunoassays (EIAs), although specificity is variable compared with culture (AAP, 2006). According to the USPSTF (2002), the sensitivities and specificities of nucleic acid amplification tests are all high, ranging from 82 to 100 %. The sensitivity of antigen detection tests (EIA, DFA) is slightly lower (70 to 80 %) but specificity remains high (96 to 100 %). The AAP states that tests for detection of chlamydial antigen or nucleic acid are useful for evaluating urethral specimens from males, cervical specimens from females, and conjunctival specimens from infants. The PCR and LCR tests are useful for evaluating urine specimens from either sex. The Scottish Intercollegiate Guidelines Network (2000) states that nucleic acid amplification tests (LCR or PCR) are the recommended laboratory test for *Chlamydia trachomatis*. The British Association of Sexual Health and HIV (BASHH, 2006) makes similar recommendations about PCR testing for *Chlamydia trachomatis*.

*Chlamydophila* (formerly *Chlamydia*) *pneumoniae* is a species of Chlamydia that is antigenically, genetically, and morphologically distinct from Chlamydia species. *C. pneumoniae* has been found in 5 to 10 % of older adults with community-acquired pneumonia and often produces disease severe enough to require hospitalization. This organism has also been implicated in 5 to 10 % of cases of nosocomial pneumonia, but relatively little is known about its epidemiology. Transmission of *C. pneumoniae* is presumably by respiratory aerosol transmission between humans.

Clinical features of *C. pneumoniae* resemble those of mycoplasmal pneumonia, including pharyngitis, bronchitis, and pneumonitis, primarily in older children and young adults. Most patients have cough, fever, and sputum production but are not seriously ill.

In addition to acute respiratory tract disease, some investigators have associated *C. pneumoniae* with atherosclerotic cardiovascular disease. This association is based on the increased frequency of serum antibodies in patients compared with controls, the detection of antigen or DNA in atheromatous plaques, the production of arterial lesions in experimentally infected animals, and small human trials demonstrating that treatment of high-risk patients with macrolides decreases the risk of subsequent cardiovascular events. According to the AAP, large, prospective, randomized trials are underway to further explore this association and to determine whether treatment is beneficial (AAP, 2003). Other investigators have associated *C. pneumoniae* with asthma, Alzheimer disease, multiple sclerosis, and Kawasaki disease, but the AAP has concluded that the evidence supporting any of these associations is limited.

*C. pneumoniae* may be detected by cultivating it in embryonated egg cultures (as with other chlamydiae), by using direct stains with immunofluorescence or a polymerase chain reaction, or by using serial serologic tests to show seroconversion. However, these tests are usually unavailable in most clinical laboratories. The diagnosis is suspected in a patient who has typical symptoms, has no established alternative diagnosis, and does not respond to beta-lactam antibiotics. *C. pneumoniae* may be treated with erythromycin or tetracycline.

Polymerase chain reaction is not available routinely but may be used to establish a probable diagnosis of psittacosis and distinguish *Chlamydophila psittaci* from other chlamydial infections (AAP, 2009; NASPHV, 2010).

#### Chronic Lymphocytic Leukemia: IgVH Mutation Analysis

Chronic lymphocytic leukemia (CLL) patients can be divided into 2 basic groups on the basis of the mutational status of the immunoglobulin heavy-chain, variable-region (IgVH) gene in leukemic cells (Chin et al., 2006). Patients with IgVH mutations have longer survival than those without IgVH mutations. Thus, mutation analysis may be useful for assessing prognosis of patients with CLL and planning management strategies.

#### Chronic Myelogenous Leukemia and Acute Lymphocytic Leukemia: bcr/abl Gene Rearrangement

The bcr/abl fusion gene, formed by rearrangement of the breakpoint cluster region (bcr) on chromosome 22 with the c-abl proto-oncogene on chromosome 9, is present in 95 % of patients with chronic myelogenous leukemia (Chin et al, 2006). It is also identified in 30 % of patients with acute lymphocytic leukemia, in which it is associated with poor prognosis. The t(9;22)(q34;q11) translocation associated with bcr/abl leads to a cytogenetic aberration known as Philadelphia chromosome, although this rearrangement may also be detected in the absence of cytogenetically defined Philadelphia chromosome. The bcr/abl rearrangement causes production of an abnormal tyrosine kinase molecule with increased activity, postulated to be responsible for the development of leukemia. Increasing levels of

bcrabl are associated with clinical progression. Identification of the bcrabl rearrangement is important for the diagnosis of CML, whereas in ALL, the presence of bcrabl is associated with poor prognosis and may warrant more aggressive therapy. In both diseases, increasing levels of bcrabl may be associated with clinical progression.

#### Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a clonal disease of multi-potent hematopoietic cells associated with specific cytogenetic changes involving a translocation t(9;22) (q34;q11), more commonly known as Philadelphia Chromosome (Ph1). Ph1-negative CML is a poorly defined entity that is less clearly distinguished from other myeloproliferative syndromes. Patients with Ph1-negative CML generally have a poorer response to treatment and shorter survival than Ph1-positive patients. However, Ph1-negative patients who have bcr-abl gene rearrangement detectable by Southern blot analysis have prognoses equivalent to Ph1-positive patients. A small subset of patients has bcr-abl detectable only by RT-PCR, which is the most sensitive technique currently available. Patients with RT-PCR evidence of the bcr-abl fusion gene appear clinically and prognostically identical to patients with a classic Ph1; however, patients who are bcr-abl-negative by RT-PCR have a clinical course more consistent with CML, a distinct clinical entity related to myelodysplastic syndrome. Fluorescent in-situ hybridization of the bcr-abl translocation can be performed on the bone marrow aspirate or on the peripheral blood of patients with CML.

#### Chromosome 18q for Colorectal Carcinoma

Colorectal cancer patients with tumors with chromosome 18 deletions are more likely to have disease recurrence and have a shorter disease-free survival period when compared to patients with 2 copies of this chromosome (Chin et al, 2006). The chromosome 18q assay is used in the diagnosis of colorectal cancer, and in predicting recurrence of disease.

#### Clostridium Difficile

*Clostridium difficile* is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis, which have significant morbidity and mortality. Accurate and timely diagnosis is critical. Repeat enzyme immunoassay testing or PCR testing for *C. difficile* toxin has been recommended because of less than 100 % sensitivity. Aichinger and colleagues (2008) reported that the diagnostic gains of repeat testing for *C. difficile* by enzyme immunoassay and PCR (i.e., initial negative result followed by positive result) within a 7-day period were 1.9 % and 1.7%, respectively. The authors concluded that there is little value of repeat testing for *C. difficile* by enzyme immunoassay or PCR.

According to the AAP Committee on Infectious Diseases (2009), "[e]ndoscopic findings of pseudomembranes and hyperemic, friable rectal mucosa suggest pseudomembranous colitis. To diagnose *C difficile* disease, stool should be tested for presence of *C difficile* toxins. Commercially available enzyme immunoassays detect toxins A and B, or an enzyme immunoassay for toxin A may be used in conjunction with cell culture cytotoxicity assay, the 'gold standard' for toxin B detection.".

According to United Kingdom National Health Services' guidelines on management of *C difficile* (2009), "although PCR has been described, its diagnostic role remains to be determined". The guidelines also note that a positive PCR test does not necessarily mean that toxin has been produced.

New guidelines from the Infectious Diseases Society of America (Cohen et al, 2010) indicate PCR testing as a preferred method of diagnosing *C. difficile*. Although more than 90 % of U.S. laboratories use commercial enzyme immunoassays to diagnose *C. difficile* infection, these tests are considered suboptimal because of their relatively low sensitivity (63 % to 94 %). The ISDA guidelines stated that they should be supplemented with either toxin testing or molecular assays (e.g., PCR).

The Committee on Infectious Diseases of the AAP's position statement on "Clostridium difficile infection in infants and children" (Schutze and Willoughby, 2013) stated that

(i) testing for *C difficile* colonization or toxin should only be performed in children with diarrhea who meet the clinical and age-related conditions, (ii) test of cure is not recommended, and (iii) testing for recurrence less than 4 weeks after initial testing is only useful when the results of repeat testing are negative.

#### Colorado Tick Fever

Colorado tick fever is an acute viral infection transmitted from the bite of an infected wood tick. The disease is found almost exclusively in the western United States and Canada, mostly in high mountain areas such as Colorado and Idaho. Colorado tick fever can be confirmed by measurement of virus-specific antibody in serum or CSF. The assay, with complement fixation or immunofluorescent techniques, must be performed in a laboratory with experience in performing this test. Serologic tests are often not positive for 10 to 14 days after symptom onset. In comparison, diagnostic PCR may be positive from the first day of symptoms (AAP, 2009).

#### Coronaviruses

Severe acute respiratory syndrome (SARS) is a viral respiratory illness caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV). SARS was first reported in Asia in February 2003. Over the next few months, the illness spread to more than 2 dozen countries in North America, South America, Europe, and Asia before the SARS global outbreak of 2003 was contained. According to guidelines from the Infectious Disease Society of America (Mandell et al, 2003), diagnostic criteria for SARS include clinical and epidemiologic features and may include diagnostic studies for SARS-CoV. Recommended virologic studies for laboratory confirmation are:

(i) culture for SARS-CoV, (ii) detection of antibody during the acute phase of illness or any time after onset, or (iii) detection of SARS-CoV ribonucleic acid (RNA) by RT-PCR confirmed by second PCR assay by using a second aliquot of the specimen or a different set of primers. Coronaviruses are also a cause of the "common cold" and other upper respiratory illnesses (AAP, 2009). Although PCR tests for coronaviruses have been developed, such testing is not necessary as infection is self-limited and treatment is symptomatic.

The Centers for Disease Control and Prevention (CDC) encourages clinicians to use an authorized nucleic acid assay that has received an FDA emergency use authorization (EUA) to test persons with signs and symptoms compatible with COVID-19. Clinicians are also encouraged to consider testing for other causes of respiratory illness, for example influenza, in addition to testing for SARS-CoV-2 depending on person's age, season, or clinical setting (CDC, 2020).

An UpToDate review on "Coronavirus disease 2019 (COVID-19): Diagnosis" (Caliendo and Hanson, 2020) state that nucleic acid amplification testing (NAAT), with a reverse-transcription PCR assay, to detect SARS-CoV-2 RNA from the upper respiratory tract is the preferred initial diagnostic test for COVID-19. The test is

typically performed on nasopharyngeal swabs, but can also be performed on other respiratory tract specimens (eg, oropharyngeal swabs, lower respiratory tract samples). Sensitivity and specificity are generally high, although performance varies based on the specific assay used, specimen quality, and duration of illness. Furthermore, if influenza and respiratory syncytial virus (RSV) are circulating in the community, it is reasonable to also test for these viruses when testing for SARS-CoV-2, as this could have management implications.

#### Cryptococcus

Primary *Cryptococcus neoformans* infection is acquired by inhalation of aerosolized fungal elements and often is unapparent or mild (AAP, 2006). Pulmonary disease, when symptomatic, is characterized by cough, hemoptysis, chest pain, and constitutional symptoms. Hematogenous dissemination to the central nervous system, bones and joints, skin, and mucous membranes can occur, but dissemination is rare in persons without defects in cell-mediated immunity (e.g., transplantation, malignant neoplasm, collagen-vascular disease, long-term corticosteroid administration, or sarcoidosis). Cryptococcal meningitis, the most common and serious form of cryptococcal disease, often follows an indolent course. Cryptococcal fungemia, without apparent organ involvement, occurs in patients with human immunodeficiency virus (HIV). Cryptococcosis is one of the acquired immunodeficiency syndrome (AIDS)-defining diseases.

Encapsulated yeast cells can be visualized by India ink or other stains of CSF. Definitive diagnosis requires isolation of the organism from body fluid or tissue. The lysis-centrifugation method is the most sensitive technique for recovery of *C. neoformans* from blood cultures. According to the AAP (2006), the latex agglutination and enzyme immunoassay tests for detection of cryptococcal capsular polysaccharide antigen in serum or CSF are excellent rapid diagnostic tests. Antigen detection in CSF or serum is positive in 90 % of patients with cryptococcal meningitis. The AAP guidelines (2006) stated that cryptococcal antibody testing is useful, but skin testing is of no value. The AAP guidelines (2009) stated that PCR tests for cryptococcus are investigational.

#### Cyclosporiasis (Cyclospora infection)

*Cyclospora* infection is diagnosed by examination of stool specimens. Microscopic methods for diagnosis involving ultraviolet light and acid fast staining are used (CDC, 2009; CDC, 2010). "Investigational" molecular diagnostic assays (e.g., PCR) are available at the CDC and some other reference laboratories (AAP, 2009). Trimethoprim/sulfamethoxazole is the usual treatment for Cyclospora infection. No highly effective alternative antibiotic regimen has been identified yet for patients who do not respond to the standard treatment or have a sulfa allergy (CDC, 2010).

#### Cryptosporidiosis (Cryptosporidium infection)

Diagnosis of cryptosporidiosis is made by examination of stool samples. Most often, stool specimens are examined microscopically using different techniques (e.g., acid-fast staining, direct fluorescent antibody, and/or enzyme immunoassays for detection of *Cryptosporidium* sp. antigens) (CDC, 2010). Molecular methods (e.g., PCR) are increasingly used in reference diagnostic labs to identify *Cryptosporidium* spp. at the species level. Although PCR assays can be used to identify species and genotyp, immunocompetent people need no specific therapy for Cryptosporidiosis (AAP, 2009). Thus, the results of such PCR testing would not alter clinical management.

#### Cytomegalovirus

Cytomegalovirus (CMV) causes various infections, occurring congenitally, postnatally, or at any age, ranging from inconsequential silent infection to disease manifested by fever, hepatitis, pneumonitis, and, in newborns, severe brain damage, stillbirth, or perinatal death.

Especially in the immunocompromised host, CMV may be isolated from urine, other body fluids, or tissues. However, CMV can be excreted for months or years after infection without causing active disease, and a positive CMV culture must be interpreted with regard to the particular host and disease manifestation.

Examination of cells shed in urine for intranuclear inclusions is an insensitive test.

Biopsy showing CMV-induced pathology is often important in demonstrating invasive disease.

Recovery of virus from a target organ provides unequivocal evidence that the disease is caused by CMV infection. However, according to the AAP (2006), a presumptive diagnosis can be made on the basis of a 4-fold antibody titer rise in paired serum samples or by demonstration of virus excretion.

Complement fixation is the least sensitive serologic method for diagnosis of CMV infection and should not be used to establish previous infection or passively acquired maternal antibody. Various immunofluorescence assays, indirect hemagglutination, latex agglutination, and enzyme immunoassays are preferred for this purpose.

Techniques for detection of viral DNA in tissues and some fluids, especially CSF, by PCR or hybridization are available from reference laboratories. Detection of pp65 antigen in white blood cells is used to detect infection in immunocompromised hosts.

Cytomegalovirus viral load tests using quantitative PCR are used to monitor disease progression.

Recently updated guidelines from the AAP Committee on Infectious Diseases (2009) commented on the use of PCR testing to detect intrauterine CMV infection: "Amniocentesis has been used in several small series of patients to establish the diagnosis of intrauterine infection. Proof of congenital infection requires isolation of CMV from urine, stool, respiratory tract secretions, or CSF obtained within 2 to 4 weeks of birth. Recent findings suggest that congenital CMV infection also might be diagnosed by using polymerase chain reaction assay to detect CMV DNA in newborn dried blood spots, although additional work is needed to define the sensitivity and feasibility of this approach. Differentiation between intrauterine and perinatal infection is difficult later in infancy unless clinical manifestations of the former, such as chorioretinitis or intracranial calcifications, are present. A strongly positive CMV-specific IgM is suggestive during early infancy, but IgM antibody assays vary in accuracy for identification of primary infection".

Boppana et al (2010) stated that reliable methods to screen newborns for congenital cytomegalovirus (CMV) infection are needed for identification of infants at increased risk of hearing loss. Since dried blood spots (DBS) are routinely collected for metabolic screening from all newborns in the United States, there has been interest in using DBS polymerase chain reaction (PCR)-based methods for newborn CMV screening. These researchers determined the diagnostic accuracy of DBS real-time PCR assays for newborn CMV screening. Between March 2007 and May 2008, infants born at 7 U.S. medical centers had saliva specimens tested by rapid culture for early antigen fluorescent foci. Results of saliva rapid culture were compared with a single-primer (March 2007 to December 2007) and a 2-primer DBS real-time PCR (January 2008 to May 2008). Infants whose specimens screened positive on rapid culture or PCR had congenital infection confirmed by the reference standard method with rapid culture testing on saliva or urine. Main outcome

measures were sensitivity, specificity, and positive and negative likelihood ratios (LRs) of single-primer and 2-primer DBS real-time PCR assays for identifying infants with confirmed congenital CMV infection. Congenital CMV infection was confirmed in 92 of 20,448 (0.45 %; 95 % CI: 0.36 % to 0.55 %) infants. Ninety-one of 92 infants had positive results on saliva rapid culture. Of the 11,422 infants screened using the single-primer DBS PCR, 17 of 60 (28 %) infants had positive results with this assay, whereas, among the 9,026 infants screened using the 2-primer DBS PCR, 11 of 32 (34 %) screened positive. The single-primer DBS PCR identified congenital CMV infection with a sensitivity of 28.3 % (95 % CI: 17.4 % to 41.4 %), specificity of 99.9 % (95 % CI: 99.9 % to 100 %), positive LR of 803.7 (95 % CI: 278.7 to 2,317.9), and negative LR of 0.7 (95 % CI: 0.6 to 0.8). The positive- and negative-predictive values of the single-primer DBS PCR were 80.9 % (95 % CI: 58.1 % to 94.5 %) and 99.6 % (95 % CI: 99.5 % to 99.7 %), respectively. The 2-primer DBS PCR assay identified infants with congenital CMV infection with a sensitivity of 34.4 % (95 % CI: 18.6 % to 53.2 %), specificity of 99.9 % (95 % CI: 99.9 % to 100.0 %), positive LR of 3,088.9 (95 % CI: 410.8 to 23,226.7), and negative LR of 0.7 (95 % CI: 0.5 to 0.8). The positive- and negative-predictive values of the 2-primer DBS PCR were 91.7 % (95 % CI: 61.5 % to 99.8 %) and 99.8 % (95 % CI: 99.6 % to 99.9 %), respectively. The authors concluded that among newborns, CMV testing with DBS real-time PCR compared with saliva rapid culture had low sensitivity (since they missed approximately 2/3 of the infections), limiting its value as a screening test.

#### Donovanosis

Donovanosis, or granuloma inguinale, is one cause of genital ulcer disease (WHO, 1999; AGM-MSSVD, 2002). The disease occurs rarely in the United States, although it is endemic in certain tropical and developing areas, including India; Papua, New Guinea; central Australia; and southern Africa. The disease is caused by *Klebsiella granulomatis* (previously known as *Calymmatobacterium granulomatis*), which can be seen in infected tissue as intracellular bacterial inclusions known as Donovan bodies but can not be cultured on artificial media. Diagnosis of donovanosis is made by direct visualization under microscope of Donovan bodies. Alternatively, a piece of clean granulation tissue is removed from the leading edge of the genital ulcer with a scalpel, smeared on a slide, and stained. According to the WHO (1999), the sensitivity and specificity of tissue microscopy or swab is 60 to 80 % and 100 %, respectively. The AAP (2009) notes that diagnosis by PCR assay and serologic testing is available on a research basis. The sensitivities and specificities of antibody detection and PCR have not been determined (WHO, 1999).

#### Ehrlichiosis

Ehrlichiosis is a febrile illness resembling Rocky Mountain spotted fever caused by rickettsial-like bacteria of the genus Ehrlichia and transmitted to humans by ticks (Merck Manual). Most cases have been identified in the southeastern and south-central parts of the United States. Two species of Ehrlichia are human pathogens in the United States: *E. chaffeensis* causes human monocytic ehrlichiosis and *E. phagocytophilia* or a related organism causes human granulocytic ehrlichiosis. *E. canis* is now regarded as human monocytic ehrlichiosis.

Regardless of the species causing the infection, the symptoms and signs are similar. Although some infections are asymptomatic, most cause an abrupt onset of illness with fever, chills, headache, and malaise, usually beginning about 12 days after the tick bite. Some patients develop a maculopapular or petechial rash involving the trunk and extremities, although rash is rare with *E. canis*. Abdominal pain, vomiting and diarrhea, disseminated intravascular coagulation, seizures, and coma may occur. Hematologic and hepatic abnormalities include leukopenia, thrombocytopenia, and abnormal liver function tests, especially elevated levels of transaminases.

According to guidelines from the Infectious Disease Society of America, ehrlichiosis may be suspected in patients with Lyme disease symptoms who have a very high fever (greater than 38 degrees C) (Wormser, 2000), or patients who develop fever in the absence of erythema migrans after an *Ixodes* tick bite in areas where these infections are endemic.

The CDC defines a confirmed case of ehrlichiosis as a 4-fold or greater change in antibody titer by IFA between acute and convalescent serum samples (ideally collected 3 to 6 weeks apart), PCR amplification of ehrlichial DNA from a clinical sample, or detection of intraleukocyttoplasmic *Ehrlichia* microcolonies (morulae) and a single IFA titer of more than 64. A probable case is defined as a single IFA titer of more than 64 or the presence of morulae within infected leukocytes. Examination of peripheral blood smears to detect morulae in peripheral blood monocytes or granulocytes is insensitive, but this test is warranted for patients for whom a high index of suspicion exists. The PCR test is useful in detecting ehrlichiosis in the acute phase before immunohistochemical tests are positive (AAP, 2006). The use of the PCR test to amplify nucleic acid from acute phase peripheral blood of patients with ehrlichiosis seems sensitive, specific, and promising for early diagnosis but currently is unstandardized and is available only in research laboratories and at the CDC (AAP, 2006; CDC, 2000; AAP, 2009).

Doxycycline is the drug of choice for treatment of human ehrlichiosis and is also effective against Lyme disease. Ehrlichiosis may be severe or fatal in untreated patients, and initiation of therapy early in the course of the disease helps minimize complications of the illness.

Polymerase chain reaction testing is also recommended for diagnosis of human granulocytic anaplasmosis (*Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophilum*)) (AAP, 2009).

#### Enteroviruses

Enteroviruses are a subgroup of the Picornaviridae family. They are subclassified into polioviruses, Group A and B coxsackieviruses, and echoviruses (Dua and Berkowicz, 2003). Enteroviruses cause a wide range of infections. Poliovirus infections can be subclinical or can cause mild illness, aseptic meningitis, or poliomyelitis. Coxsackie virus, an RNA virus, is one of several non-polio enteroviruses that are responsible for significant and frequent illnesses in infants and children and result in protean clinical manifestations. Coxsackievirus infections are the most common cause of viral heart disease. Group A viruses cause flaccid paralysis, while group B viruses cause spastic paralysis. Other diseases associated with coxsackievirus infections are hand-foot-and-mouth (HFM) disease and hemorrhagic conjunctivitis, caused by group A, while group B coxsackievirus is associated with herpangina, pleurodynia, myocarditis, pericarditis, and meningoencephalitis. Aseptic meningitis and colds are associated with both group A and group B. Echovirus infections range from the common cold and fever to aseptic meningitis and acute hemorrhagic conjunctivitis (AHC). The enteroviruses are spread from person to person via the fecal-oral route (Marx, 2002).

All enteroviruses enter the body through the oropharynx and multiply in the tissues around the oropharynx (Marx, 2002). Most enteroviral infections are unapparent. The most common clinical manifestation is that of a nonspecific febrile illness. Young children may be admitted to hospitals with enteroviral fevers that simulate bacterial sepsis. Coxsackie B virus and some of the echoviruses may cause severe perinatal infection associated with fever, meningitis, myocarditis, and hepatitis. Immunocompromised patients with humoral deficiencies can have persistent central nervous system infections lasting for several months or more.

Polymerase chain reaction assays for detection of enterovirus RNA is more rapid and more sensitive than cell culture and can detect all enteroviruses, including enteroviruses that are difficult to culture (AAP, 2009). Enteroviruses can be detected by PCR assay and culture from stool, rectal swab, throat specimens, urine, and blood during acute illness and from CSF when meningitis is present. Polymerase chain reaction testing is most useful for detecting enterovirus RNA in the CSF (AAP, 2009; Dua and Berkowicz, 2003).

No specific treatments for the enteroviruses exist (Marx, 2002; AAP, 2009). Thus, care is supportive and the results of diagnostic testing will not direct clinical management in most cases. An antiviral agent, pleconaril, is undergoing clinical evaluation. For chronic enteroviral meningoencephalitis in an immunodeficient patient, intravenous immunoglobulin (IVIG) containing high antibody titer to the infecting virus has been used for treatment of persistent enterovirus infection (AAP, 2009).

#### EoGenius (a 96-Gene Quantitative PCR Array and an Associated Dual Algorithm) for Eosinophilic Esophagitis

Lu and Rothenberg (2013) stated that allergic inflammation is accompanied by the coordinated expression of a myriad of genes and proteins that initiate, sustain, and propagate immune responses and tissue remodeling. MicroRNAs (miRNAs) are a class of short single-stranded RNA molecules that post-transcriptionally silence gene expression and have been shown to fine-tune gene transcriptional networks because single miRNAs can target hundreds of genes. Considerable attention has been focused on the key role of miRNAs in regulating homeostatic immune architecture and acquired immunity. Recent studies have identified miRNA profiles in multiple allergic inflammatory diseases, including asthma, eosinophilic esophagitis (EoE), allergic rhinitis, and atopic dermatitis. Specific miRNAs have been found to have critical roles in regulating key pathogenic mechanisms in allergic inflammation, including polarization of adaptive immune responses and activation of T cells (e.g., miR-21 and miR-146), regulation of eosinophil development (e.g., miR-21 and miR-223), and modulation of IL-13-driven epithelial responses (e.g., miR-375). The authors discussed recent advances in the understanding of the expression and function of miRNAs in patients with allergic inflammation, their role as disease biomarkers, and perspectives for future investigation and clinical utility.

Zahm et al (2014) noted that the incidence of EoE has increased in the past several years, yet the understanding of its pathogenesis remains limited. To test the hypothesis that miRNAs are altered in children with EoE, miRNAs were profiled in esophageal mucosa biopsies obtained from patients with active disease ( $n = 5$ ) and healthy control subjects ( $n = 6$ ). 14 miRNAs were significantly altered between groups; 4 of these miRNAs were decreased in EoE patients. A panel of 5 miRNAs (miR-203, miR-375, miR-21, miR-223, and miR-142-3p) were selected for validation in an independent set of samples from control ( $n = 22$ ), active disease ( $n = 22$ ), inactive disease ( $n = 22$ ), and gastro-esophageal reflux disease (GERD;  $n = 6$ ) patients. Each panel miRNA was significantly altered among groups. miRNA changes in esophageal biopsies were not reflected in the circulating RNA pool, as no differences in panel miRNA levels were observed in sera collected from the 4 patient groups. In addition, in contrast to previous studies, no change in esophageal miRNA levels was detected following treatment that resolved esophageal eosinophilia. In an effort to identify the ramifications of reduced esophageal miR-203, miR-203 activity was inhibited in cultured epithelial cells via expression of a tough decoy miRNA inhibitor. Luciferase reporter assays demonstrated that miR-203 did not directly regulate human IL-15 through targeting of the IL-15 3'-untranslated region. The authors concluded that miRNAs are

perturbed in the esophageal mucosa, but not the serum, of pediatric EoE patients. They stated that further investigation is needed to decipher pathologically relevant consequences of miRNA perturbation in this context.

Lexmond et al (2015) stated that quantification of tissue eosinophils remains the golden standard in diagnosing EoE, but this approach suffers from poor specificity. It has been recognized that histopathological changes that occur in patients with EoE are associated with a disease-specific tissue transcriptome. These researchers hypothesized that digital mRNA profiling targeted at a set of EoE-specific and Th2 inflammatory genes in esophageal biopsies could help differentiate patients with EoE from those with reflux esophagitis (RE) or normal tissue histology (NH). The mRNA expression levels of 79 target genes were defined in both proximal and distal biopsies of 196 patients with nCounter® (Nanostring) technology. According to clinicopathological diagnosis, these patients were grouped in a training set (35 EoE, 30 RE, 30 NH) for building of a 3-class prediction model using the random forest method, and a blinded predictive set ( $n = 47$ ) for model validation. A diagnostic model built on 10 differentially expressed genes was able to differentiate with 100 % sensitivity and specificity between conditions in the training set. In a blinded predictive set, this model was able to correctly predict EoE in 14 of 18 patients in distal (sensitivity 78 %, 95 % confidence interval [CI]: 52 to 93 %) and 16 of 18 patients in proximal biopsies (sensitivity 89 %, 95 % CI: 64 to 98 %), without false-positive diagnosis of EoE in RE or NH patients (specificity 100 %, 95 % CI 85 to 100 %). Sensitivity was increased to 94 % (95 % CI: 71 to 100 %) when either the best predictive distal or proximal biopsy was used. The authors concluded that mRNA profiling of esophageal tissue is an accurate diagnostic strategy in detecting EoE. These findings need to be validated in well-designed studies.

Sawant et al (2015) stated that microRNAs (miRs) have emerged as useful biomarkers for different disease states, including allergic inflammatory diseases such as asthma and EoE. Serum miRs are a possible non-invasive method for diagnosis of such diseases. These researchers focused on microRNA-21 (miR-21) levels in serum, in order to assess the feasibility of using this gene as a non-invasive biomarker for these diseases in the clinic, as well as to better understand the expression pattern of miR-21 in allergic inflammation. They used qPCR to assay miR-21 and other control miRs in esophageal biopsies from EoE patients and serum samples from EoE and asthma patients. Serum levels of miR-21 were significantly elevated in patients with asthma, whereas serum miR-21 levels were not associated with the presence of allergen-specific IgE (i.e., atopy). Esophageal biopsies showed a large elevation of miR-21 in EoE and an increase in miR-21 in EoE serum. Control U6 miR did not vary between asthma and control patients, however EoE serum had significantly decreased U6 microRNA compared to controls. The decreased U6 in EoE sera did not completely account for the relative increase in miR-21 in the sera of EoE patients. The authors reported for the first time that miR-21 is elevated in the sera of both asthma and EoE patients. They found no relation between serum miR-21 levels and atopy; they stated that the results thus suggested miR-21 is a novel biomarker for human allergic inflammatory diseases.

Dellon et al (2015) noted that a new gene expression profile test may distinguish EoE and GERD, but the optimal tissue preparation and biopsy location are unknown. These researchers determined if formalin-fixed paraffin-embedded (FFPE) and RNA-later (RNAL) preserved specimens from newly diagnosed EoE patients have equivalent gene expression scores and whether scores vary by esophageal biopsy location. These investigators analyzed prospectively collected and banked esophageal biopsies from EoE patients and GERD controls. Paired FFPE and RNAL samples from the distal, mid, and proximal esophagus were used; RNA was extracted, and gene expression for a previously constructed 96-gene panel was quantified with a summary expression score. Scores were compared between EoE and GERD patients, between FFPE and RNAL samples, and between the different esophageal locations. A total of 72 samples, representing paired FFPE and RNAL

specimens from 9 EoE cases and 3 GERD controls, were analyzed. Overall median gene expression scores were similar between FFPE and RNAL (238 versus 227; p = 0.64), correlation was excellent between FFPE and RNAL (Spearman's rho = 0.90; p < 0.001), and there were no differences by biopsy level. Median gene scores distinguished EoE from controls (134 versus 402; p = 0.02), and overall agreement between preservation methods and EoE case status was perfect ( $\kappa$  = 1.0; p < 0.001). The authors concluded that gene expression scores were equivalent in FFPE and RNAL, and were also similar across 3 esophageal locations. They stated the theses findings implied that a single biopsy in either FFPE or RNAL from anywhere in the esophagus may have the potential for genetic diagnosis of EoE.

An UpToDate review on "Clinical manifestations and diagnosis of eosinophilic esophagitis" (Bonis and Furuta, 2017) does not mention the use of RNA/microRNA as a diagnostic tool. Moreover, it states that "Assessment of eotaxin-3 and major basic protein levels in esophageal biopsy specimens (by immunohistochemistry or real-time PCR) has been suggested to help differentiate GERD from eosinophilic esophagitis, but further studies are needed".

#### Epidemic Typhus

*Rickettsia prowazekii* can be isolated from acute blood specimens by animal passage or through tissue culture but can be hazardous. Definitive diagnosis requires immunohistochemical visualization of rickettsiae in tissues, isolation of the organism, detection of the DNA of rickettsiae by PCR assay, or antibody detection in paired serum specimens obtained during the acute and convalescent phases of disease (AAP, 2009).

#### Epstein Barr Virus (EBV)

Current guidelines do not indicate a role for PCR testing in the routine diagnosis of EBV infection. According to available guidelines, PCR testing for EBV may be considered in immunocompromised persons. Polymerase chain reaction testing for EBV may also be indicated in persons with lymphoma when CNS involvement is suspected in the presence of focal neurologic deficits, seizures, or changes in mental status and when CT scan or magnetic resonance imaging (MRI) reveals a mass lesion.

Epstein-Barr virus, a DNA virus, is a B-lymphotropic herpesvirus and is the most common cause of infectious mononucleosis. Replication of EBV in B lymphocytes and the resulting lymphoproliferation usually is inhibited by natural killer and T-cell responses, but in patients who have congenital or acquired cellular immune deficiencies, fatal disseminated infection or B-cell lymphomas can occur.

Epstein-Barr virus causes several other distinct disorders, including the X-linked lymphoproliferative syndrome (also known as Duncan syndrome), post-transplantation lymphoproliferative disorders, Burkitt lymphoma, nasopharyngeal carcinoma, and undifferentiated B-cell lymphomas of the CNS. The X-linked lymphoproliferative syndrome occurs in persons with an inherited, maternally derived, recessive genetic defect characterized by several phenotypic expressions, including occurrence of infectious mononucleosis early in life among boys, nodular B-cell lymphomas often with CNS involvement, and profound hypogammaglobulinemia.

Epstein-Barr virus-associated lymphoproliferative disorders result in a number of complex syndromes associated with immunosuppression, including HIV infection, and occur in approximately 2 % of graft recipients. The highest incidence occurs after heart transplantation.

Other EBV syndromes are of greater importance outside the United States, including Burkitt lymphoma (a B-cell tumor), found primarily in Central Africa, and nasopharyngeal carcinoma, found in Southeast Asia.

According to the AAP (2006), the chronic fatigue syndrome is not related specifically to EBV infection. A small group of patients with recurring or persistent symptoms have abnormal serologic test results for EBV, as well as for other viruses.

Isolation of EBV from oropharyngeal secretions is possible, but techniques for performing this procedure usually are not available in routine diagnostic laboratories, and viral isolation does not necessarily indicate acute infection (AAP, 2006). Hence, diagnosis depends on serologic testing. Non-specific tests for heterophil antibody, including the Paul-Bunnell test and slide agglutination reaction, are most commonly available. The results of these tests are often negative in infants and children younger than 4 years of age with EBV infection, but they identify approximately 90 % of cases (proven by EBV-specific serology) in older children and adults. An absolute increase in atypical lymphocytes in the 2nd week of illness with infectious mononucleosis is a characteristic but not specific finding.

Multiple specific serologic antibody tests for EBV are available in diagnostic virology laboratories. The most commonly performed test is for antibody against the viral capsid antigen (VCA). Since immunoglobulin (Ig) G antibody against VCA occurs in high titers early after onset of infection, testing of acute and convalescent serum samples for anti-VCA may not be useful for establishing the presence of infection. Testing for IgM anti-VCA antibody and for antibodies against early antigen is useful for identifying recent infections. Since serum antibody against EBV nuclear antigen (EBNA) is not present until several weeks to months after onset of the infection, a positive anti-EBNA antibody test excludes acute infection.

Serologic tests for EBV are particularly useful for evaluating patients who have heterophil-negative infectious mononucleosis. Testing for other viral agents, especially CMV, is indicated for these patients. In research studies, culture of saliva or peripheral blood mononuclear cells for EBV, in-situ DNA hybridization, or PCR can determine the presence of EBV or EBV DNA and may implicate EBV with a syndrome, such as lymphoproliferation (AAP, 2006).

The AAP guidelines (2009) stated that detection by DNA PCR assay of serum, plasma, and tissue and RNA PCR assay of lymphoid cells or tissue are available commercially and may be useful in evaluation of immunocompromised patients and in complex clinical problems.

According to available guidelines, PCR testing of CSF for EBV is necessary in immunosuppressed persons and persons with lymphoma when CNS involvement is suspected. CNS involvement is suspected in the presence of focal neurologic deficits, seizures, or changes in mental status and when CT scan or MRI reveals a mass lesion (New York State Department of Health, 2003). Persons with lymphoma detected outside the CNS should be vigorously assessed for possible intra-cranial involvement. Lumbar puncture for EBV PCR and cytology (assuming no evidence of mass effect on neuroimaging studies), and functional neuroimaging (SPECT scan) are non-invasive methods by which to diagnose lymphoma. A brain biopsy may be necessary to confirm diagnosis of lymphoma.

According to available guidelines, all patients who have had solid organ transplants should be monitored for evidence of EBV viral replication by measuring whole blood quantitative EBV PCR at regular intervals for 6 months after transplantation (Cincinnati Children's Hospital Medical Center, 2003).

Supportive therapy should include rest in the acute stages of illness (AAP, 2006). Corticosteroid use is considered only for cases with complications such as marked tonsillar inflammation with impending airway obstruction, massive splenomegaly, myocarditis, hemolytic anemia, and hemophagocytic syndrome. Although acyclovir has in-vitro anti-viral activity against EBV, the clinical benefits of treatment have not been demonstrated, with the possible exception of HIV-infected patients with hairy leukoplakia.

A 2012 revision of the Cincinnati Children's Hospital Medical Center guidelines on monitoring of EBV following transplantation recommend that all patients be monitored for evidence of increased EBV-induced B-cell proliferation or EBV reactivation ( by measuring blood quantitative EBV PCR at regular intervals after transplantation. The suggested frequency for renal transplantation is at baseline and with presentation of symptoms. The suggested frequency for liver transplantation is every 2 weeks for 3 months, then monthly for 9 months, then yearly and with presentation of symptoms. The suggested frequency for heart transplantation is at baseline, then every 3 months and with presentation of symptoms or rising PCR. The suggested frequency for small intestine transplantation is every 2 weeks for 3 months followed by monthly for 9 months, then yearly and with presentation of symptoms (Cincinnati Children's Hospital Medical Center, 2012).

In a single-institution study (Omar et al, 2009), a total of 131 consecutive stem cell transplant recipients were divided into 2 groups based on prior risk factors, with high-risk patients undergoing EBV load measurement weekly during the first 3 months, while standard-risk patients underwent testing only when they were suspected to have EBV infection (which turned out to be a common scenario); 40 % of high-risk patients had at least 1 positive EBV result, compared to 24 % of standard-risk patients, and median values were elevated in the high-risk group. Rituximab was given when the EBV load exceeded 10,000 copies per ml of serum or when symptoms suggested EBV disease, which happened in 9 high-risk and 3 standard-risk patients. Four patients developed biopsy-proven PTLD, 3 in the high-risk group (6 %) and 1 in the standard-risk group (1 %), at a median of 70 days post-transplant. None of the PTLD cases were missed by the routine monitoring strategy; 2 of the 4 affected patients survived, and 1 of those survivors also received cytotoxic T cell infusion. The authors concluded that a targeted monitoring strategy among patients at a high risk of EBV-associated PTLD might be helpful to decrease the risk of development of PTLD. However, they stated that larger prospective studies are needed to verify this hypothesis.

Gulley and Tang (2010) stated that post-transplant lymphoproliferative disorder (PTLD) typically occurs in the 1st year after transplantation, sometimes within weeks of the onset of immunosuppression. The median onset of PTLD is 2 months after marrow transplant or 6 months after solid organ transplant. Onset is delayed occasionally beyond a year and rarely beyond a decade after transplantation.

Nearly all transplant recipients are infected or eventually become infected by EBV, yet only a fraction will develop PTLD. Risk factors for PTLD are as follows: EBV seronegativity at the time of transplant, active primary EBV infection at the time of transplant, underlying disease leading to transplantation, prior splenectomy, 2nd transplant, patient age (children and older adults), co-infection by cytomegalovirus and other viruses, acute or chronic graft-versus-host disease (GVHD), immunosuppressive drug regimen and intensity, cytokine polymorphisms, HLA type and extent of HLA mismatch, and the presence of multiple risk factors on this list.

In a study by Landgren et al (2009), of 21,686 stem cell transplant patients, a low incidence of PTLD (0.2 %) was found in patients with no risk factors, while the incidence was 8.1 % when there were 3 or more risk factors for PTLD.

Patients at high-risk for PTLD (e.g., those who are intensely immunosuppressed and who were seronegative at the time of transplant) tend to be monitored frequently (e.g., weekly in the first few months after transplant and then monthly) so that preemptive therapy may be considered. Preemptive therapies include reducing immunosuppression and infusing anti-CD20 antibody or donor T cells.

Optimally designed trials should measure EBV load once-monthly during the first year, with some patients continuing to be frequently monitored beyond the 1st year if they have a history of high EBV loads, if their drug regimen is particularly immunosuppressive, or in the aftermath of discontinuing antiviral prophylaxis.

The Second European Conference on Infections in Leukemia issued guidelines calling for routine EBV load testing of high-risk allogeneic stem cell transplant recipients. Screening should begin the day of transplantation and continue at least weekly for the first 3 months and even longer if the patient (i) is being treated for graft-versus-host disease, (ii) has a haploidentical graft, or (iii) has already experienced EBV viremia. More frequent testing is worth considering if the EBV load is rising. The threshold for intervention varies by local experience; a level of 100 g eq/ml of whole blood or plasma was suggested in one study. It is difficult to discern how this threshold corresponds to levels measured by another testing laboratory, further reinforcing the need for a universal calibrator. For high-risk patients, screening should begin the day of transplantation and continue at least weekly for the first 3 months and even longer if the patient (i) is being treated for graft-versus-host disease, (ii) has a haploidentical graft, or (iii) has already experienced EBV viremia. More frequent testing is worth considering if the EBV load is rising. (Per the guidelines from the Second European Conference on Infections in Leukemia).

For standard-risk patients, testing should be done only when they are suspected to have EBV infection.

#### Escherichia Coli

*Escherichia coli* is a gram negative bacterium that is commonly found in the intestine. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for hemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia. Microscopy shows gram negative rods. *E. coli* can be cultured using specialized media. Rapid methods for detecting *E. coli* in stool include ELISA tests, colony immunoblots, and direct immunofluorescence microscopy. The role of PCR testing in *E. coli* infection has not been established.

#### Fragile X Syndrome

Fragile X syndrome (a.k.a. Martin-Bell Syndrome, FRAX) is an X-linked syndrome of mental retardation associated in a proportion of cases with dysmorphic features including large everted ears, coarse facies, elongated face and macro-orchidism. Behavioral disturbances including hyperactivity or autistic-like behavior may be present. Females can also be affected. Approximately 1/3 of female carriers of full mutations will have mild to moderate mental retardation. Most recent publications on the prevalence of FRAX estimate that the disease affects approximately 1 in 5,000 males.

The gene involved (termed FMR-1 for Fragile X mental retardation) is located in chromosomal band Xq27.3 and encodes an RNA-binding protein. FRAX is caused in the vast majority of cases by expansions at a (CGG)n repeat sequence in the promoter region of the FMR-1 gene. Expansion of the array above about 200 repeats, accompanied by methylation of the adjacent CpG island, extinguishes expression of the gene. It appears that FRAX is due to absence of functional FMR-1 gene product, as deletions and point mutations in the FMR-1 coding sequence have also been reported to cause the syndrome.

According to available guidelines (McIntosh et al, 2000), Fragile site mental retardation 1 (FMR1) gene analysis by PCR/Southern Blot is medically necessary to confirm the diagnosis of fragile X syndrome (McIntosh et al, 2000). If the diagnosis of fragile X syndrome was made by cytogenetic testing, FMR1 gene analysis by PCR is recommended to confirm the diagnosis and to rule out the presence of other fragile sites in the same region of the X chromosome (i.e., FRAXE and FRAXF).

#### Gastrointestinal Pathogen Panel

Gastrointestinal pathogen panels have been developed that use multiplex PCR to test for multiple gastrointestinal infectious agents. GI pathogen panels are generally a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of nucleic acids from multiple pathogens (e.g., bacteria, viruses and parasites). Studies of gastrointestinal pathogen panels have focused on accuracy diagnostic yield (Buss et al, 2015; Perry et al, 2014; Khare et al, 2014; Beckmann et al, 2014; Zboromyska et al, 2014; Enserink et al, 2014; Wessels et al, 2014; Mengelle et al, 2013; Claas et al, 2013; Navidad et al, 2013; Liu et al, 2013), with a paucity of data on clinical utility. Current World Gastroenterology Organisation guidelines on acute diarrhea in adults and children (WGO, 2012) have no recommendations for multiplex PCR testing.

Stockman et al (2015) compared the etiologic yield of standard-of-care microbiologic testing ordered by physicians with that of a multiplex PCR platform. Stool specimens obtained from children and young adults with gastrointestinal illness were evaluated by standard laboratory methods and a developmental version of the FilmArray Gastrointestinal (GI) Diagnostic System (FilmArray GI Panel), a rapid multiplex PCR platform that detects 23 bacterial, viral and protozoal agents. Results were classified according to the microbiologic tests requested by the treating physician. A median of 3 (range of 1 to 10) microbiologic tests were performed by the clinical laboratory during 378 unique diarrheal episodes. A potential etiologic agent was identified in 46 % of stool specimens by standard laboratory methods and in 65 % of specimens tested using the FilmArray GI Panel ( $p < 0.001$ ). For those patients who only had Clostridium difficile testing requested, an alternative pathogen was identified in 29 % of cases with the FilmArray GI Panel. Notably, 11 (12 %) cases of norovirus were identified among children who only had testing for Clostridium difficile ordered. Among those who had C. difficile testing ordered in combination with other tests, an additional pathogen was identified in 57 % of stool specimens with the FilmArray GI Panel. For patients who had no C. difficile testing performed, the FilmArray GI Panel identified a pathogen in 63 % of cases, including C. difficile in 8 %.

Recognizing the need for a rapid diagnostic assay to distinguish infectious from non-infectious diarrhea in hospitalized patients for purposes of triaging isolation, Pankhurst et al (2014) evaluated the ability of 2 multiplex PCR assays, the Luminex xTag gastrointestinal panel and the MassCode multiplex PCR assay, to detect 4 common important enteropathogens in stool: (i) Clostridium difficile, (ii) Campylobacter spp., (iii) Salmonella spp. and (iv) norovirus. The authors compared the performance of these multiplex PCR assays on samples positive for C. difficile ( $n = 200$ ), Campylobacter spp. ( $n = 200$ ), Salmonella spp. ( $n = 100$ ) and norovirus ( $n = 200$ ) plus samples negative for all these pathogens ( $n = 300$ ) by standard microbiological testing. The authors found that, overall, the Luminex xTag gastrointestinal panel showed similar or superior sensitivity and specificity to the MassCode assay. However, on fresh extracts, this test had low sensitivity to detect a key enteric pathogen, S. enterica; making it an unrealistic option for most microbiology laboratories. The authors concluded that extraction efficiency appeared to be a major obstacle for nucleic acid-based tests for this organism, and possibly the whole Enterobacteriaceae family.

Guidelines from the American College of Gastroenterology (ACG) (Riddle, et al., 2016) state that stool diagnostic studies may be used if available in cases of dysentery, moderate-severe disease, and symptoms lasting >7 days to clarify the etiology of the patient's illness and enable specific directed therapy.(Strong recommendation, very low level of evidence). The guidelines state that traditional methods of diagnosis (bacterial culture, microscopy with and without special stains and immunofluorescence, and antigen testing) fail to reveal the etiology of the majority of cases of acute diarrheal infection. The guidelines state that, if available, the use of FDA-approved culture-independent methods of diagnosis can be recommended at least as an adjunct to traditional methods. (Strong recommendation, low level of evidence).

The ACG guidelines (Riddle, et al., 2016) explain that molecular diagnostic tests can provide a more comprehensive assessment of disease etiology by increasing the diagnostic yield compared with conventional diagnostic tests. They are also faster, providing results in hours rather than days. The guidelines state that "the new diagnostics' best applicability is for the clinician in practice, seeing one patient at a time rather than in the public health setting, e.g., in outbreak investigations. The guidelines note that one potential drawback of molecular technologies is the need to predefine the particular microbes being sought. In addition the significance of an identified organism may not be clear as these molecular technologies, which involve nucleic acid amplification, are limited to our existing knowledge of a microbes' genome. Nucleic acid amplification techniques do not discriminate between viable and non-viable organisms, and as a result, they can detect microbes at nonpathogenic levels. The guidelines point out that, given the high rates of asymptomatic carriage of enteropathogens, this can be a considerable problem. "To confound matters, further multiplex techniques are more commonly associated with increased detection of mixed infections and the relative importance of each pathogen may be unclear." Multiplex diagnostics do not test for antimicrobial sensitivity, and do not yield isolates that can be forwarded to public health laboratories.

According to a Medicare LCD, gastrointestinal pathogen multiplex nucleic acid amplification tests (NAATs) of up to 5 bacterial targets represent the top 90-95% of foodborne infections in immune competent persons. In addition, when there is a clinical concern for *Clostridium difficile* colitis, up to 11 targets may be necessary. Testing for 12 or more organisms is only necessary in critically ill or immunosuppressed patients.

The TaqPath Enteric Bacterial Select Panel (Thermo Fisher Scientific) is a gastrointestinal (GI) pathogen test that analyzes a stool sample using PCR technology to detect and differentiate common GI bacteria which include *Shigella* spp/enteroinvasive *E. coli* (EIEC), *Salmonella* spp, *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter upsaliensis*. The test is a multiplex real-time PCR diagnostic test that allows for rapid results in approximately 2 hours with a specificity and sensitivity of more than 98%. The test can be performed in a single test tube and scaled up to accommodate testing of 93 samples in a single run. The panel compares favorably with the culturing of samples in a laboratory, which can take up to two weeks. The TaqPath Enteric Bacterial Select Panel received European CE marking for In-Vitro Diagnostic Devices Directive (IVDD) in May 2022. The test enables clinicians to identify the root cause of an infection and administer the most appropriate treatment to their patients more quickly (AMA, 2023; Thermo Fisher Scientific, 2022).

#### Genital Mycoplasma Infections: Ureaplasma urealyticum and Mycoplasma hominis

Because detection of mycoplasma or ureaplasma is currently impractical, guidelines from the AAP and CDC recommend performing diagnostic tests for mycoplasmas and ureaplasmas when a patient presents with a clinical condition known to be

caused by or associated with these organisms and when more common etiologies are excluded (AAP, 2006; CDC, 2002). The correct microbiological diagnosis takes on greater importance in patients who are immunosuppressed and at greater risk for disseminated infection with a poor outcome.

The standard method of diagnosing genital mycoplasma infections is by cell culture. Specialized culture media and growth conditions are necessary. Both *M. hominis* and *U. urealyticum* can be detected in culture within 2 to 5 days. According to the CDC, serologic studies are not useful for evaluating genital mycoplasma infections (CDC, 2002). Molecular techniques such as PCR are not needed when culture is available for *M. hominis* and *Ureaplasma* species.

The AAP (2009) noted that sensitive PCR methods for *U. urealyticum* have been developed, but are not available routinely.

*Mycoplasma genitalium* has been identified as a potential cause of nongonococcal urethritis and a possible cause of genital tract inflammation in women. Guidelines on sexually transmitted diseases from the Centers for Disease Control and Prevention (Workowski, et al., 2015) note that *M. genitalium* is a slow-growing organism, and culture can take up to 6 months. Therefore, PCR testing is the preferred method for *M. genitalium* detection.

Evidence is emerging on other mycoplasma organisms, including *Mycoplasma fermentans* (see below) and *Mycoplasma penetrans*, and their possible roles in certain pathologic conditions in humans. Although PCR testing can be used to detect these organisms, relatively little is known about their pathogenic importance.

#### Genital Ulcer Diseases

According to guidelines from the CDC (2002), a diagnosis of genital ulcer disease based only on the patient's medical history and physical examination often is inaccurate. Therefore, evaluation of all patients who have genital ulcers should include a serologic test for syphilis and a diagnostic evaluation for genital herpes; in settings where chancroid is prevalent, a test for *Haemophilus ducreyi* should also be performed.

According to the CDC, specific tests for evaluation of genital ulcers include:

- Serology, and either darkfield examination or direct immunofluorescence test for *T. pallidum*;
- Culture or antigen test for herpes simplex virus (HSV); and
- Culture for *H. ducreyi*.

According to CDC guidelines, no FDA-approved PCR test for these organisms is available in the United States, but such testing can be performed by commercial laboratories that have developed their own PCR tests.

#### Gonorrhea

Infection of the genital tract with *Neisseria gonorrhoeae* can cause urethritis, cervicitis, proctitis, or bartholinitis (WHO, 1999; AGM-MSSVD, 2002). Complications of untreated disease include epididymitis, prostatitis, and infertility in men and pelvic inflammatory disease and infertility in women. Because most cases in females are asymptomatic, detection of infection using laboratory tests is needed to prevent sequelae and transmission to sexual partners and, for pregnant women, to neonates (WHO, 1999).

According to the AAP (2006), microscopic examination of Gram-stained smears of exudate from the eyes, the endocervix of postpubertal females, the vagina of prepubertal girls, male urethra, skin lesions, synovial fluid, and, when clinically warranted, cerebrospinal fluid (CSF) is useful in the initial evaluation. According to the WHO, the sensitivity and specificity of Gram-stain for detection in urethral samples in symptomatic men is 90 to 95 % and the specificity is 98 to 100 % (WHO, 1999). However, Gram stain is not as useful for endocervical smears (sensitivity of 50 to 70 %) because the presence of other Gram-negative diplococci makes interpretation difficult.

*Neisseria gonorrhoeae* can be cultured from normally sterile sites, such as blood, CSF, or synovial fluid, using specialized culture media. Selective media that inhibit normal flora and nonpathogenic *Neisseria* organisms are used for culture from non-sterile sites, such as the cervix, vagina, rectum, urethra, and pharynx. According to the WHO (1999), the sensitivity of culture for *N. gonorrhoeae* ranges from 81 to 100 % with a specificity of 100 %. An advantage of culture is that isolates are available for further testing. Disadvantages of culture are the need for stringent handling and up to 3 days for results.

The AAP (2006) notes that nucleic acid amplification methods by PCR, mediated amplification (TMA), and strand-displacement assays are highly sensitive and specific when used on urethral (males) and endocervical swab assays. They also can be used with good sensitivity and specificity on first-void urine specimens, which has led to increased compliance with testing and follow-up in hard-to-access populations, such as adolescents. These techniques also permit dual testing of urine for *C. trachomatis* and *N. gonorrhoeae*. According to the WHO, the sensitivity and specificity of DNA hybridization assays for gonorrhea are 86 to 100 % and 99 %, respectively. The sensitivity of PCR testing ranges from 89 to 97 % and the specificity ranges from 94 to 100 %. The sensitivity of LCR ranges from 95 to 100 % and the specificity ranges from 98 to 100 %.

According to guidelines from the CDC (2002), PCR tests for *Neisseria gonorrhoeae* are recommended for testing urethral swabs from males and endocervical swabs when conditions during holding and transport of inoculated culture media are not adequate to maintain the viability of organisms. The CDC guidelines note that commercial PCR assays have cross-reacted with non-gonococcal *Neisseria*; such cross-reactivity has not been reported for commercial LCR and un-amplified probe assays.

Polymerase chain reaction testing of the urine may be indicated for urinary screening in women when pelvic examination is not indicated, and in men (CDC, 2002). The CDC guidelines note that the sensitivity of urine testing with PCR may be lower than with urethral (males) or endocervical swabs. Polymerase chain reaction tests may be indicated in screening vaginal swabs of prepubescent children for possible sexual abuse if culture is not available (CDC, 2002). According to CDC guidelines, additional review is needed before a recommendation can be made for use of PCR tests in vaginal swabs of post-menarcheal adolescents and adults.

According to guidelines from the CDC, PCR tests for gonorrhea are not recommended for vaginal, rectal, conjunctival or pharyngeal swabs, or for detecting disseminated gonococcal infection (CDC, 2002; see also AAP, 2006; AAP, 2009).

#### Haemophilus Influenzae

*Haemophilus influenzae* type b (Hib) causes pneumonia, occult febrile bacteremia, meningitis, epiglottitis, septic arthritis, cellulitis, otitis media, and purulent pericarditis. The mode of transmission is person to person by inhalation of respiratory tract droplets or by direct contact with respiratory tract secretions.

According to current guidelines for diagnosis (AAP, 2009), CSF, blood, synovial fluid, pleural fluid, and middle-ear aspirates should be cultured on a specialized medium such as chocolate agar. Gram stain of an infected body fluid specimen can facilitate presumptive diagnosis. Latex particle agglutination for detection of type b capsular antigen in CSF can be helpful, but a negative test result does not exclude the diagnosis, and false-positive results have been recorded. All *H. influenzae* isolates associated with an invasive infection should be serotyped.

#### Hantavirus

Diagnosis of Sin Nombre virus (SNV) RNA has been detected uniformly by RT-PCR assay of peripheral blood mononuclear cells and other clinical specimens from the first few days of hospitalization up to 10 to 21 days after symptom onset, and the duration of viremia is unknown (AAP, 2009). According to the CDC (2011), RT-PCR can be used to detect hantaviral RNA in fresh frozen lung tissue, blood clots, or nucleated blood cells. However, "RT-PCR is very prone to cross-contamination and should be considered an experimental technique." Differences in viruses in the United States complicate the use and sensitivity of RT-PCR for the routine diagnosis of hantaviral infections (CDC, 2011).

#### Hepatitis B Virus

Hepatitis B virus (HBV) causes a wide spectrum of manifestations, ranging from asymptomatic sero-conversion, subacute illness with nonspecific symptoms (e.g., anorexia, nausea, or malaise) or extra-hepatic symptoms, and clinical hepatitis with jaundice, to fulminant fatal hepatitis (AAP, 2009). Chronic HBV infection with persistence of hepatitis B surface antigen (HBsAg) occurs in as many as 90 % of infants infected by peri-natal transmission, in an average of 30 % of children 1 to 5 years of age infected after birth, and in 2 % to 6 % of older children, adolescents, and adults with HBV infection. Chronically infected persons are at increased risk for developing chronic liver disease (e.g., cirrhosis, chronic active hepatitis, or chronic persistent hepatitis) or primary hepatocellular carcinoma in later life.

Hepatitis B virus is transmitted through blood or body fluids, such as wound exudates, semen, cervical secretions, and saliva of people who are HBsAg-positive.

Commercial serologic antigen tests are available to detect hepatitis B surface antigen (HbsAg) and hepatitis B e antigen (HbeAg). Assays also are available for detection of antibody to HBsAg (anti-HBs), total antibody to hepatitis B core antigen (anti-HBc), IgM anti-HBc, and antibody to HBeAg. In addition, hybridization assays and gene amplification techniques (e.g., PCR, branched DNA methods) are available to detect and quantitate HBV DNA. Tests for HBeAg and HBV DNA are useful in the selection of candidates to receive antiviral therapy and to monitor the response to therapy. Quantitative PCR viral load tests are used to monitor response to therapy.

#### Helicobacter Pylori

Current guidelines do not provide any indication for PCR testing in the diagnosis of *Helicobacter pylori*. To establish the presence of *H. pylori*, tests for antibody to *H. pylori* in blood are greater than 90 % specific and sensitive. Other tests include a urea breath test or rapid urease test, and histology of antral biopsies obtained at endoscopy.

*Helicobacter pylori* infection can be diagnosed by culture of gastric biopsy tissue (AAP 2006). Organisms usually can be visualized on histologic sections using special stains. Because of production of urease by the organisms, urease testing of a gastric specimen can give a rapid and specific microbiologic diagnosis. Each of these tests requires endoscopy and biopsy. Non-invasive, commercially available tests include the breath test, which detects labeled carbon dioxide in expired air after oral administration of isotopically labeled urea, and serology for the presence

of immunoglobulin G to *H. pylori*. According to the AAP (2006), each of the diagnostic tests has a sensitivity and specificity of 95 % or more. A stool antigen test is also available commercially.

#### Hemorrhagic Fevers and Related Syndromes Caused by Viruses of the Family Bunyaviridae

Polymerase chain reaction assay performed in containment laboratories can be a useful complement to serodiagnostic assays on samples obtained during the acute phase of Crimean-Congo hemorrhagic fever, Rift Valley fever, or hemorrhagic fever with renal syndrome (AAN, 2009).

#### Hepatitis A Virus

Hepatitis A characteristically is an acute, self-limited illness associated with fever, malaise, jaundice, anorexia, and nausea. The most common mode of transmission is person to person, resulting from fecal contamination and oral ingestion. Current guidelines (AAP, 2009) recommend for the diagnosis of hepatitis A virus serologic tests for HAV-specific total antibody. The presence of serum IgM anti-HAV indicates current or recent infection. IgG anti-HAV is detectable shortly after the appearance of IgM. A positive total anti-HAV test result and a negative IgM anti-HAV test result indicate past infection and immunity.

#### Hepatitis C Virus

The signs and symptoms of hepatitis C virus (HCV) infection usually are indistinguishable from those of hepatitis A or B. Persistent infection with HCV occurs in 75 % to 85 % of infected persons. Chronic hepatitis develops in approximately 60 % to 70 % of chronically infected patients, and cirrhosis develops in 10 % to 20 %; primary hepatocellular carcinoma can occur in these patients. Infection with HCV is the leading reason for liver transplantation in the United States.

The prevalence of HCV infection in the general population of the United States is estimated at 1.8 %. Sero-prevalence rates vary among individuals according to their associated risk factors. Infection is spread primarily by parenteral exposure to blood and blood products from HCV-infected persons.

The highest sero-prevalence rates of infection (60 % to 90 %) occur in persons with large or repeated direct percutaneous exposure to blood or blood products, such as injection drug users and persons with hemophilia who were treated with clotting factor concentrates produced before 1987. Rates are moderately high among those with frequent but smaller direct percutaneous exposures, such as patients receiving hemodialysis (10 % to 20 %). Lower rates are found among persons with unapparent percutaneous or mucosal exposures, such as persons with high-risk sexual behaviors (1 % to 10 %), or among persons with sporadic percutaneous exposures, such as health care personnel (1 %).

In persons with no risk factors, sero-prevalence rates are less than 0.5 %. For most infected children and adolescents, no specific source of infection can be identified.

According to the AAP (2009), there are 2 major types of tests available for the laboratory diagnosis of HCV infections:

(i) antibody assays for anti-HCV, and (ii) assays to detect HCV nucleic acid (RNA). Diagnosis by antibody assays involves an initial screening enzyme immunoassay (EIA); repeated positive results are confirmed by a recombinant immunoblot assay (RIBA), analogous to testing for HIV infection. The current EIA and RIBA assays are at least 97 % sensitive and more than 99 % specific. False-negative results early in the course of acute infection result from the prolonged interval between exposure or

onset of illness and sero-conversion that may occur. Within 15 weeks after exposure and within 5 to 6 weeks after the onset of hepatitis, 80 % of patients will have positive test results for serum HCV antibody.

According to the AAP (2009), highly sensitive FDA-licensed PCR assays for detection of HCV RNA are available from several commercial laboratories. Hepatitis C virus RNA can be detected in serum or plasma within 1 to 2 weeks after exposure to the virus and weeks before onset of liver enzyme abnormalities or appearance of anti-HCV. Polymerase chain reaction assays for HCV infection are used commonly in clinical practice in the early diagnosis of infection, for identifying infection in infants early in life (i.e., peri-natal transmission) when maternal serum antibody interferes with the ability to detect antibody produced by the infant, and for monitoring patients receiving anti-viral therapy (AAP, 2009; CDC, 1998). However, false-positive and false-negative results can occur from improper handling, storage, and contamination of the test samples. Viral RNA may be detected intermittently, and, thus, a single negative PCR assay result is not conclusive. The AAP (2009) guidelines noted that quantitative assays for measuring the concentration of HCV RNA also are available but are less sensitive than qualitative assays. These quantitative assays have primarily been used a prognostic indicator for patients undergoing or about to undergo antiviral therapy. A Consensus Conference convened by the Health Canada Laboratory Centre for Disease Control (1999) concluded that pretreatment quantitative HCV RNA assays provide important information with respect to the risks and benefits of treatment and duration of therapy and should be made available. In addition, the LCDC Consensus Conference concluded that pretreatment genotyping provides important information with respect to the risks/benefits and duration of treatment. Interferon given alone or in combination with ribavirin is FDA-approved for treatment of chronic HCV infection in adults.

#### Hepatitis G Virus

Although hepatitis G virus (HGV) can cause chronic infection and viremia, it is a rare cause of hepatic inflammation, and most infected persons are asymptomatic (AAP, 2003). Histologic evidence of HGV infection is rare, and serum aminotransferase concentrations usually are normal. Although high levels of HGV RNA are found in blood, the liver is not a significant site of replication. Currently, no conclusive evidence indicates that HGV causes fulminant or chronic disease, and co-infection does not seem to worsen the course or severity of concurrent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV).

The HGV has been reported in adults and children throughout the world and is found in about 1.5 % of blood donors in the United States. Infection has been reported in 10 % to 20 % of adults with chronic HBV or HCV infection, indicating that co-infection is a common occurrence. The primary route of spread is thought to be through transfusions, but HGV also can be transmitted by organ transplantation. Other important risk factors for infection include injection drug use, hemodialysis, and homosexual and bisexual relationships, indicating that sexual transmission also may occur.

Although PCR testing can detect HGV testing, such testing would not influence management because the disease is mild, and there is no known method to treat or prevent it. Currently, HGV infection can be diagnosed only by identifying viral genomes by using polymerase chain reaction assay, which is not widely available (AAP, 2003). No serologic test is available.

According to the AAP (2003), no treatment is indicated for this virus that causes mild, if any, disease. No method to prevent infection with HGV is known.

Herpes simplex virus (HSV) is one of the major causes of genital ulcer disease. Primary infection is followed by latency and variable periods of reactivation. Although clinical diagnosis may be accurate if based on the presence of typical vesicles, up to 2/3 of individuals acquire HSV asymptotically, and most infected persons shed virus during latent periods. Laboratory diagnosis is necessary to detect HSV in asymptotically infected people to prevent transmission to sexual partners and to children born to infected mothers.

The Association for Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases (2002) stated that the clinical utility of HSV serologic tests has not been fully assessed, and that virus detection remains the method of choice. According to the CDC's current guidelines for diagnosing HSV infection, type-specific serology for HSV type 2 may be helpful in identifying persons with genital herpes (CDC, 2002). Biopsy of ulcers may be helpful in identifying the cause of unusual ulcers or ulcers that do not respond to initial therapy.

The British Association for Sexual Health and HIV (BASHH, 2007) recommends real-time PCR as the preferred diagnostic method for genital herpes. BASHH guidelines stated that HSV DNA detection by PCR increases HSV detection rates by 11 % to 71 % compared with virus culture. The guidelines state that PCR-based methods allow less stringent conditions for sample storage and transport than virus culture and new real-time PCR assays are rapid and highly specific.

According to guidelines from the AAP Committee on Infectious Diseases (AAP, 2009), a PCR assay often can detect HSV DNA in CSF from patients with CNS infection during the neonatal period (neonatal HSV CNS disease) and with herpes simplex encephalitis in older children and adults and is the diagnostic method of choice for CNS HSV involvement. The AAP (2009) also stated that blood PCR may be of benefit in the diagnosis of neonatal HSV disease, but its use should not supplant the standard work-up of such patients (which includes surface cultures and CSF PCR).

Current guidelines indicate no role for quantification of HSV viral load in the diagnosis or management of herpes simplex virus infection (AAP, 2009; CDC, 2002). The AAP (2009) stated that there is no role for serial blood PCR in monitoring response to therapy.

#### Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), and Varicella Zoster Virus (VZV) Assay

The herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV) can cause life-threatening infections of the central nervous system (CNS), specifically in newborns and people who are immunocompromised. Thus, rapid laboratory diagnosis is urgently needed to distinguish HSV from VZV infections when the CNS is involved, especially in cases with clinically confusing dermal manifestations and in neonates to prevent a lethal outcome. Molecular diagnostic assays using PCR are the standard for detecting herpesvirus infections of the CNS. Modifications of the basic PCR technique have been used to increase the sensitivity of detection of the viruses, such as using nested PCR assays (Weidmann et al, 2003).

>Weidmann and colleagues (2003) developed three different real-time PCR assays based on TaqMan chemistry for the LightCycler instrument to detect HSV-1, HSV-2, and VZV. When the TaqMan assays were compared to their in-house nested PCR assays, the test systems had equal sensitivities of  $\leq 10$  plasmid copies per assay. When clinical samples were investigated by TaqMan PCR to detect HSV-1, HSV-2, and VZV DNA, 95, 100, and 96% of the samples determined to be positive by nested PCR, respectively, were positive by the real-time PCR assays. The specificities of all PCR assays were almost 100%. Furthermore, the TaqMan PCR

assays could be performed within 2.5 hours, whereas nested PCR results were available after 9 hours. In addition to offering more rapid results, the TaqMan PCR assays appear to be less expensive than nested PCR assays due to less hands-on time. The authors acknowledged that although 106 clinical samples were investigated, only 4 samples turned out to be positive for HSV-2. Thus, the low number of HSV-2-positive clinical samples does not really permit a valid assessment of the HSV-2 assay. Nonetheless, the authors concluded that the TaqMan PCR is an excellent alternative to conventional nested PCR assays for the rapid detection of HSV-1, HSV-2, and VZV in clinical samples.

The Abbott Alinity m HSV 1 & 2 / VZV assay (Abbott Molecular, Inc) is an FDA-cleared in vitro multiplex, reverse-transcription real-time PCR test that uses TaqMan chemistry to qualitatively detect the presence of HSV-1, HSV-2, and VZV DNA from clinician-collected cutaneous or mucocutaneous lesion swab specimens. The assay is intended for use as an aid in the diagnosis of HSV-1, HSV-2 and/or VZV infections in symptomatic patients. Each pathogen is reported as detected or not detected. However, negative results do not preclude HSV-1, HSV-2, or VZV infections and are not to be used as the sole basis for diagnosis, treatment or other management decisions. The assay is not intended for use with cerebrospinal fluid (CSF) or to aid in the diagnosis of HSV or VZV infections of the CNS. The Abbott Alinity m HSV 1 & 2 / VZV assay is not intended for use in prenatal screening (FDA, 2024).

>In an UpToDate review on "Neonatal herpes simplex virus infection: Clinical features and diagnosis", Demmler-Harrison (2022) states that "The laboratory diagnosis of neonatal HSV infection may be established through isolation of HSV in traditional or enhanced viral culture, detection of viral DNA using qualitative or quantitative polymerase chain reaction (PCR) assays, and detection of viral antigens using rapid direct immunofluorescence assays (DFA). Serology is generally not helpful in the diagnosis of neonatal HSV at the time of presentation".

In an UpToDate review on "Varicella-zoster virus (VZV) infection in the newborn", Speer (2024) states that the diagnosis of neonatal varicella is confirmed by detection of the virus using polymerase chain reaction (PCR). "PCR is the test of choice for diagnosis of neonatal varicella because it is highly sensitive and specific. PCR can detect VZV from vesicular swabs or scrapings, scabs from crusted lesions, blood samples, cerebral spinal fluid, and/or tissue from a biopsy specimen. PCR can also distinguish between wild VZV and vaccine strains."

In a 2024 update by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM), Miller et al state that "Clinicians need to recognize that HSV-1 is now commonly seen as a genital pathogen, especially in young women and MSM [men who have sex with men] and that atypical VZV presentations occur, especially in children. Most laboratories performing NAATs [nucleic acid amplification tests] usually offer HSV-1 and -2 as well as VZV from the same specimen. Typically, a generic lesion swab specimen is acceptable, that includes mucocutaneous, vesicular or even crusted lesions, and collected in universal transport medium (UTM) which can be transported at room temperature. Several FDA-cleared NAATs and laboratory developed tests (LDTs) exist. Consultation with the laboratory before specimen collection is appropriate as there may be limitations as to specimen source able to be tested and/or patient age depending on the test being used. NAATs are the preferred diagnostic method because they provide typing to specify HSV type 1 or 2, are the most sensitive, especially where suboptimal collection or non-ulcerative or vesicular lesions may be present".

Wheat and Kauffman (2012) stated that the role of PCR for diagnosis of histoplasmosis is uncertain. In one study, 2 of 6 culture-positive broncho-alveolar lavage (BAL) fluid specimens and 9 of 9 other respiratory or tissue specimens were positive using a PCR assay developed in a commercial laboratory, but comparison to microscopy was not reported, leaving open the question of whether PCR improves the sensitivity for diagnosis. In another report, PCR was positive only if organisms were seen by microscopy. Other studies noted a sensitivity of 8 % of urine and 22 % of BAL specimens that were positive for Histoplasma antigen, whereas results in cerebrospinal fluid (CSF) and serum were uniformly negative.

#### Human Bocavirus

Human bocavirus (HBoV) has been identified in 5 % to 10 % of all children with acute respiratory tract infections. Cough, rhinorrhea, and fever are the most prominent symptoms. Transmission is presumed to be from respiratory tract secretions. HBoV circulates worldwide and throughout the year. No commercial test is available to diagnose HBoV infection (AAP, 2009). HBoV PCR and detection of HBoV-specific antibody are used by research laboratories to detect the presence of virus and infection, respectively. However, there is no evidence that these tests alter management, and no specific therapy is available.

#### Human Herpesvirus Type 6

Human herpesvirus 6 (HHV-6) is a T-cell lymphotropic virus with high affinity for CD4 lymphocytes. HHV-6 has 2 variants, A and B. Variant B causes the childhood illness roseola infantum, while variant A has been isolated mainly from immunocompromised hosts. The disease manifestations of variant A still are undefined, but both variants may turn out to be pathogenic in the settings of transplantation and AIDS.

Primary HHV-6 infection usually occurs in infants and is the most common cause of febrile-induced seizures in children aged 6 to 24 months. Acute infection in immunocompetent adults is rare but may present as a mononucleosis-like illness with fever, lymphadenopathy, hepatitis or encephalitis, and negative test results for CMV or Epstein-Barr virus (EBV).

Infection remains latent in lymphocytes and monocytes and can persist in some tissues at low levels. In the immunocompetent host, this persistent infection generally is of no consequence. In the immunosuppressed host, HHV-6 may be associated with opportunistic disease. Whether this represents reactivation of latent infection or superinfection is unclear.

In transplant patients, HHV-6 infection has been linked with infection/reactivation and increased severity of CMV disease. In HIV patients, HHV-6 infection may up-regulate HIV replication and hasten the progression towards AIDS. HHV-6 also has been implicated in the pathogenesis of white matter demyelination in AIDS dementia complex. Causality has yet to be demonstrated clearly.

HHV-6 infection often is asymptomatic. Symptomatic disease occurs predominately after primary infection in infants and after either primary or reactivation disease in immunocompromised adults.

An Expert Working Group convened by the Health Canada Laboratory Centre for Disease Control (LCDC, 2000) concluded that the most appropriate clinical scenarios in which HHV-6 laboratory diagnosis may be indicated appear to be:

- primary infection in febrile children less than 3 years of age;
- primary infection or viral reactivation in immunocompromised individuals such as AIDS patients or transplant patients; and
- mononucleosis-like syndrome in patients without heterophile antibodies or antibodies

specific to EBV.

The diagnosis of primary HHV-6 infection currently necessitates use of research techniques to isolate the virus from a peripheral blood specimen (AAP, 2006). A 4-fold increase in serum antibody alone does not necessarily indicate new infection, as an increase in titer also may occur with reactivation and in association with other infections. However, sero-conversion from negative to positive in paired sera is good evidence of recent primary infection. The LCDC Expert Working Group (2000) concluded that serologic and PCR tests have been developed to diagnose an active or recent HHV-6 infection, "further evaluation in the clinical context (specificity, sensitivity, predictive values) needs to be done to improve confidence in and reliability of HHV-6 laboratory testing." Guidelines from the AAP (2009) stated that PCR tests for HHV-6 are available in reference laboratories. However, chromosomal integration of HHV-6 DNA always will result in a positive PCR test result with a high viral load, potentially confounding the interpretation of a positive test result. Chromosomal integration has been reported in 0.8 % of adult blood donors. The diagnosis of active HHV-6 infection should not be made without first excluding chromosomal integration by measuring DNA load in serum or CSF and comparing it with the DNA load in whole blood.

According to the AAP (2009), treatment for HHV-6 infection is supportive. Therapy usually is unnecessary with primary infection of immunocompetent patients.

Therefore, HHV-6 testing in immunocompetent individuals is not necessary. For immunocompromised patients with serious HHV-6 disease, some experts recommend a course of ganciclovir. Therefore, HHV-6 testing may be necessary in immunocompromised patients such as AIDS patients and transplant recipients. In addition, HHV-6 testing may be necessary to rule out other potential diagnoses in patients presenting with a mononucleosis-like illness with fever, lymphadenopathy, hepatitis or encephalitis, and negative test results for CMV or EBV.

#### Human Herpesvirus Type 7

According to the AAP (2006), recognition of the varied clinical manifestations of human herpesvirus 7 (HHV-7) infection is evolving. Many, if not most, primary infections with HHV-7 may be asymptomatic or mild; some may present as typical roseola and may account for second or recurrent cases of roseola. Febrile illnesses associated with seizures also have been reported. Some investigators suggest that the association of HHV-7 with these clinical manifestations results from the ability of HHV-7 to reactivate HHV-6 from latency.

An Expert Working Group convened by the Health Canada Laboratory Centre for Disease Control (LCDC, 2000) concluded that "presumably" the most appropriate clinical scenarios in which HHV-7 laboratory diagnosis may be indicated appear to be similar to those for HHV-6 - children with febrile illness and immunocompromised individuals. However, the LCDC Expert Working Group stated that HHV-7 "has not been linked to any specific clinical scenarios."

The AAP (2009) concluded that "[d]iagnostic tests for HHV-7 are also limited to research laboratories, and reliable differentiation between primary infection and reactivated is problematic." The LCDC Working Group stated that HHV-7 serologic assays must be carefully selected to avoid cross-reaction with antibodies to HHV-6. The LCDC Working Group commented that, while PCR-based assays can differentiate between HHV-6 and HHV-7, "further studies are required to determine the most appropriate samples and the most appropriate PCR method format (i.e., qualitative or quantitative) for detecting an active HHV-7 infection." Diagnosis of HHV-7 will not alter the patient's management because no effective treatment is known for HHV-7; treatment of HHV-7 is supportive (AAP, 2009).

## Human Herpesvirus Type 8

Human herpesvirus 8 (HHV-8) is the most recently discovered member of the herpesvirus family. In adults, HHV-8 is etiologically associated with Kaposi's sarcoma. Evidence of HHV-8 infection in children is rare, and no clinical associations are known.

Diagnostic tests for detection of HHV-8 infections are limited to research laboratories, and reliable differentiation of primary versus latent infection is problematic. According to the AAP (2009), both serologic and nucleic acid amplification tests for HHV-8 are available, but no HHV-8 screening method has been approved by the FDA. The AAP stated that screening for HHV-8 may be advisable for blood transfusion and organ transplantation procedures once a suitable method is available, but existing diagnostic tests are of limited clinical utility.

No effective treatment is known for HHV-8. Thus, diagnosis of HHV-8 will not alter the patient's management.

## Human Immunodeficiency Virus (HIV)

Most individuals can be diagnosed as infected with human immunodeficiency virus (HIV) based on the detection of HIV specific IgG antibodies (WHO, 1999). For detection of early infection before seroconversion occurs, or to detect HIV infection in neonates, assays that detect HIV p24 protein or HIV DNA or RNA are used. Quantitative HIV RNA assays are not necessary for diagnosis of infection but are useful for monitoring treatment.

The laboratory diagnosis of HIV infection during infancy depends on detection of virus or virus nucleic acid. The transplacental transfer of antibody complicates the serologic diagnosis of infant infection. According to the AAP (2006), HIV nucleic acid detection by PCR of DNA extracted from peripheral blood mononuclear cells is the preferred test for diagnosis of HIV infection in infants.

Plasma HIV RNA PCR may be used to diagnose HIV infection if the result is positive. However, this test result may be negative in HIV-infected persons. The test is licensed by the Food and Drug Administration only in quantitative format and, according to the AAP (2006), currently is used for quantifying the amount of virus present as a measurement of disease progression, not for diagnosis of HIV infection in infants.

According to the AAP (2006), EIAs are used most widely as the initial test for serum HIV antibody in adults, adolescents and children older than 18 months of age. These tests are highly sensitive and specific. Repeated EIA testing of initially reactive specimens is required to reduce the small likelihood of laboratory error. Western blot or immunofluorescent antibody tests should be used for confirmation, which will overcome the problem of a false-positive EIA result. A positive HIV antibody test result in a child 18 months of age or older usually indicates infection. In adults, adolescents, and children infected by other than peri-natal exposure, plasma viral RNA nucleic acid tests should not be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay). In addition, a negative (i.e., undetectable) plasma HIV-1 RNA test result does not rule out the diagnosis of HIV infection.

Human immunodeficiency virus nucleic acid detection by PCR of DNA extracted from peripheral blood mononuclear cells is the preferred test for diagnosis of HIV infection in infants up to 18 months of age. Although HIV culture can be used for this purpose, it is more complex and expensive to perform and is less well standardized than nucleic acid detection tests. The use of p24 antigen testing to exclude infection in children aged less than 18 months is not recommended

because of its lack of sensitivity. Although plasma HIV RNA PCR may be used to diagnose HIV infection if the result is positive, this test result may be negative in HIV-infected persons. The test is licensed by the FDA only in quantitative format and currently is used for quantifying the amount of virus present as a measurement of disease progression, not for diagnosis of HIV infection in infants.

#### Human Metapneumovirus (hMPV)

The human metapneumovirus (hMPV) is a newly reported respiratory virus belonging to the Paramyxoviridae family that appears to be one of the leading causes of bronchiolitis in infants and also causes some cases of pneumonia and croup (AAP, 2009). Otherwise healthy young children infected with hMPV usually have mild or moderate symptoms, but some young children have severe disease requiring hospitalization. Risk factors for severe hMPV infection include immunodeficiency disease or therapy causing immunosuppression at any age. Serologic studies suggest that all children are infected at least once by 5 years of age. Recurrent infection appears to occur throughout life and, in healthy people, usually is mild or asymptomatic. According to the AAP Committee on Infectious Diseases (AAP, 2009), rapid diagnostic immunofluorescent assays based on hMPV antigen detection by monoclonal antibodies are available commercially. The assays for hMPV developed and used by research laboratories include PCR and viral isolation from nasopharyngeal secretions using cell culture. However, only about 50 % of nasopharyngeal cultures that have positive results for hMPV by PCR yield cultivable virus by current techniques. There is no specific treatment for hMPV. Treatment is supportive and includes hydration, careful clinical assessment of respiratory status, including measurement of oxygen saturation, use of supplemental oxygen, and if necessary, mechanical ventilation (AAP, 2009).

#### Human Papillomavirus (HPV)

Human papillomaviruses (HPVs) produce epithelial tumors (warts) of the skin and mucous membranes (AAP, 2006). Cutaneous non-genital warts include common skin warts, plantar warts, flat warts, thread-like (filiform) warts, and epidermodysplasia verruciformis. Those affecting the mucous membranes include anogenital, oral, nasal, and conjunctival warts, as well as respiratory papillomatosis.

More than 100 types of HPV exist, more than 40 of which can infect the genital area (Workowski et al, 2010). Most HPV infections are asymptomatic, unrecognized, or subclinical. Oncogenic, or high-risk HPV types (e.g., HPV types 16 and 18), are the cause of cervical cancers. These HPV types are also associated with other anogenital cancers in men and women, including penile, vulvar, vaginal, and anal cancer, as well as subset of oropharyngeal cancers. Non-oncogenic, or low-risk HPV types (e.g., HPV types 6 and 11), are the cause of genital warts and recurrent respiratory papillomatosis.

Most cutaneous and anogenital warts are diagnosed by clinical inspection. Detection of cervical HPV infection may be enhanced by use of colposcopy with application of acetic acid (vinegar), which causes the lesion to turn white. This characteristic, however, is not specific for HPV infection, and false-positive test results are common. When the diagnosis is questionable, histologic examination of a biopsy specimen can be diagnostic.

Human papillomavirus can not be cultured. According to the American College of Obstetricians and Gynecologists (ACOG, 2002), the Hybrid Capture II is FDA-approved for HPV DNA. This test uses nucleic acid amplification and hybridization to assess exfoliated cervical cells for the presence of one or more of 13 high- and intermediate-risk HPV types. Although this test appears to be very sensitive, rare cross-reactivity with low-risk HPV types and HPV types of undetermined significance has been reported.

According to the CDC (Workowski et al, 2010), HPV tests are available for women aged greater than 30 years undergoing cervical cancer screening. These tests should not be used for men, for women less than 20 years of age, or as a general test for sexually transmitted diseases. These HPV tests detect viral nucleic acid (i.e., DNA or RNA) or capsid protein. Four tests have been approved by the FDA for use in the United States: the HC II High-Risk HPV test (Qiagen), HC II Low-Risk HPV test (Qiagen), Cervista HPV 16/18 test, and Cervista HPV High-Risk test (Hologic). Since the updated CDC guidelines were published, an additional test, the Roche cobas 4800 HPV test was approved by the FDA; this PCR test identifies genotypes 16 and 18 plus 12 other high-risk genotypes.

Given the prevalence of HPV infection, the clinical benefit of testing for the presence of HPV, other than as an adjunct to cancer screening, is of unknown clinical benefit. According to the CDC, "[n]o data support the use of type-specific HPV nucleic acid tests in the routine diagnosis or management of visible genital warts." The Advisory Committee on Immunization Practices does not recommend HPV testing to select persons for HPV vaccination. The AAP (2009) explained that testing for HPV types is used in combination with Pap test to determine whether patients need to be sent for colposcopy; otherwise, screening for clinically inapparent HPV infection or evaluating anogenital warts using HPV DNA or RNA tests is not recommended.

The National Cancer Institute's (2002) interim guidelines for managing abnormal cervical cytology and the American Society of Colposcopy and Cervical Pathology recommend human papilloma virus (HPV) DNA testing for women with ASCUS (atypical cervical squamous cells of undetermined significance) using the a hybridization assay. The hybridization assay can distinguish low-risk HPV (not usually found in pre-cancerous lesions) from high-risk HPV (found in pre-cancerous and cancerous lesions) in ASCUS lesions. According to the CDC, typing HPV DNA is not useful in types of cervical abnormalities other than ASCUS lesions. See [CPB 0443 - Cervical Cancer Screening and Diagnosis \(./400\\_499/0443.html\)](#). The CDC (2002) noted that screening for subclinical genital HPV infection using DNA or RNA tests is not recommended.

#### Human T-Cell Leukemia Virus

Human T-Cell Lymphotropic Virus Type I (HTLV-I) is a retrovirus that is endemic in Japan, the Caribbean, and parts of South America, and is associated with development of malignant neoplasms and neurologic disorders among adults (Armstrong, 2000). HTLV-I can be transmitted by sexual intercourse, inoculation of infected blood or blood products and peri-natal exposure.

Only a small proportion of those infected with HTLV-I develop adult T-cell leukemia or HTLV-I-associated myelopathy: the lifetime risk of these diseases in HTLV-I-infected Japanese is estimated at 2 to 4 % and 0.25 %, respectively. The usual age at onset is the fifth decade of life and more women than men are affected.

The myeloradiculopathy produced by HTLV-I mainly affects the pyramidal tracts and, to a lesser extent, the sensory system. HTLV-I-associated myelopathy is clinically characterized by a chronic syndrome with a combination of upper- and lower-motor neuron signs. Patients often complain of difficulty walking, dragging pains and stiffness of the legs, together with numbness and paresthesia, urinary retention and/or incontinence and impotence. About 1/3 of patients have weakness in the upper limbs, but the cranial nerves are only very rarely involved. Examination reveals a symmetric spastic paraparesis with mild sensory abnormalities indicative of posterior column involvement (diminished vibration and proprioception). Most patients progress gradually over months or years.

There may be confusion of HTLV-I-associated myelopathy with multiple sclerosis.

There is, however, a lack of optic neuritis or ocular movement problems in the former and the latter tends to run a relapsing-remitting course. The WHO has published diagnostic guidelines for HTLV-I myelopathy.

The diagnostic hallmark of HTLV-I infection is the presence of 'flower lymphocytes' (T-helper cells with multi-lobulated nuclei that are similar to the cells of ATL) in the blood. These cells only comprise about 1 % of the circulating white cells, however, and the diagnosis of HTLV-I infection requires the demonstration of specific antibodies in the serum.

In HTLV-I CNS disease, the CSF examination may be normal or show a slightly elevated protein concentration and a mild lymphocytosis. Flower lymphocytes are found in a minority of cases. A definitive diagnosis of HTLV-I-associated myelopathy requires detection of HTLV-I DNA in the CSF by polymerase chain reaction or evidence of intra-thecal synthesis of HTLV-I antibody.

No therapy has been proven to be of benefit in HTLV-I-associated myelopathy. At present the management of HTLV-I-associated myelopathy is similar to that of myelopathies of any cause, with supportive therapy of spasticity and urinary sphincter disturbance. Occasional patients have improved while receiving oral corticosteroids or systemic a-interferon, and plasmapheresis has also been claimed to lead to a temporary benefit.

Human T-cell lymphotropic virus type II (HTLV-II), also a retrovirus, has been detected among American and European injection drug users and some indigenous Native American groups. Limited data are available regarding the association of clinical disease with HTLV-II infection. In contrast to the clear association of adult T-cell leukemia with HTLV-I infection, no convincing link of HTLV-II to malignancy has been observed (Feigin, 1998). Although HTLV-II has been isolated in some patients with myeloneuropathies resembling HTLV-1 myelopathy, there is also no clear link between HTLV-II and myeloradiculopathies. Feigin (1998) concluded that "[t]he natural history and clinical manifestations of HTLV-II need further delineation in the context of ongoing prospective natural history studies."

Trevino et al (2012) noted that although most HTLV infections in Spain have been found in native intravenous drug users carrying HTLV-2, the large immigration flows from Latin America and Sub-Saharan Africa in recent years may have changed the prevalence and distribution of HTLV-1 and HTLV-2 infections, and hypothetically open the opportunity for introducing HTLV-3 or HTLV-4 in Spain. These investigators assessed the current sero-prevalence of HTLV infection in Spain; a national multi-center, cross-sectional, study was conducted in June 2009. A total of 6,460 consecutive outpatients attending 16 hospitals were examined. Overall, 12 % were immigrants, and their main origin was Latin America (4.9 %), Africa (3.6 %) and other European countries (2.8 %). Nine individuals were sero-reactive for HTLV antibodies (overall prevalence, 0.14 %). Evidence of HTLV-1 infection was confirmed by Western blot in 4 subjects (prevalence 0.06 %) while HTLV-2 infection was found in 5 (prevalence 0.08 %). Infection with HTLV types 1, 2, 3 and 4 was discarded by Western blot and specific PCR assays in another 2 specimens initially reactive in the enzyme immunoassay. All but 1 HTLV-1 cases were Latin-Americans while all persons with HTLV-2 infection were native Spaniards. The authors conclude that the overall prevalence of HTLV infections in Spain remains low, with no evidence of HTLV-3 or HTLV-4 infections so far.

Pinto et al (2012) noted that the sero-prevalence and geographic distribution of HTLV-1/2 among blood donors are extremely important to transfusion services. These investigators evaluated the sero-prevalence of HTLV-1/2 infection among first-time blood donor candidates in Ribeirao Preto city and region. From January 2000 to December 2010, 1,038,489 blood donations were obtained and 301,470

were first-time blood donations. All samples were screened with serological tests for HTLV-1/2 using enzyme immunoassay (EIA). In addition, the frequency of co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), Chagas disease (CD) and syphilis was also determined. In-house PCR was used as confirmatory test for HTLV-1/2. A total of 296 (0.1 %) first-time donors were serologically reactive for HTLV-1/2. Confirmatory PCR of 63 samples showed that 28 were HTLV-1 positive, 13 HTLV-2 positive, 19 negative and 3 indeterminate. Regarding HTLV co-infection rates, the most prevalent was with HBV (51.3 %) and HCV (35.9 %), but co-infection with HIV, CD and syphilis was also detected. The authors stated that the real number of HTLV-infected individual and co-infection rate in the population is under-estimated and epidemiological studies like theirs are very informative.

An UpToDate review on "Human T-lymphotropic virus type I: Disease associations, diagnosis, and treatment" (Scadden et al, 2013) states that "Polymerase chain reaction (PCR)-based testing to detect proviral DNA in peripheral blood mononuclear cells is an alternative diagnostic test. This test will also differentiate HTLV-I from HTLV-II infection. Advantages of this type of analysis are its ability to provide quantitation of proviral load in the blood [62], and its applicability in detecting proviral DNA in tumor cells or other tissue samples".

#### Influenza Virus

Influenza is characterized by the sudden onset of fever, frequently with chills or rigors, headache, malaise, diffuse myalgia, and a nonproductive cough (AAP, 2009). Subsequently, the respiratory tract signs of sore throat, nasal congestion, rhinitis, and cough become more prominent.

Influenza is spread from person to person by inhalation of small particle aerosols, by direct contact, by large droplet infection, or by contact with articles recently contaminated by nasopharyngeal secretions. In temperate climates, epidemics usually occur during the winter months and, within a community, peak within 2 weeks of onset and last 4 to 8 weeks or longer.

When viral cultures are performed, specimens should be obtained during the first 72 hours of illness because the quantity of virus shed subsequently decreases rapidly. Rapid diagnostic tests for identification of influenza A and B antigens in nasopharyngeal specimens are available commercially, although their sensitivity and specificity have been variable. Serologic diagnosis can be established retrospectively by a significant change in antibody titer between acute and convalescent serum samples, as determined by complement fixation, hemagglutination inhibition, neutralization, or enzyme immunoassay tests. The AAP guidelines (2009) noted that reverse transcriptase-PCR (RT-PCR) testing of respiratory tract specimens may be available at some institutions, and offers potential for high sensitivity and specificity.

Amantadine and rimantadine are approved for treatment of influenza A; treatment with either drug diminishes the severity of influenza A infection when administered within 48 hours of onset of illness. Neither amantadine nor rimantadine is effective against influenza B infections. Two neuraminidase inhibitors, zanamivir and oseltamivir, have been approved for treatment of influenza A and B.

According to the CDC (2003), testing for highly pathogenic Avian influenza A (H5N1) virus can be performed by PCR. Testing of hospitalized patients for influenza A (H5N1) infection is indicated when both of the following exist: (i) radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternative diagnosis has not been established; and (ii) a history of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza infections in poultry or humans. Ongoing listings of countries affected

by avian influenza are available from the World Organization for Animal Health at [OIE from the World Organization for Animal Health \(http://www.oie.int/eng/en\\_index.htm\)](http://www.oie.int/eng/en_index.htm).

Testing for influenza A (H5N1) also should be considered on a case-by-case basis in consultation with state and local health departments for hospitalized or ambulatory patients with all of the following: (i) documented temperature of greater than 100.4° F (greater than 38° C); (ii) cough, sore throat, or shortness of breath; and (iii) history of contact with poultry or domestic birds (e.g., visited a poultry farm, a household raising poultry, or a bird market) or a known or suspected patient with influenza A (H5N1) in an H5N1-affected country within 10 days of symptom onset (CDC, 2003).

According to guidelines from the CDC (2009), a confirmed case of H1N1 influenza A (swine flu) virus infection is defined as a person with an acute respiratory illness with laboratory confirmed H1N1 influenza A virus infection at CDC by either real-time PCR or by viral culture. According to the CDC (2009), a suspected case of H1N1 influenza A virus infection is defined as: (i) a person with acute respiratory illness who was a close contact to a confirmed case of H1N1 influenza A virus infection during the case's infectious period; or (ii) a person with an acute respiratory illness who traveled to or resides in an area where there are confirmed cases of H1N1 influenza A virus infection. The CDC defines "close contact" as being within about 6 feet of an ill person who is a confirmed or suspected case of H1N1 influenza A virus infection during the case's infectious period. The infectious period for a confirmed case of H1N1 influenza A virus infection is defined as 1 day prior to the case's illness onset to 7 days after onset. "Acute respiratory illness" is defined as recent onset of at least 2 of the following: rhinorrhea or nasal congestion, sore throat, cough (with or without fever or feverishness).

According to CDC guidelines for the 2009 to 2010 influenza season, clinicians should consider 2009 H1N1 influenza A (swine flu) virus infection in the differential diagnosis of patients with febrile respiratory disease and who (i) live in areas in the U.S. with confirmed human cases of H1N1 influenza A virus infection; or (ii) who traveled recently to Mexico or were in contact with persons who had febrile respiratory illness and were in the areas of the U.S. with confirmed H1N1 influenza cases or Mexico in the 7 days preceding their illness onset. Ongoing information about areas of the world affected by H1N1 influenza Avirus is available at the WHO's Influenza A (H1N1) website: [Pandemic \(H1N1\) \(http://www.who.int/csr/disease/swineflu/en/index.html\)](http://www.who.int/csr/disease/swineflu/en/index.html).

#### Joint Effusion

UpToDate reviews on "Synovial fluid analysis" (Sholter and Russell, 2017), "Overview of monoarthritis in adults" (Helfgott, 2017) and "Clinical manifestations and diagnosis of osteoarthritis" (Doherty and Abhishek, 2017) do not mention PCR testing as a management tool.

#### Kawasaki Disease

Kawasaki disease, previously known as mucocutaneous lymph node syndrome, is an acute, typically self-limited, illness that commonly occurs in childhood and is characterized by fever, rash and lymphadenopathy. However, for some, serious complications such as coronary artery (CA) aneurysms, depressed myocardial contractility and heart failure, myocardial infarction, arrhythmias, and peripheral arterial occlusion may develop and lead to significant morbidity and mortality. No laboratory values are included in the classical diagnostic criteria. Diagnosis is based on clinical findings/symptoms. Concurrent viral infections are common, and, therefore, the presence of respiratory symptoms or positive respiratory viral PCR testing does not exclude the diagnosis of Kawasaki disease (Sundel, 2022).

KawasakiDx (mProbe) is a quantitative assessment that uses reverse transcription polymerase chain reaction (RT-qPCR) method for testing the absence or presence of Kawasaki disease from a blood sample. Per mProbe website, there are testing

limitations, as it is possible that the results will not provide any benefit, as proteomics in health remains unknown and the interpretation and reporting of results are based on available information and technology.

#### Klebsiella Pneumoniae

*Klebsiella pneumoniae* is a member of the *Klebsiella* genus of Enterobacteriaceae and belongs to the normal flora of human mouth and intestine. Infections with *K. pneumoniae* are usually hospital-acquired and occur primarily in patients with diminished resistance. The diagnosis of *K. pneumoniae* infection is confirmed by culture of blood, sputum, urine, or aspirated body fluid, including pleural effusion, pericardial effusion, synovial fluid, CSF, and abscess material. In the setting of bacterial pneumonia, sputum Gram stain may provide a presumptive identification for an etiologic agent. Serology results are not useful for detection of infection with *Klebsiella* organisms. There is a lack of reliable evidence of the clinical performance and utility of PCR testing for *K. pneumoniae*.

An UpToDate review on "Overview of carbapenemase-producing gram-negative bacilli" (Quale and Spelman, 2017) states that "Identification of specific carbapenemases can be accomplished utilizing molecular techniques. These include multiplex polymerase chain reaction (PCR) assays and DNA microarrays that can screen at once for several different types of enzymes, including *K. pneumoniae* carbapenemases, specific MBLs, and OXA-type carbapenemases. Detection of organisms harboring these enzymes will be greatly improved as these technologies become incorporated into clinical practice. They are generally used for infection control purposes". Thus, this information is not used for management of patients.

#### Legionella Pneumophila

According to available guidelines, the diagnosis of *Legionella pneumophila* is confirmed by urinary antigen detection, change in antibody titers (with a 4-fold increase at 2 to 5 weeks) and immunofluorescent staining of the organism in the pleural fluid, sputum or bronchial washings. Available guidelines provide no specific indication for PCR testing in the diagnosis of *L. pneumophila*. To date, clinical experience has not shown PCR to be more sensitive than culture, and therefore the CDC does not recommend the routine use of PCR for the detection of *Legionella* in clinical samples. Guidelines from the Infectious Diseases Society of America and the American Thoracic Society do not include recommendations for PCR testing for *Legionella* (Mandell et al, 2007).

An UpToDate review on "Pulmonary infections in immunocompromised patients" (Fishman, 2017) states that "The differential diagnosis of pulmonary infections in the immunocompromised host is broad and includes bacteria, fungi, viruses, and parasites". However, the review does not mention the use of PCR as a management tool.

#### Leishmaniasis

The diagnosis of cutaneous leishmaniasis relies on the demonstration of Leishmania in tissue biopsy, scraping or impression preparations by microscopy and/or culture in a specialized medium (CDC, 2010). Species identification is recommended because management may vary depending on the infecting species. Recently, assays based on the use of PCR, including multiplex assays that can distinguish among several species simultaneously, have become more widely available. For visceral leishmaniasis, definitive diagnosis requires the demonstration of the parasite by smear or culture in tissue, usually bone marrow or spleen, and thus entails an invasive procedure (CDC, 2010). Parasites can be detected in tissue samples by light microscopy of stained slides, culture in a specialized medium, or by specific PCR assays. Serological tests can be used to demonstrate anti-leishmanial antibodies. These assays have high sensitivity for visceral

leishmaniasis in patients without HIV infection, but may show positive results due to subclinical infection or cross-reactions, and are therefore less specific than tissue sampling.

#### Leptospirosis

Clinical features and routine laboratory findings of leptospirosis are not specific, and a high index of suspicion must be maintained for the diagnosis. The organism can be cultured, but the diagnosis is more frequently made by serologic testing.

Polymerase chain reaction assays for detection of *Leptospira* organisms are being explored for the diagnosis of leptospirosis, and are available only in research laboratories (AAP, 2009; Everett, 2011).

#### Lyme Disease

According to available evidence-based guidelines, PCR has not been validated for either the diagnosis of Lyme disease or monitoring response to therapy.

American College of Physicians-American Society of Internal Medicine guidelines (1997) stated that PCR of serum or cerebrospinal fluid "need[s] further validation" and that "[p]ublished experience with these techniques [PCR] is insufficient to allow development of guidelines for their use."

The CDC (2001) stated that "PCR has not been standardized for routine diagnosis of Lyme Disease."

The National Institute of Arthritis and Infectious Disease (2001) has explained the reasons why PCR has limited utility in the diagnosis of Lyme disease: "To be sure, the polymerase chain reaction (PCR) is an extremely sensitive laboratory test that is capable of detecting very few molecules of bacterial DNA. However, the numbers of *Borrelia* likely to be present -- if at all -- in patients suspected of having Lyme disease are too small to generate sufficient amounts of bacterial DNA to be detected by this procedure."

The AAP's Committee on Infectious Diseases (2003) stated: "New, more sensitive and more specific diagnostic tests such as the polymerase chain reaction assay, which may be able to identify the presence of even small quantities of spirochetal DNA, are in development. However, physicians should be cautious when interpreting the results of these investigational tests until their clinical usefulness has been proven."

The American Lyme Disease Foundation (2002) stated: "The polymerase chain reaction (PCR) test is a very sensitive assay that detects the DNA of *B. burgdorferi*. However, certain limitations prevent the PCR from being widely used. First, *B. burgdorferi* bacteria do not persist in easily obtainable fluids such as blood, synovial (joint) fluid or spinal fluid, but typically bind to joint and nerve tissues. A PCR done on spinal fluid may be positive in early neurologic disease (e.g., Lyme meningitis) but is usually negative in a patient with long-term central nervous system damage. Second, a PCR can be easily contaminated, producing false positive results. For this reason, a positive PCR in a patient whose standard blood tests (ELISA and Western blot) are negative must be viewed with skepticism."

Puotinen et al (2002) concluded that "[t]he Lyme multiplex polymerase chain reaction (PCR) has not been standardized; therefore, it is not employed currently in routine testing." Edlow (2001) explained that PCR remains a research technique, in part because labs performing PCR tests must be meticulous in technique to minimize the likelihood of false-positive results". In addition, Edlow explained, "no large clinical series have been reported that assess the performance of the test in the non-research setting."

The AAP (2006) stated that PCR tests for spirochete DNA have no role in diagnosis of Lyme disease. The AAP (2009) noted that PCR testing "has been used in to detect *B. burgdorferi* DNA in joint fluid", but make no specific recommendation for its use.

Thus, the clinical utility of PCR in the diagnosis or monitoring of Lyme disease has not been established. In addition, current guidelines do not indicate any role for PCR quantification of spirochete load in the diagnosis or management of patients with Lyme disease.

#### Lymphogranuloma Venereum

Lymphogranuloma venereum (LGV) is caused by *C. trachomatis* serovars L1, L2, or L3. The disease occurs rarely in the United States. The most common clinical manifestation of LGV among heterosexuals is tender inguinal and/or femoral lymphadenopathy that is most commonly unilateral (CDC, 2002). Women and homosexually active men may have proctocolitis or inflammatory involvement of peri-rectal or peri-anal lymphatic tissues resulting in fistulas and strictures.

According to the CDC (2002), the diagnosis of LGV is usually made by complement fixation and by exclusion of other causes of inguinal lymphadenopathy of genital ulcers. The diagnostic utility of serologic methods other than complement fixation is unknown (CDC, 2002). The British Association of Sexual Health and HIV (BASHH, 2006) states that detection of nucleic acid (DNA) by amplification techniques such as the ligase chain reaction (LCR) or PCR are becoming established for routine testing of urethral, cervical, or urine specimens but have rarely been used in the context of LGV, until recent outbreaks in Western Europe. BASHH (2006) notes that these methods are highly sensitive and specific, and have now widely become available commercially. Positive samples should be confirmed by real-time PCR for LGV specific DNA.

#### Malaise and Fatigue (including Chronic Fatigue Syndrome)

"Shotgun" testing for a variety of infectious etiologies in patients with symptoms of fatigue is not medically necessary or appropriate. Testing for specific individual infectious etiologies is only appropriate when the patient exhibits signs or symptoms suggestive of active infection with that virus. See also [CPB 0369 - Chronic Fatigue Syndrome \(./300\\_399/0369.html\)](#).

According to the CDC (2000), in clinical practice, no tests can be recommended for the specific purpose of diagnosing chronic fatigue syndrome. Tests should be directed toward confirming or excluding other possible clinical conditions.

In a statement on the "Theoretical and experimental tests" for chronic fatigue syndrome, the CDC (2000) states: "No diagnostic tests for infectious agents, such as Epstein-Barr virus, enteroviruses, retroviruses, human herpesvirus 6, *Candida albicans*, and *Mycoplasma incognita*, are diagnostic for CFS and as such should not be used (except to identify an illness that would exclude a CFS diagnosis, such as mononucleosis)."

A Clinical Practice Guideline from the Collège des médecins du Québec (1998) reached the same conclusion about the value of diagnostic testing for infectious agents in patients with chronic fatigue syndrome.

A review of the literature on the diagnosis of viruses in patients suspected of having chronic fatigue syndrome from Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association of America (2001) states that viral tests are only appropriate when a specific active viral infection is suspected based on clinical signs: "Because research has documented no clear association between a virus and CFIDS, testing patients for viral infection has limited use unless clinical signs indicate that an

active viral infection may be present and requiring treatment. The results are also difficult to interpret, because the immune system in CFIDS may be up-regulated and latent viruses may not be fully suppressed. Tests to determine elevated antibody titers for EBV and other viruses are not considered diagnostic for CFIDS by most physicians, and are usually performed only when a specific viral infection is suspected as a cause of the patient's symptoms."

A review of diagnosis and management of chronic fatigue syndrome (2002) published in the journal *American Family Physician* explains that there is no clear evidence that chronic fatigue syndrome is caused by an infection: "Although a number of other viral pathogens (such as the Coxsackie virus, human herpes virus 6, cytomegalovirus, measles, and the human T-cell lymphotropic virus [HTLV-II]) have also been implicated as etiologic agents for CFS, there is no consistent or conclusive data to suggest any causal relationships. It is now believed that CFS is not specific to one pathogenic agent but could be a state of chronic immune activation, possibly of polyclonal activity of B-lymphocytes, initiated by a virus."

Regarding laboratory tests, the authors stated that "[l]aboratory tests should be limited to complete blood cell counts and tests specific for the patient's symptoms. For example, serologic and neurologic analyses for Lyme disease or multiple sclerosis need only be conducted if the patient presents with appropriate symptoms."

Thus, panels of PCR tests to detect various infections are not indicated in patients with symptoms suggestive of chronic fatigue syndrome. It is only appropriate for individual tests to be selected to detect particular infectious agents if the patient's clinical presentation suggests active infection with that infectious agent.

#### Malaria

Malaria is infection with any of 4 different species of Plasmodia, causing periodic paroxysms of chills, fever and sweating, anemia, and splenomegaly. Malaria is endemic in Africa, much of South and Southeast Asia, Central America, and northern South America. Malaria once was endemic in the United States but has been virtually eliminated from North America. The 4 important Plasmodium species are *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.

*P. falciparum* infection is a medical emergency. Recurrent attacks of chills and fever without apparent cause should always suggest malaria, particularly if the patient has been in an endemic area within 3 to 5 yrs, has an enlarged spleen, or has been recently transfused. Finding Plasmodium in a blood smear is diagnostic. The infecting species must be identified, since this influences therapy and prognosis.

According to the CDC (2010), parasite nucleic acids are detected using PCR. The CDC states that, although this technique may be slightly more sensitive than smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. Polymerase chain reaction results are often not available quickly enough to be of value in establishing the diagnosis of malaria infection. It is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or rapid diagnostic test.

#### Mantle Cell Lymphoma: bcl-1 Translocation

Mantle cell lymphoma is an aggressive non-follicular small B-cell lymphoma that is associated with significantly shorter survival despite a low-grade histology. Because of the aggressive nature of mantle cell lymphoma, accurate diagnosis is important for prognosis and management.

The t(11;14)(q13;q32) translocation causes deregulation of the bcl-1 gene and over-expression of cyclin D1, which may in turn lead to lymphoma genesis (Chin et al, 2006). The bcl-1 translocation is specific for mantle cell lymphoma and occurs in the majority of cases. The bcl-1 translocation is specific for mantle cell lymphoma. Detection of the bcl-1/JH gene rearrangement [t(11;14)(q13;q32)] can be helpful in the differential diagnosis of mantle cell lymphoma and in following up patients during and after treatment. However, because this translocation occurs in only about 2/3 of cases, a negative result does not preclude the diagnosis of mantle cell lymphoma.

#### Measles

Measles virus infection can be diagnosed by a positive serologic test result for measles immunoglobulin (Ig) M antibody, a significant increase in measles IgG antibody concentration in paired acute and convalescent serum specimens by any standard serologic assay, or isolation of measles virus or identification of measles RNA (by RT-PCR assay) from clinical specimens, such as urine, blood, throat, or nasopharyngeal secretions (AAP, 2009; CDC, 2009). The simplest method of establishing the diagnosis of measles is testing for IgM antibody on a single serum specimen obtained during the first encounter with a person suspected of having disease.

#### Metastatic Melanoma

The use of reversed transcriptase and PCR to detect circulating melanoma cells was described in 1991 as the first example of detecting hematogenous spread of melanoma cells from a solid tumor in peripheral blood.

Because of the higher rate of treatment failure in the subset of clinical stage I melanoma patients with occult nodal disease, clinical trials are evaluating new techniques to detect submicroscopic sentinel lymph node metastasis, in order to identify those patients who may benefit from regional lymphadenectomy with or without adjuvant therapy (NCI, 2002). One of the objectives of the phase III "Sunbelt Melanoma Trial" is to determine the effects of lymphadenectomy with or without adjuvant high-dose interferon alfa-2b versus observation on disease-free and overall survival in patients with submicroscopic sentinel lymph node metastasis detected only by PCR (i.e., sentinel lymph node negative by histology and immunohistochemistry) (Urist, 2001). No survival data have been reported from this study. An ongoing diagnostic study is testing the combination of reverse transcription and PCR (RT-PCR) in the detection of melanoma tumor antigen transcripts in lymph nodes and peripheral blood samples (Gajewski, 2000). Currently, the use of PCR testing to detect melanoma micrometastases in the serum is considered experimental and investigational.

#### Methicillin-Resistant Staphylococcus Aureus (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) must be differentiated from other strains of *S. aureus* because special infection control precautions are recommended for patients with MRSA, but are not necessary for patients with low-level oxacillin-resistant (so-called "borderline-resistant") *S. aureus* that do not contain the *mecA* gene characteristic of MRSA.

Methicillin resistance is mediated by the *mecA* gene, which encodes an abnormal low-affinity binding protein, PBP-2a, that permits the organism to grow and divide in the presence of methicillin and other beta-lactam antibiotics, thus rendering the antibiotics ineffective.

According to available guidelines, the most accurate methods of detecting MRSA are PCR methods for detection of the *mecA* gene and latex agglutination tests for PBP-2a.

## Microsporidia

Microsporidia are obligate intracellular, spore-forming protozoa. Patients with intestinal infection have watery, non-bloody diarrhea, generally without fever, although asymptomatic infection may be more common than originally suspected (AAP, 2006). Intestinal infection is most common in immunocompromised people, especially people who are infected with HIV, and often results in chronic diarrhea. Chronic infection in immunocompetent people is rare. According to the AAP (2009), infection with gastrointestinal Microsporidia species can be documented by identification of organisms in biopsy specimens from the small intestine.

Microsporidia species spores also can be detected in stool specimens or duodenal aspirates. Polymerase chain reaction assay also can be used for diagnosis (AAP, 2009).

## Molluscum Contagiosum

Molluscum contagiosum is a benign, usually asymptomatic viral infection of the skin with no systemic manifestations (AAP, 2009). It usually is characterized by discrete, flesh-colored to translucent papules. Lesions commonly occur on the trunk, face, and extremities but rarely are generalized. People with eczema, immunocompromising conditions, and HIV infection tend to have more widespread and prolonged eruptions. The diagnosis usually can be made clinically from the characteristic appearance of the lesions (AAP, 2009). Wright or Giemsa staining of cells expressed from the central core of a lesion reveals characteristic intracytoplasmic inclusions. There is a lack of reliable evidence for PCR testing in the diagnosis and management of molluscum contagiosum. Lesions usually regress spontaneously, but curettage of the central core of each lesion may result in more rapid resolution.

## Moraxella Catarrhalis

*Moraxella catarrhalis* is a gram-negative aerobic diplococcus that is part of the normal flora of the upper respiratory tract. Common infections include acute otitis media and sinusitis. Bronchopulmonary infection occurs predominantly among patients with chronic lung disease or impaired host defenses. Rare manifestations are bacteremia and conjunctivitis or meningitis in neonates. The organism can be isolated on blood or chocolate agar culture media after incubation in air or with increased carbon dioxide (AAP, 2009). Culture of middle ear or sinus aspirates is indicated for patients with unusually severe infection, patients with infection that fails to respond to treatment, and immunocompromised children. The role of PCR testing for *M. catarrhalis* has not been established.

## Mosquito-Borne Arboviruses

Mosquito-borne arboviruses are viral diseases that are spread to humans through the bite of infected mosquitos. These viruses do not normally infect humans but if they do, they usually cause a mild infection such as a fever or a rash. Others however are epidemic and can cause serious infections such as meningitis and encephalitis. St. Louis encephalitis is found throughout much of the United States, as well as parts of Canada, the Caribbean, and South America. Eastern equine encephalitis virus is a rare illness in humans, and only a few cases are reported in the United States each year. Most cases occur in the Atlantic and Gulf Coast states. Western equine encephalitis virus is a mosquito-borne virus closely related to eastern and Venezuelan equine encephalitis viruses, and is found mainly in the plains regions of the western and central United States. Most cases of La Crosse encephalitis virus disease occur in the upper Midwestern and mid-Atlantic and southeastern states.

According to the CDC (2007), a presumptive diagnosis of an arboviral disease is often based on the patient's clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where infection occurred. Laboratory diagnosis of arboviral infections is generally accomplished by testing of serum or CSF to detect virus-specific IgM and neutralizing antibodies. According to the CDC, in fatal cases, nucleic acid amplification [PCR], histopathology with immunohistochemistry, and virus culture of biopsy or autopsy tissues can also be useful. Only a few state laboratories or other specialized laboratories, including those at CDC, are capable of doing this specialized testing.

#### Mucosa-Associated Lymphoid Tissue (MALT) Lymphomas and Marginal Zone Lymphomas

Mucosa-Associated Lymphoid Tissue lymphomas have recently been reclassified as extra-nodal marginal-zone lymphomas of MALT-type. The most common and well-studied MALT lymphoma is gastric MALToma. This neoplasm is intimately associated with *H. pylori*, with the organism being present in more than 90 % of pathologic specimens of MALTomas. This etiologic factor serves as the basis for treatment. Treatment of gastrointestinal MALT lymphomas is with antibiotics designed to eradicate *H. pylori*.

Guidelines from the National Comprehensive Cancer Network (NCCN, 2003) on non-Hodgkin's lymphoma indicated that PCR testing is useful in evaluating individuals with MALT lymphomas and marginal zone lymphomas who have non-diagnostic atypical lymphoid infiltrates that are positive for *H. pylori* infection. According to the NCCN guidelines, detection by PCR of a t(11;18) gene rearrangement, a specific translocation of genes 11 and 18, in these persons predicts no response to antibiotic therapy for *H. pylori* infection, and alternative treatment should be considered.

#### Multiplex PCR Testing

Multiplex polymerase chain reaction (mPCR) refers to the simultaneous amplification of multiple targets (DNA fragments) using a different set of primers in a single PCR reaction. Thus, several genomic DNA sequences are amplified in a single step.

#### Mycobacterium Species

According to the AAP (2009), one PCR tests for rapid diagnosis of *Mycobacterium tuberculosis* is licensed by the FDA only for acid-fast stain positive respiratory tract specimens, and another for any respiratory tract specimen. The AAP noted that the PCR assay has decreased sensitivity for gastric aspirate, CSF, and tissue specimens, with false-negative and false-positive results reported.

Current guidelines do not indicate any role for PCR testing for diagnosis of *Mycobacterium avium intracellulare* or other Mycobacteria species (NYS DOH, 2006). According to the AAP (2009), definitive diagnosis of non-tuberculous Mycobacteria disease requires isolation of the organism.

#### Mycoplasma Fermentans

*M. fermentans* originally was isolated from the genital tract of men and women 45 years ago, but it has not been established as a cause of genitourinary disease (Feigin 1998). This organism has been isolated from the blood of leukemia patients, from joint fluid of patients with arthritis, and from the blood and urine of patients with AIDS. In recent studies, *M. fermentans* has been identified by PCR in

peripheral blood mononuclear cells and lymph nodes of HIV-infected patients. The organism also has been recovered from the blood of homosexual men without HIV infection.

*M. fermentans* has been identified in synovial fluid samples from patients with inflammatory arthritis diseases, including rheumatoid arthritis. The detection of *M. fermentans* in joints of rheumatic patients incriminates this microorganism as a cause of arthritis in humans. Although *M. fermentans* is a human pathogen suspected to be involved in the induction of arthritis since 1970, its pathogenesis mechanisms are poorly understood (Rivera et al, 2002). It is not known how *M. fermentans* reaches the joints and induces arthritis.

*M. fermentans* has been frequently found in normal healthy persons. This organism has been detected in the saliva samples from 44 % of healthy people (Chingbingyoung et a., 1996), and in 55 % of healthy people (Shibata et al, 1999).

This organism has also been detected in the throat, urine, or peripheral blood mononuclear cells of 33 % of HIV-seronegative patients attending a venereal diseases clinic (Katseni et al, 1993), in 11 % of the peripheral blood mononuclear cells from HIV-seronegative subjects (Kovacic et al, 1996), and in the synovial fluid of 14 % of patients with rheumatoid and other inflammatory arthritides (Schaeverbeke et al, 1996), although the organism was present in 40 % of the biopsy specimens of the RA patients' synovial lining cells.

Cultivation of these organisms directly from patient material on cell-free medium has proved to be difficult, and prior animal and tissue culture passage is required. This difficulty in direct culturing has fueled a controversy over whether these organisms are pathogens or contaminants in these patients. A further controversy exists regarding the causative or disease-enhancing role of these organisms in AIDS versus their role as only one of the many opportunistic infectious agents in this condition.

In summary, although *M. fermentans* has been found in rheumatoid arthritis, AIDS, and other conditions, *M. fermentans* is also frequently found in normal healthy persons. *M. fermentans* role in the pathogenesis of disease has not been established. Although PCR assays have been developed to detect *M. fermentans*, there are no prospective studies demonstrating that diagnosis and treatment of *M. fermentans* infections improves patient outcomes in rheumatoid arthritis, AIDS, chronic fatigue syndrome, or other conditions. Therefore, PCR testing for *M. fermentans* is considered experimental and investigational.

#### Mycoplasma Pneumonia

According to available guidelines, the diagnosis of Mycoplasma pneumonia may be established by a rise of specific antibody titer. Although this occurs in most instances, it requires paired samples separated by 1 week or more, and is therefore not useful in the initial diagnosis.

Other methods of diagnosis include cold hemagglutination serology, which is present in about 50 % of cases but may produce false-positives in measles, infectious mononucleosis, adenovirus pneumonias, and certain tropical diseases and collagen vascular disease.

The diagnosis may also be established by isolation of *Mycoplasma pneumoniae*.

The AAP (2009) reported, that, where available, PCR has replaced other tests, because PCR enables more rapid diagnosis in acutely ill patients. However, no PCR kits are available commercially in the United States, and tests prepared at different

institutions use different primer sequences and target different genes, which precludes generalizations about sensitivity and specificity.

#### Nanobacteria

Nanobacteria are tiny bacteria (less than 0.5 μm) recently discovered in human and bovine blood samples and in commercial batches of cell culture quality serum (Pitcher and Fry, 2000). The extraordinary property possessed by nanobacteria is their ability to excrete calcium phosphate in the form of a crystalline apatite shell. This ability to deposit minerals in media and blood has aroused considerable speculation about their possible role in the formation of kidney stones, and also to other pathologies involving abnormal mineral deposition. The existence of nanobacteria and their potential role in the pathogenesis of disease remains controversial. Pitcher and Fry (2000) cited evidence that the nanobacteria may be a PCR artifact. Because the existence of nanobacteria and their role in disease have not been established, PCR testing for nanobacteria is considered experimental and investigational.

#### *Neisseria Meningitidis*

*Neisseria meningitidis* is a gram-negative diplococcus with at least 13 serogroups based on capsule type. Strains belonging to groups A, B, C, Y, and W-135 are implicated most commonly in invasive disease worldwide. Invasive infection usually results in meningococcemia, meningitis, or both. According to current guidelines (AAP, 2009; CDC, 2009), cultures of blood and CSF are indicated for patients with suspected invasive meningococcal disease. Bacterial antigen detection in CSF supports the diagnosis of a probable case if the clinical illness is consistent with meningococcal disease. Polymerase chain reaction testing is available in some research and public health laboratories. A serogroup-specific PCR test to detect N meningitidis from clinical specimens is used routinely in the United Kingdom, where up to 56 % of cases are confirmed by PCR testing alone. This test particularly is useful in patients who receive antimicrobial therapy before cultures are obtained.

#### Onychomycosis

Guidelines from the AAP Committee on Infectious Diseases (2018) state that tinea corporis is diagnosed by clinical manifestations and can be confirmed by microscopic examination of a potassium hydroxide wet mount of skin scrapings or fungal culture. "Polymerase chain reaction and periodic acid-Schiff stain evaluation of specimens are available but are expensive and generally are not necessary." The guidelines state that confirmatory diagnostic tests for tinea pedis are similar to those for tinea corporis. The guidelines state that fungal infection of the nail (tinea unguium or onychomycosis) can be verified by direct microscopic examination with potassium hydroxide, fungal culture of desquamated subungual material, or fungal stain of a nail clippings fixed in formalin.

#### Orthopoxvirus (e.g., Cowpox Virus, Mpox Virus, Vaccinia Virus)

The genus Orthopoxvirus contains a number of species that can infect animals and humans. The most well-known member of the genus is variola virus, the causative agent of smallpox. Other notable members include cowpox virus, mpox virus, and vaccinia virus (smallpox vaccine) (CDC, 2015).

#### Cowpox

Cowpox infection occurs after contact with infected animals; person-to-person transmission has not been observed. Human infections with cowpox and cowpoxlike viruses have been reported in Europe and the Caucasus (cowpox and Akhmeta virus

in Georgia). Travelers with direct, hands-on contact with affected bovines, felines, rodents, or captive exotics (zoo animals) may be at risk for cutaneous infection (CDC, 2019).

Human infections with vaccinia, wild vaccinalike viruses, cowpox, and cowpoxlike viruses are most often self-limited, characterized by localized vesicular-pustular (and in cowpox, occasionally ulcerative) lesions. Fever and other constitutional symptoms may occur briefly after lesions first appear. Lesions can be painful and can persist for weeks. Immunocompromised patients or those with exfoliative skin conditions (such as eczema or atopic dermatitis) are at higher risk of severe illness or death (CDC, 2019)

PCR testing or virus isolation confirms orthopoxvirus infection (CDC, 2019).

#### Mpox

Mpox, formerly known as monkeypox, is a relatively rare viral zoonotic infection that is caused by the mpox virus. Mpox virus belongs to the Orthopoxvirus genus which includes variola (causative agent of smallpox) and vaccinia viruses (the virus used in smallpox vaccine). Mpox symptoms are similar to smallpox symptoms, but are considered milder and rarely fatal (CDC, 2022; Isaacs and Shenoy, 2022).

Mpox was discovered in the late 1950's when there were two outbreaks of a "pox-like disease" that occurred in colonies of monkeys kept for research. Per the CDC, despite being named "monkeypox," the source of the disease remains unknown. However, African rodents and non-human primates (like monkeys) might harbor the virus and infect people. The first human case of mpox was identified in 1970 in the Democratic Republic of the Congo. Subsequent cases have historically been identified in several central and western African countries. Mpox cases are rare in nonendemic countries such as Europe and the United States. Prior to 2022, almost all mpox cases in people outside of Africa were linked to international travel to countries where the disease commonly occurs or through imported animals. However, in May 2022, an outbreak of mpox was reported in Europe, in which some cases were identified as nontravel-related. The United States also reported their first case in May 2022. Cases related to this outbreak have continued to be reported in nonendemic countries worldwide, providing evidence of community spread. As of July 2022, thousands of confirmed mpox cases in dozens of countries have been reported. In July 2022, the World Health Organization (WHO) declared the outbreak a public health emergency of international concern (CDC, 2022; Isaacs and Shenoy, 2022).

There are two types (or clades) of mpox virus: West African and Congo Basin. Infections in the 2022 outbreak are from the West African type. Infections with the type of mpox virus identified in this outbreak, the West African type, are rarely fatal. Over 99% of people who get this form of the disease are likely to survive. However, people with weakened immune systems, children under 8 years of age, people with a history of eczema, and people who are pregnant or breastfeeding may be more likely to get seriously ill or die. The Congo Basin type of mpox virus has a fatality rate around 10% (CDC, 2022).

Per the CDC, mpox spreads in different ways. The virus can spread from person-to-person through:

- direct contact with the infectious rash, scabs, or body fluids
- respiratory secretions during prolonged, face-to-face contact, or during intimate physical contact, such as kissing, cuddling, or sex
- touching items (such as clothing or linens) that previously touched the infectious rash or body fluids
- pregnant people can spread the virus to their fetus through the placenta.

It's also possible for people to get mpox from infected animals, either by being scratched or bitten by the animal or by preparing or eating meat or using products from an infected animal. Mpox can spread from the time symptoms start until the rash has fully healed and a fresh layer of skin has formed. The illness typically lasts 2 to 4 weeks. People who do not have mpox symptoms cannot spread the virus to others (CDC, 2022).

The diagnosis of mpox should be suspected in individuals who present with a rash or other symptoms that could be consistent with mpox and epidemiologic risk factors for infection (e.g., recent travel to Central or West Africa or other areas where large outbreaks of mpox have been reported; close or intimate in-person contact with individuals who have suspected or confirmed mpox or are part of a social network experiencing mpox activity). The diagnosis should also be suspected in patients who do not fall in groups previously described but present with genital ulcer disease or proctitis that does not respond to empiric treatment for typical sexually transmitted infections. Patients with mpox typically present with a systemic illness that includes fevers, chills, and myalgias, followed by a characteristic rash that appears similar to smallpox. The rash typically begins as macules and evolves to papules, vesicles, and then pustules. The lesions eventually crust over, and these crusts dry up and then fall off. However, during the outbreak of mpox disease in 2022, some patients have presented with genital, rectal, and/or oral lesions without the initial prodrome (Isaacs and Shenoy, 2022).

The CDC provides case definitions for use in the 2022 m>pox response. Suspect cases include new characteristic rash or meets one of the epidemiologic criteria and has a high clinical suspicion for mpox. Note, the characteristic rash associated with mpox lesions involve the following: deep-seated and well-circumscribed lesions, often with central umbilication; and lesion progression through specific sequential stages—macules, papules, vesicles, pustules, and scabs; this can sometimes be confused with other diseases that are more commonly encountered in clinical practice (e.g., secondary syphilis, herpes, and varicella zoster). Historically, sporadic accounts of patients co-infected with mpox virus and other infectious agents (e.g., varicella zoster, syphilis) have been reported, so patients with a characteristic rash should be considered for testing, even if other tests are positive. Also note, clinical suspicion may exist if presentation is consistent with illnesses confused with mpox (e.g., secondary syphilis, herpes, and varicella zoster).

CDC's epidemiologic criteria (within 21 days of illness onset) include:

- Reports having contact with a person or people with a similar appearing rash or who received a diagnosis of confirmed or probable mpox; or
- Had close or intimate in-person contact with individuals in a social network experiencing mpox activity, this includes men who have sex with men (MSM) who meet partners through an online website, digital application ("app"), or social event (e.g., a bar or party); or
- Traveled outside the US to a country with confirmed cases of mpox or where Mpox virus is endemic; or
- Had contact with a dead or live wild animal or exotic pet that is an African endemic species or used a product derived from such animals (e.g., game meat, creams, lotions, powders, etc.)

Probable cases include no suspicion of other recent Orthopoxvirus exposure (e.g., Vaccinia virus in ACAM2000 vaccination) and demonstration of the presence of Orthopoxvirus DNA by polymerase chain reaction of a clinical specimen, or Orthopoxvirus using immunohistochemical or electron microscopy testing methods, or demonstration of detectable levels of anti-orthopoxvirus IgM antibody during the period of 4 to 56 days after rash onset. Confirmed cases include demonstration of

the presence of Mpox virus DNA by polymerase chain reaction testing or next-generation sequencing of a clinical specimen or isolation of Mpox virus in culture from a clinical specimen (CDC, 2022)

Polymerase chain reaction (PCR) testing should be performed on samples of the lesion (CDC, 2022; Isaacs and Shenoy, 2022). In the United States, this testing can be done at a Laboratory Response Network (LRN) site or certain commercial laboratories. In the 2022 global outbreak, the CDC considers a patient with a positive orthopoxvirus PCR result to have a probable case of mpox. Viral testing of a throat swab may also be performed for epidemiologic purposes but is generally not used to confirm the diagnosis in the clinical setting (Isaacs and Shenoy, 2022).

The U.S. Food and Drug Administration (FDA) is advising people to use swab samples taken directly from a lesion (rash or growth) when testing for the mpox virus. The FDA is not aware of clinical data supporting the use of other sample types, such as blood or saliva, for mpox virus testing. Testing samples not taken from a lesion may lead to false test results (FDA, 2022).

For additional information, see

[CPB 0644 - Orthopoxvirus Vaccines \(./600\\_699/0644.html\)](#).

#### Vaccinia Virus

The smallpox vaccine is made from a live virus called vaccinia, which is a poxvirus similar to smallpox, but considered less harmful. For that reason, people who are vaccinated must take precautions when caring for the place on their arm where they were vaccinated, so they can prevent the vaccinia virus from spreading. For most people with healthy immune systems, live virus vaccines are effective and safe. Sometimes a person getting a live virus vaccine experiences mild symptoms such as rash, fever, and head and body aches. In certain groups of people, complications from the vaccinia virus can be severe. Routine smallpox vaccination among the American public stopped in 1972 after the disease was eradicated in the United States (CDC, 2017). See [CPB 0644 - Smallpox Vaccine \(./600\\_699/0644.html\)](#).

#### Parainfluenza Virus

Parainfluenza viruses are the major cause of laryngotracheobronchitis (croup), but they also commonly cause upper respiratory tract infection, pneumonia, and/or bronchiolitis. Virus may be isolated from nasopharyngeal secretions by culture inoculation or by staining for viral antigen (shell viral assay) (AAP, 2009). Confirmation is made by rapid antigen detection, usually immunofluorescent. Rapid antigen identification techniques, including immunofluorescent assays, enzyme immunoassays, and fluoroimmunoassays, can be used to detect the virus in nasopharyngeal secretions, but the sensitivities of the tests vary. In addition, sensitive and specific PCR tests are available; however, the role of PCR testing in the management of persons with parainfluenza virus infection has not been established. Treatment of parainfluenza virus infection is supportive, and no specific antiviral therapy is available.

#### Parvovirus

Infection with parvovirus B19 is recognized most often as erythema infectiosum (EI), which is characterized by mild systemic symptoms, fever in 15 % to 30 % of patients, and, frequently, a distinctive rash (Cunningham and Rennels, 2002). Before onset of these manifestations, a brief, mild, non-specific illness consisting of fever, malaise, myalgias, and headache, followed approximately 7 to 10 days later by the characteristic exanthema, may occur in some patients. The facial rash is intensely red with a "slapped cheek" appearance and often accompanied by circumoral pallor.

Infection with the causative agent of EI, human parvovirus B19, also can cause asymptomatic infection, a mild respiratory tract illness with no rash, a rash atypical for EI that may be rubelliform or petechial, arthritis in adults (in the absence of manifestations of EI), chronic bone marrow failure in immunodeficient patients, and transient aplastic crisis lasting 7 to 10 days in patients with hemolytic anemias (e.g., sickle cell disease, and autoimmune hemolytic anemia) and other conditions associated with low hemoglobin levels, including hemorrhage, severe anemia, and thalassemia (Cunningham and Rennels, 2002). Chronic parvovirus B19 infection has been detected in some human immunodeficiency virus (HIV)-infected patients with severe anemia. In addition, parvovirus B19 infection has been associated with thrombocytopenia and neutropenia. Patients with aplastic crisis may have a prodromal illness with fever, malaise, and myalgia, but rash usually is absent. The red blood cell aplasia is related to lytic infection in erythrocyte precursors.

Parvovirus B19 infection occurring during pregnancy can cause fetal hydrops and death but is not a proven cause of congenital anomalies. The risk of fetal death is probably between 2 % and 6 %, with the greatest risk when infection occurs during the first half of pregnancy.

Parvovirus B19 is distributed worldwide and is a common cause of infection in humans, who are the only known hosts. Modes of transmission include contact with respiratory tract secretions, percutaneous exposure to blood or blood products, and vertical transmission between a mother and her fetus. Parvovirus B19 infections are ubiquitous, and cases of EI can occur sporadically or as part of community outbreaks, which often occur in elementary or junior high schools during the late winter and early spring.

According to the AAP (2006), the most feasible methods of diagnosis are direct detection of parvovirus B19 antigen or DNA in clinical specimens and serologic tests. In the immunocompetent host, detection of serum parvovirus B19-specific immunoglobulin (Ig) M antibody is preferred, and detection indicates infection probably occurred within the previous 2 to 4 months. By using a radioimmunoassay or enzyme immunoassay, antibody may be detected in 90 % or more of patients at the time of the EI rash and by the 3rd day of illness in patients with transient aplastic crisis. Serum IgG antibody indicates previous infection and immunity. These assays are available through commercial laboratories and through some state health and research laboratories. However, their sensitivity and specificity may vary, particularly for IgM antibody. The optimal method for detecting chronic infection in the immunocompromised patient is demonstration of virus by nucleic acid hybridization or PCR assay, because parvovirus B19 antibody is variably present in persistent infection. Since parvovirus B19 DNA can be detected by PCR in serum after the acute viremic phase for up to 9 months in some patients, PCR detection of parvovirus B19 DNA does not necessarily indicate acute infection. Less sensitive nucleic acid hybridization assays usually are positive for only 2 to 4 days after onset of illness. For HIV-infected patients with severe anemia associated with chronic infection, dot blot hybridization of serum may be a more appropriate assay. Parvovirus B19 has not been grown in standard cell culture, but the virus has been cultivated in experimental cell culture.

For most patients, only supportive care is indicated. Patients with aplastic crises may require transfusion. For the treatment of chronic infection in immunodeficient patients, intravenous immunoglobulin therapy has been used. Some cases of B19 infected hydrops fetalis have been treated successfully with intra-uterine blood transfusions.

Farruggia and Dufour (2015) stated that autoimmune neutropenia of infancy (AIN), also called primary autoimmune neutropenia, is a disease in which antibodies recognize membrane antigens of neutrophils, mostly located on immunoglobulin G (IgG) Fc receptor type 3b (Fc<sub>γ</sub>IIIb receptor), causing their peripheral destruction. It is the most frequent type of neutropenia in children under 3 to 4 years of age and in most cases shows a benign, self-limited course. The diagnosis is based on evidence of indirect anti-neutrophil antibodies, whose detection frequently remains difficult. These researchers analyzed the literature regarding AIN and presented their personal experience in diagnosis and management.

While there is some evidence that some cases of AIN may be a consequence of parvovirus B19 infection. However, it is unclear how the detection (or lack of detection) of parvovirus B19 would affect management. Furthermore, an UpToDate review on "Immune neutropenia" (Coates, 2015) does not mention parvovirus PCR/polymerase chain reaction as a management tool.

#### Plesiomonas Shigelloides Infections

An UpToDate review on "Plesiomonas shigelloides infections" (Morris and Homeman, 2015) does not mention PCR testing as a management tool.

#### Pleuropulmonary Coccidioidomycosis

Thompson et al (2013) stated that in patients with positive serum serology for coccidioidomycosis, the differential diagnosis of concurrent pleural effusions can be challenging. These researchers sought to clarify the performance characteristics of biochemical, serologic, and nucleic-acid-based testing in an attempt to avoid invasive procedures. The utility of adenosine deaminase (ADA), coccidioidal serology, and PCR in the evaluation of pleuropulmonary coccidioidomycosis has not been previously reported. A total of 40 consecutive patients evaluated for pleuropulmonary coccidioidomycosis were included. Demographic data, pleural fluid values, culture results, and clinical diagnoses were obtained from patient chart review. Testing of ADA was performed by ARUP Laboratories, coccidioidal serologic testing was performed by the University of California-Davis coccidioidomycosis serology laboratory, and PCR testing was performed by the Translational Genomics Research Institute using a previously published methodology. Fifteen patients were diagnosed with pleuropulmonary coccidioidomycosis by European Organization for the Research and Treatment of Cancer/Mycoses Study Group criteria. Pleural fluid ADA concentrations were less than 40 IU/L in all patients (range of less than 1.0 to 28.6 IU/L; median of 4.7). The sensitivity and specificity of coccidioidal serologic testing was 100 % in this study. The specificity of PCR testing was high (100 %), although the overall sensitivity remained low, and was comparable to the experience of others in the clinical use of PCR for coccidioidal diagnostics. The authors concluded that contrary to prior speculation, ADA levels in pleuropulmonary coccidioidomycosis were not elevated in this study. The sensitivity and specificity of coccidioidal serologic testing in non-serum samples remained high, but the clinical usefulness of PCR testing in pleural fluid was disappointing and was comparable to pleural fluid culture.

#### Pneumococcal Disease

Avni and colleagues (2010) stated that the use of molecular-based methods for the diagnosis of bacterial infections in blood is appealing, but they have not yet passed the threshold for clinical practice. These researchers performed a systematic review of prospective and case-control studies assessing the diagnostic utility of PCR directly with blood samples for the diagnosis of invasive pneumococcal disease (IPD). A broad search was conducted to identify published and unpublished studies. Two reviewers independently extracted the data. Summary estimates for sensitivity and specificity with 95 % confidence intervals (CIs) were calculated by using the hierarchical summary receiver operating characteristic method. The

effects of sample processing, PCR type, the gene-specific primer, study design, the participants' age, and the source of infection on the diagnostic odds ratios were assessed through meta-regression. A total of 29 studies published between 1993 and 2009 were included. By using pneumococcal bacteremia for case definition and healthy people or patients with bacteremia caused by other bacteria as controls (22 studies), the summary estimates for sensitivity and specificity were 57.1 % (95 % CI: 45.7 to 67.8 %) and 98.6 % (95 % CI: 96.4 to 99.5 %), respectively. When the controls were patients suspected of having IPD without pneumococcal bacteremia (26 studies), the respective values were 66.4 % (95 % CI: 55.9 to 75.6 %) and 87.8 % (95 % CI: 79.5 to 93.1 %). With lower degrees of proof for IPD (any culture or serology result and the clinical impression), the sensitivity of PCR decreased and the specificity increased. All analyses were highly heterogeneous. The use of nested PCR and being a child were associated with low specificity, while the use of a cohort study design was associated with a low sensitivity. The lack of an appropriate reference standard might have caused under-estimation of the performance of the PCR. The authors concluded that currently available methods for PCR with blood samples for the diagnosis of IPD lack the sensitivity and specificity necessary for clinical practice.

#### Pneumocystis Pneumonia

*Pneumocystis jiroveci* (formerly *P. carinii*), now considered a fungus rather than a protozoan, causes disease only when defenses are compromised, most commonly when there are defects in cell-mediated immunity as in hematologic malignancies, lymphoproliferative diseases, cancer chemotherapy, and AIDS (Beers and Berkow, 1999). About 30 % of patients with HIV infection have *P. jiroveci* pneumonia as the initial AIDS-defining diagnosis, and greater than 80 % of AIDS patients have this infection at some time if prophylaxis is not given. Patients with HIV infection become vulnerable to *P. jiroveci* pneumonia when the CD4 count is less than 200/ $\mu$ L. Most patients have fever, dyspnea, and a dry, non-productive cough that may evolve subacutely over several weeks or acutely over several days.

A definitive diagnosis of PCP is made by demonstration of organisms in lung tissue or respiratory tract secretions (AAP, 2006). The most sensitive and specific diagnostic procedures have been open lung biopsy and transbronchial biopsy. However, bronchoscopy with bronchoalveolar lavage, induction of sputum in older children and adolescents, and intubation with deep endotracheal aspiration are less invasive and often diagnostic and have been sufficiently sensitive in patients with HIV infection who have an increased number of organisms compared with non-HIV-infected patients with PCP. According to the AAP (2009), "polymerase chain reaction assays for detecting *P. jiroveci* infection are experimental and are not approved by the U.S. Food and Drug Administration for diagnosis." The AAP notes that serologic tests are not useful. Guidelines from Cincinnati Children's Hospital Medical Center (2001) stated that PCR testing for *P. jiroveci* infection is investigational. Other guidelines indicate no role for PCR testing for this indication (NYSDOH, 2006).

#### Pneumonia Panels

The BioFire FilmArray Pneumonia Panel (bioMérieux) is a multiplex PCR assay that analyzes lower respiratory tract specimens for 18 bacteria, 8 viruses, and 7 genetic markers of antimicrobial resistance. The assay uses amplified probe technique, including reverse transcription for RNA targets. Each analyte is reported as detected or not detected with semi-quantitative results for 15 bacteria.

Lower respiratory tract infections, including hospital-acquired (HAP) and ventilator-associated pneumonia (VAP), are common in hospitalized patient populations. Buchan et al (2020) state that molecular diagnostics, including PCR-based tests, generate a sensitive result within hours of specimen collection and have the potential to reduce the duration of broad-spectrum empirical antibiotic therapy by

identifying pathogenic organisms or specific antibiotic resistance markers 2 to 3 days sooner than routine methods. Thus, the authors examined the impact of the multiplexed, semi-quantitative BioFire FilmArray Pneumonia panel (PN panel) test on laboratory reporting for 259 adult inpatients submitting bronchoalveolar lavage (BAL) specimens for laboratory analysis. The authors found that the PN panel demonstrated a combined 96.2% positive percent agreement (PPA) and 98.1% negative percent agreement (NPA) for the qualitative identification of 15 bacterial targets compared to routine bacterial culture. Semi-quantitative values reported by the PN panel were frequently higher than values reported by culture, resulting in semi-quantitative agreement of 43.6% between the PN panel and culture. However, all bacterial targets reported as greater than 105 CFU/ml in culture were reported as greater than or equal to 105 genomic copies/ml by the PN panel. Viral targets were identified by the PN panel in 17.7% of specimens tested, of which 39.1% were detected in conjunction with a bacterial target. A review of patient medical records, including clinically prescribed antibiotics, revealed the potential for antibiotic adjustment in 70.7% of patients based on the PN panel result, including discontinuation or de-escalation in 48.2% of patients, resulting in an average savings of 6.2 antibiotic days/patient. The authors state that the PN panel is indicated as an adjunctive test for patients with symptoms of lower respiratory tract infection. Moreover, the PN panel has the potential to provide rapid identification of bacterial and viral pathogens that can be used to aid in definitive etiologic diagnosis and positively impact efforts to meet infection prevention and antibiotic stewardship objectives.

In a systematic review and meta-analysis, Timbrook et al (2021) evaluated the impact of BioFire FilmArray Pneumonia Panel in detecting bacteria and clinical management among critically ill COVID-19 patients admitted to the ICU. Seven studies with 558 patients were included. The authors found that antibiotic use before respiratory sampling occurred in 28-79% of cases. The panel incidence of detections was 33% (95% CI 0.25 to 0.41,  $I^2=32\%$ ) while culture yielded 18% (95% CI 0.02 to 0.45;  $I^2=93\%$ ). The panel was associated with approximately a 1 and 2 day decrease in turnaround for identification and common resistance targets, respectively. The authors concluded that their study reflects the increase of bacterial co-detections among critically ill COVID-19 ICU patients using the BioFire Pneumonia Panels. Thus, overcoming the limitations of culture-based methods in terms of sensitivity and turnaround time, the BioFire Pneumonia Panels may be an important tool to frontline clinicians for improving antimicrobial use in critically ill COVID-19 patients.

Escudero and colleagues (2022) state that microbiological diagnosis by using commercial multiplex quantitative PCR systems provides great advantages over the conventional culture. Thus, the authors evaluated the Biofire FilmArray Pneumonia Panel Plus (FAPP+) to test 144 low respiratory tract samples from 105 COVID-19 patients admitted to an Intensive Care Unit (ICU). The molecular panel detected 78 pathogens in 59 (41%) samples. The molecular panel was assessed by using the conventional culture (CC) as comparator, which isolated 42 pathogens in 40 (27.7%) samples. The overall percentage of agreement was 82.6%. Values of sensitivity (93%), specificity (62%), positive predictive value (50%), and negative predictive value (96%) were obtained. The mean time elapsed from sample extraction to modification of antibiotic treatment was 7.6 h. A change in antimicrobial treatment after the FAPP+ results was performed in 27% of patients. A limitation of the study is that it was carried out in a single center on a small series of patients. The authors state that their study supports the conclusion that the FAPP+ offers a rapid diagnosis of respiratory bacterial infections with a high sensitivity and specificity. The molecular approach cannot completely replace conventional cultures since the latter continue to be the gold standard and allow the isolation of the microorganism for further antimicrobial susceptibility testing. However, the FAPP+ can be a complement for the early management of pneumonia, which proved to be an

excellent tool to rapidly identify etiological agents, to guide clinical decisions early, and to optimize the use of antimicrobials, especially in the context of diagnostic and antimicrobial stewardship initiatives.

Virk et al (2024) conducted a single-center, open-label, pragmatic, randomized controlled trial at the Mayo Clinic to compare the effect of the BioFire FilmArray Pneumonia Panel with standard of care testing on antibiotic use in a real-world hospital setting. The study included 1181 hospitalized adult patients with suspected pneumonia, from whom expectorated or induced sputum, tracheal secretions, or bronchoalveolar lavage fluid respiratory culture samples (one per individual) could be collected during index hospitalization. Samples were randomly assigned (1:1) with a computerized tool to undergo testing with either the BioFire FilmArray Pneumonia Panel, conventional culture, and antimicrobial susceptibility testing (intervention group) or conventional culture and antimicrobial susceptibility testing alone (control group). Antimicrobial stewardship review in both groups involved an assessment and recommendations for antibiotic modifications based on clinical data and the results from the BioFire FilmArray Pneumonia Panel, conventional culture, or both. The primary outcome was median time to first antibiotic modification (i.e., escalation or de-escalation of antibiotics against Gram-negative and Gram-positive bacteria) within 96 hours of randomization, assessed with the Wilcoxon rank-sum test and analyzed in a modified intention-to-treat population. In total, 1152 participants were included in the modified intention-to-treat analysis, 589 (51.1%) in the control group and 563 (48.9%) in the intervention group. For the modified intention-to-treat population, median time to any first antibiotic modification was 20.4 hours (95% CI 18.0-20.4) in the intervention group and 25.8 hours (22.0-28.7) in the control group ( $p=0.076$ ). Median time to any antibiotic escalation was 13.8 hours (9.2-19.0) in the intervention group and 24.1 hours (19.5-29.6) in the control group ( $p=0.0022$ ). Median time to escalation of antibiotics against Gram-positive organisms was 10.3 hours (6.2-30.9) in the intervention group and 24.6 hours (19.5-37.2) in the control group ( $p=0.044$ ); median time to escalation of antibiotics against Gram-negative organisms was 17.3 hours (10.8-23.3) in the intervention group and 27.2 hours (21.3-33.9) in the control group ( $p=0.010$ ). Median time to any antibiotic de-escalation did not differ between groups ( $p=0.37$ ). Median time to first de-escalation of antibiotics against Gram-positive organisms was 20.7 hours (17.8-24.0) in the intervention group and 27.8 hours (22.9-33.0) in the control group ( $p=0.015$ ); median time to first de-escalation of antibiotics against Gram-negative organisms did not differ between groups ( $p=0.46$ ). The authors concluded that clinical use of the BioFire FilmArray Pneumonia Panel might lead to faster antibiotic escalations, including for Gram-negative or Gram-positive bacteria, and faster antibiotic de-escalations directed at Gram-positive bacteria. However, additional research is needed regarding antimicrobial de-escalation, especially when antibiotics with broad Gram-negative spectrum are being used, by use of rapid diagnostics in patients with lower respiratory tract infection.

Zhang and colleagues (2024) evaluated the optimal timing for implementing the BioFire FilmArray Pneumonia Panel (FA-PP) in the medical intensive care unit (MICU). Respiratory samples from 135 MICU-admitted patients with acute respiratory failure and severe pneumonia were examined using FA-PP. The cohort had an average age of 67.1 years, and 69.6% were male. Notably, 38.5% were smokers, and the mean acute physiology and chronic health evaluation-II (APACHE-II) score at initial MICU admission was 30.62, and the mean sequential organ failure assessment score (SOFA) was 11.23, indicating severe illness. Furthermore, 28.9, 52.6, and 43% of patients had a history of malignancy, hypertension, and diabetes mellitus, respectively. Community-acquired pneumonia accounted for 42.2% of cases, whereas hospital-acquired pneumonia accounted for 37%. The average time interval between pneumonia diagnosis and FA-PP implementation was 1.9 days, and the mean MICU length of stay was 19.42 days. The mortality rate was 50.4%. Multivariate logistic regression analysis identified two variables as significant

independent predictors of mortality: APACHE-II score ( $p = 0.033$ , OR = 1.06, 95% CI 1.00-1.11), history of malignancy (OR = 3.89, 95% CI 1.64-9.26). The Kaplan-Meier survival analysis indicated that early FA-PP testing did not provide a survival benefit. The study suggested that the FA-PP test did not significantly impact the mortality rate of patients with severe pneumonia with acute respiratory failure. However, a history of cancer and a higher APACHE-II score remain important independent risk factors for mortality.

Kling et al (2024) conducted a single-center retrospective study in which residual bronchoalveolar lavage (BAL) specimens were retrieved from critically ill hospitalized patients and compared the results from the Respiratory Pathogen Illumina Panel (RPIP) to culture and BioFire FilmArray Pneumonia Panel (BioFire PN). In total, 47 BAL specimens from 47 unique patients were included. All BAL samples were tested with culture and multiplex PCR. In total, 38 of the 47 BALs were consistent with a clinical picture of pneumonia per chart review. Additional testing of the 38 samples with the RPIP identified a new bacterium in 20 patients, a new virus in 4 patients, a new bacterium plus virus in 4 patients, and no additional organisms in 10 patients. In 17 (44.5%) of these patients, the RPIP results could have indicated an antibiotic addition. Compared with cultures, the RPIP had an overall sensitivity of 64% and specificity of 98%, with a 0% sensitivity for fungus and 14% sensitivity for mycobacteria. Compared with BioFire PN, the RPIP was 70% sensitive and 99% specific, with a 74% sensitivity for bacteria and 33% sensitivity for viruses. The RPIP was 29% more sensitive for HAP/VAP bacterial targets compared with CAP. The authors state that emerging next-generation sequencing (NGS) technologies such as the RPIP may have a role in identifying the etiology of pneumonia, even when patients have BAL culture and multiplex PCR results available. Similar to prior studies evaluating RPIP, their study showed this platform lacked sensitivity when compared with cultures, particularly for fungi and mycobacteria. They concluded, however; that the high specificity of the test can be leveraged when clinicians are seeking to rule out certain infections.

A 2024 update by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM) state, "Two molecular pneumonia syndromic panel tests are available for the evaluation of patients with HAP and VAP, the FilmArray Pneumonia panel (BioFire, Inc., Salt Lake City, Utah) and Unyvero HPN (OpGen, Inc. Rockville, Maryland). Data on potential clinical impact are largely derived from retrospective studies. These studies show early de-escalation of antimicrobial agents in 39%-48% of patients and escalation in 21%-22%. At the time of writing, several randomized controlled trials are in progress to assess the clinical impact of these pneumonia panels on patients with HAP/VAP. Laboratories that implement pneumonia syndromic panel testing may wish to work with antimicrobial stewardship and other key stakeholders to ensure appropriate utilization and interpretation of test results. For example, these tests are most useful in the hospitalized patient at risk for *S. aureus* or *P. aeruginosa* who is not receiving antimicrobial therapy and who has clear documentation of a change in respiratory status and radiographic evidence of a new infiltrate".

#### Prostate Cancer Micrometastases

Reverse transcription of messenger RNA followed by the PCR (RT-PCR) can identify circulating prostate cancer cells that express PSA or prostate-specific membrane antigen (PSMA) (Sieden et al, 1994). Studies in clinically localized patients have shown very variable results, which are at least partly due to significant technical differences in how separate laboratories have performed the assay. Hematogenous PSA-expressing cells have been identified by RT-PCR in 0 to 81 % of patients prior to radical prostatectomy. In patients documented to have metastatic disease, RT-PCR detected circulating cancer cells in 31 to 100 % of patients (de la Taille et al, 1999). In a pooled analysis of available data of RT-PCR in peripheral blood, 174 of 757 (23 %) patients with pathologic T1 or T2 disease and 151 of 401 (38 %)

patients with pT3 disease were found to be RT-PCR positive for PSA or PSMA (Bast et al, 2000). All but 2 of the studies evaluated showed no additional advantage to the use of RT-PCR over conventional staging modalities. According to Bast et al (2000), "in its current form, RT-PCR for PSA or PSMA remains an investigational staging tool."

#### Prostatitis

An UpToDate review on "Chronic prostatitis and chronic pelvic pain syndrome" (Pontari, 2019) states that "Although bacterial infection has been suspected, particularly in the inflammatory subset of CP/CPPS, a bacterial etiology has not been consistently identified. Most experts believe that inflammatory and noninflammatory CP/CPPS are both noninfectious disorders. Studies of Chlamydia, Mycoplasma, and Ureaplasma, which have all been implicated in CP/CPPS, have generally concluded that they are not responsible for CP/CPPS. Several investigators have performed polymerase chain reaction (PCR) testing looking for evidence of bacteria in prostatic tissues, but these have yielded negative results. One study cultured prostatic biopsy specimens obtained via the transperineal approach from men with CP/CPPS and from normal volunteers. There was no difference in the number of patients from whom bacteria were cultured (38 versus 36 %, respectively).

Furthermore, UpToDate reviews on "Acute bacterial prostatitis" (Meyrier and Fekete, 2019a) and "Chronic bacterial prostatitis" (Meyrier and Fekete, 2019b) do not mention PCR as a management tool.

#### Proteus Mirabilis

*Proteus* species are part of the Enterobacteriaceae family of gram-negative bacilli. *Proteus* organisms are implicated as serious causes of infections in humans. *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, *Proteus mirabilis* causes 90 % of *Proteus* infections. *Proteus* organisms are easily recovered through routine laboratory cultures. Cultures may be indicated when patients do not respond to empiric therapy or when they have recurrent symptoms. A role for PCR testing in the management of *Proteus mirabilis* has not been established.

#### Q Fever (C. Burnetti)

According to current guidelines (AAP, 2009; CDC, 2011), confirmation of acute Q fever requires one of the following: (i) a fourfold change in immunoglobulin (Ig) G-specific antibody titer between acute and convalescent specimens taken 3 to 6 weeks apart by immunofluorescent antibody assay (IFA) or enzyme linked immunosorbent assay; (ii) detection of C burnetii DNA in a clinical sample using PCR assay; (iii) culture of C burnetii from a clinical specimen; or (iv) positive immunohistochemical staining of C burnetii in a tissue sample. Confirmation of chronic Q fever is based on a single IgG titer of 800 or more by IFA.

#### Quantitative PCR Tests

The Karius Test for infectious disease uses next-generation sequencing (NGS) to detect microbial cell free DNA (cfDNA) in plasma from bacteria, DNA viruses, fungi and protozoa. Microbial cfDNA may be found in plasma when viable microorganisms are not detected in blood by other methods. The reported microorganism(s) may or may not be the cause of patient infection. Results should be interpreted within the context of clinical data, including medical history, physical findings, epidemiological factors, and other laboratory data (Karius, 2020).

Camargo et al. (2019) state that cell-free DNA (cfDNA) sequencing technology in diagnostic evaluation of infections in immunocompromised hosts is limited. The authors conducted an exploratory study using next-generation sequencing (NGS) for detection of microbial cfDNA in a cohort of 10 immunocompromised patients with febrile neutropenia, pneumonia or intra-abdominal infection. Pathogen identification by cfDNA NGS demonstrated positive agreement with conventional diagnostic laboratory methods in 7 (70%) cases, including patients with proven/probable invasive aspergillosis, *Pneumocystis jirovecii* pneumonia, *Stenotrophomonas maltophilia* bacteremia, Cytomegalovirus and Adenovirus viremia. NGS results were discordant in 3 (30%) cases including two patients with culture negative sepsis who had undergone hematopoietic stem cell transplant in whom cfDNA testing identified the etiological agent of sepsis; and one kidney transplant recipient with invasive aspergillosis who had received > 6 months of antifungal therapy prior to NGS testing. The authors concluded that these observations support the clinical utility of measurement of microbial cfDNA sequencing from peripheral blood for rapid noninvasive diagnosis of infections in immunocompromised hosts; however, larger studies are needed.

#### Recurrent Fever

An UpToDate review on "Fever of unknown origin in children: Evaluation" (Palazzi, 2017) does not mention PCR as a diagnostic tool.

#### Respiratory Pathogen Panels

Many respiratory tract infections are caused by viral pathogens. Rhinovirus, parainfluenza virus, coronavirus, adenovirus, respiratory syncytial virus, human metapneumovirus, and influenza virus account for most cases. Some viral infections (e.g., rhinovirus) are usually self-limited, or may only require care to help relieve symptoms. However, respiratory viral pathogens can lead to serious morbidity and mortality in individuals with a weakened immune system or are at high-risk of developing complications related to the infection (e.g., cancer, transplant recipients). Early diagnosis and treatment of viral infections in immunocompromised patients continues to be paramount. These infections are more likely to be severe in this population than those at average risk. Prognosis can be grave once viral replication and invasive infections are evident (Shahani, et al., 2017). Identification of viral respiratory pathogens has typically been based on direct antigen detection or culture; however, polymerase chain reaction (PCR) has been found to be more sensitive for most respiratory viruses. To help guide therapy decisions, such as use of antiviral agents, (e.g., for influenza), RSV prophylaxis, or to provide guidance on initiating, continuing or discontinuing antibiotic therapy, multiplex PCR respiratory viral panel (RVP) assays are available to detect various respiratory pathogens and their subtypes (e.g., influenza A (H1N1, H3N3), RSV subtype A and B).

The RVP is a multiplexed nucleic acid test which uses PCR technology with multiple sets of primers to simultaneously analyze multiple targets in a single sample, thus, several genomic DNA sequences are amplified in a single step of PCR. The RVP test can detect a wide range of different respiratory pathogens, with some RVPs detecting up to 20 pathogens. The RVP PCR test was found to be faster and more sensitive than direct fluorescent-antibody assay (DFA) and culture. In addition, the RVP test detected 43% additional respiratory virus infections not detected by conventional methods used in the clinical virology laboratory (Mahony et al, 2007).

Individuals who are status post stem cell and/or solid organ transplants are at higher risk for severe illness stemming from a viral respiratory tract infection. For individuals at average-risk, or considered a "normal host", viral respiratory infections typically result in a self-limited illness. Thus, those with weakened immune systems and/or considered high-risk for developing complications (immunocompromised host) are more likely to have significant morbidity and

mortality than those at average-risk. Therefore, rapid and accurate diagnosis of viral respiratory pathogens are considered reasonable for those patients who are immunocompromised (Hammond, et al, 2012). Although, results of the multiplex RVP panel may be considered to guide decisions in immunocompromised and/or high-risk populations, there is insufficient evidence in published peer-reviewed literature to support use of RVPs in average-risk persons.

Per the Centers for Medicare & Medicaid Services (LCD L37713, 2018), the use of limited multiplex viral panels in susceptible populations may be reasonable and necessary. Panels of 3-5 pathogens are covered under some CMS LCDs under limited circumstances. "Specifically the test must be ordered either in a healthcare setting that is equipped to care for and routinely does care for critically ill patients, or it must be ordered by an infectious disease specialist, unless an infectious disease specialist is not available." Multiplex PCR respiratory viral panels of 6 or more pathogens are non-covered by Medicare, as it does not meet Medicare's "reasonable and necessary" criteria. According to the Medicare LCD, the pathogen targets that compose the panels are determined by the manufacturers that make them, and do not represent specific pathogens that cause a common syndrome, or the organisms that commonly are found in a specific sample type or patient population or reflect seasonal variations. The fixed nature of these multiplex panels includes pathogens that cause infections different enough that simultaneous testing for these pathogens should be rare. Examples include Chlamydophilia (*Chlamydia*) pneumoniae or *Bordetella* pertussis in combination with rhinovirus, influenza viruses, and respiratory syncytial virus (RSV). The multiplex PCR respiratory viral panels are effectively a "one size fits all" diagnostic approach, and do not meet Medicare's "reasonable and necessary" criteria. Non-coverage of these multiplex RCR respiratory viral panels does not deny patient access because appropriate clinician directed testing is available.

Respiratory panels now include both virus and/or bacteria targets to help aid clinicians to quickly diagnose respiratory infections which often include signs and symptoms that are indistinguishable from one another. The U.S. FDA has authorized the marketing of multiplexed nucleic acid tests (e.g., BioFire FilmArray Respiratory Panel (RP)) for use with the FilmArray instrument systems for the simultaneous qualitative in vitro detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) collected in viral transport media and obtained from individuals suspected of respiratory tract infections. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information (FDA, 2019).

BioFire Diagnostics offers a line of respiratory panels to help clinicians quickly diagnose respiratory infections at the point of care. Products include the BioFire FilmArray Respiratory Panel EZ, which tests for 14 respiratory viral and bacterial pathogens, the BioFire FilmArray Respiratory Panel (RP), which tests 20 of the most common respiratory viral and bacterial pathogens, and the BioFire FilmArray Respiratory Panel 2 (RP2), which tests 21 pathogens that includes *Bordetella parapertussis*, all via a nasopharyngeal swab and using a multiplex PCR system. BioFire Filmarray System integrates sample preparation, amplification, detection and analysis into one system that requires just 2 minutes of hands-on time, with a total run time of about an hour (BioFire, 2019).

GenMark Diagnostics, Inc. offers the ePlex Respiratory Pathogen (RP) Panel which is a qualitative nucleic acid multiplex test that targets 19 viral (e.g., adenovirus, influenza, parainfluenza, RSV) and 2 bacterial pathogens (i.e., *Chlamydia*

*pneumoniae*, *Mycoplasma pneumoniae*) commonly associated with upper respiratory infection. The test uses a sample collected using a nasopharyngeal swab.

The ePlex RP panel is a sample-to-answer multiplex assay that runs on a single-use cartridge that automates all aspects of nucleic acid testing, including extraction, amplification, and detection. Babady et al. (2018) compared the performance and reproducibility of the ePlex RP panel with the bioMerieux/BioFire FilmArray Respiratory Panel. The authors evaluated 2,908 nasopharyngeal swab specimens prospectively and retrospectively from persons with signs and/or symptoms of upper respiratory tract infection at 13 clinical sites located in the United States and Canada. Discordance analysis was performed by using target-specific PCRs and bidirectional sequencing. The reproducibility of the assay was evaluated by using reproducibility panels comprised of 6 pathogens (influenza A H3 virus, RSV-A, PIV1, hMPV, coronavirus OC43, and adenovirus species B). The authors found that the overall agreement between the ePlex RP and BioFire RP results was 95% for all targets. Positive percent agreement with the BioFire RP result for viruses ranged from 85.1% to 95.1%, while negative percent agreement values ranged from 99.5% to 99.8%. Additional testing of discordant targets (12%; 349/2,908) confirmed the results of ePlex RP for 38% (131/349) of samples tested. Reproducibility was 100% for all targets tested, with the exception of adenovirus, for which reproducibility were 91.6% at low virus concentrations and 100% at moderate virus concentrations. A notable difference between the ePlex RP panel and the BioFire RP is the absence of *Bordetella pertussis*-*Bordetella parapertussis* targets among the bacterial targets. The authors concluded that the ePlex RP panel offers a rapid, sensitive, broadly multiplexed, sample-to-results option with minimal hands-on time for the detection of the most common viral and bacterial respiratory infections. Furthermore, the assay performance was found to be equivalent to that of the BioFire FilmArray RP for all targets. This study was designed and funded by GenMark Diagnostics.

The Respiratory Pathogen with ABR (RPX) is a multiplex PCR test that analyzes upper and/or lower respiratory specimens for 17 bacteria, 8 fungus, 13 virus and 16 antibiotic resistance markers in persons with signs and symptoms of a respiratory infection. Each microbial agent is reported as either detected or not detected. In addition, antibiotic resistance markers are also reported as detected or not detected. Results are produced in approximately 1 hour with a 95% sensitivity and 99% specificity (AMA, 2023).

#### Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (RSV) is a common respiratory virus that usually causes mild, cold-like symptoms in which most persons recover within a week or two. However, RSV can be serious for infants and older adults. RSV is the most common cause of bronchiolitis and pneumonia in children younger than 1 year of age in the U.S. It is also a significant cause of respiratory illness in older adults (CDC, 2018a).

The Committee on Infectious Diseases, American Academy of Pediatrics, Red Book (AAP, 2018) states that "rapid diagnostic assays, including direct fluorescent antibody (DFA) assay and enzyme or chromatographic immunoassay techniques for detection of viral antigen in nasopharyngeal specimens, are available commercially for RSV and generally are reliable in infants and young children. In children, the sensitivity of these assays in comparison with culture varies between 53% and 96%, with most in the 80% to 90% range. The sensitivity may be lower in older children and is quite poor in adults, because adults typically shed low concentrations of RSV. As with all antigen detection assays, the predictive value is high during the peak season, but false-positive test results are more likely to occur when the incidence of disease is low, such as in the summer in temperate areas. Therefore, antigen detection assays should not be the only basis on which the beginning and end of

monthly RSV immunoprophylaxis is determined. Molecular diagnostic tests using reverse transcriptase-polymerase chain reaction (RT-PCR) are cleared by the US Food and Drug Administration (FDA) and available widely and increase RSV detection rates over viral isolation or antigen detection assays, especially in older children and adults. Many tests are designed as multiplex assays to facilitate testing for multiple respiratory viruses with a single assay. Because of the increased sensitivity of RT-PCR assay, these tests may be preferred. However, results of these tests should be interpreted with caution, especially when a multiplex assay identifies more than one virus, because some viruses (eg, RSV, rhinovirus, adenovirus, and bocavirus) may persist in the airway for many weeks after the acute infection has resolved. As many as 25% of asymptomatic children test positive for respiratory viruses using RT-PCR assays in population-based studies. Up to 30% of children with RSV bronchiolitis may be coinfected with another respiratory tract pathogen, such as human metapneumovirus, rhinovirus, bocavirus, adenovirus, coronavirus, influenza virus, or parainfluenza virus. Whether children with bronchiolitis who are coinfected with more than one virus experience more severe or even less severe disease is not clear."

The Centers for Disease Control and Prevention (CDC, 2018b) state that "healthcare professionals should use highly sensitive rRT-PCR assays when testing older children and adults for RSV. rRT-PCR assays are now commercially available for RSV. The sensitivity of these assays often exceeds the sensitivity of virus isolation and antigen detection methods. Antigen tests are not sensitive for older children and adults because they may have lower viral loads in their respiratory specimens".

UpToDate review on "Respiratory syncytial virus infection: Clinical features and diagnosis" (Barr and Graham, 2019) state that the "laboratory diagnosis of RSV is made by analysis of respiratory secretions. In healthy children, a nasal wash usually provides the best yield, but a nasopharyngeal swab or nasal swab may be adequate if a nasal wash is not possible". When confirmation of RSV infection is necessary, the authors "prefer PCR-based assays if they are available. PCR-based assays have high sensitivity and are not affected by passively administered antibody to RSV. If PCR-based assays are not available, rapid antigen detection tests (RADTs) are a reasonable alternative, although false negative results may occur, particularly in adult patients who shed less virus from infected cells than infants. Some laboratories perform initial RADT and confirm negative results with PCR".

#### Rhinovirus

Rhinoviruses are the most frequent causes of the common cold or rhinosinusitis. Transmission occurs predominantly by person-to-person contact with self-inoculation by contaminated secretions on hands. Inoculation of nasopharyngeal secretions in appropriate cell cultures for viral isolation has been the primary means to diagnose infection but is insensitive for many strains (AAP, 2009). Although PCR assays have been developed, they are not commercially available. Treatment of rhinovirus infection is symptomatic, and diagnostic testing does not alter management such that clinical outcomes are improved.

#### Rocky Mountain Spotted Fever

Rocky Mountain spotted fever (RMSF) is an acute febrile disease caused by *Rickettsia rickettsii* and transmitted by ixodid ticks, producing high fever, cough, and rash (Beers and Berkow, 1999). Rocky Mountain spotted fever is limited to the Western Hemisphere. Initially recognized in the Rocky Mountain states, it occurs in practically all states (except Maine, Hawaii, and Alaska) in the United States, especially the Atlantic states. In humans, infection occurs mainly from May to September, when adult ticks are active and persons are most likely to be in tick-infested areas. In southern states, cases occur throughout the year. The incidence is high in children less than 15 years of age and in others who frequent tick-infested areas for work or recreation.

Serologic tests, isolation and identification of *Rickettsia rickettsii* from blood or tissues, and identification of the agent in skin or other tissues by immunofluorescence help confirm the diagnosis, particularly in RMSF. To be useful, serologic tests require 3 serum samples, taken during the 1st, 2nd, and 4th to 6th weeks of illness. Polymerase chain reaction is useful in early identification of specific rickettsial nucleic acids.

According to the AAP (2006), the diagnosis of RMSF can be established by one of the multiple rickettsial group-specific serologic tests. A 4-fold or greater change in titer between acute- and convalescent-phase serum specimens is diagnostic when determined by IFA, enzyme immunoassay (EIA), complement fixation (CF), latex agglutination (LA), indirect hemagglutination (IHA), or microagglutination (MA) tests. The IFA is the most widely available confirmatory test. Antibodies are detected by IFA 7 to 10 days after onset of illness. According to the AAP, the non-specific and insensitive Weil-Felix serologic test (Proteus vulgaris OX-19 and OX-2 agglutinins) is not recommended (AAP, 2006).

The AAP guidelines state that culture of *R. rickettsii* usually is not attempted because of the danger of transmission to laboratory personnel (AAP, 2009). *Rickettsia rickettsii* have been identified by immunofluorescent staining or PCR testing of tissue specimens. The AAP guidelines stated that PCR for detection of *R. rickettsii* in blood and biopsy specimens during the acute phase of the illness confirms the diagnosis and is available from CDC reference laboratories.

#### Rotavirus

Guidelines indicate no specific role for PCR testing in the diagnosis of rotavirus infection. Rotaviruses (Rv) are RNA viruses. Infection can result in diarrhea, usually preceded or accompanied by emesis and fever (AAP, 2009). In severe cases, dehydration, electrolyte abnormalities, and acidosis may occur. In immunocompromised children, including those with HIV infection, persistent infection can develop.

The AAP (2009) notes that EIA and latex agglutination assays for group A Rv antigen detection in stool are available commercially. However, EIAs are more sensitive for the detection of antigen late in the course of illness. Both assays have high specificity, but false-positive and non-specific reactions can occur in neonates and in persons with underlying intestinal disease. These non-specific reactions can be distinguished from true positive ones by the performance of confirmatory assays. Virus also can be identified in stool by electron microscopy and by specific nucleic acid amplification techniques.

The ISDA (2001) noted, however, that routine fecal testing to diagnose rotavirus infection is not necessary. "Rotavirus infection, a leading cause of diarrhea in young children (especially in winter months in temperate climates) can be diagnosed with commercial assays, and Norwalk-like virus infections can be diagnosed with research assays, but these tests are usually not necessary for managing an individual case."

No specific antiviral therapy is available (AAP, 2009). Oral or parenteral fluids are given to prevent and correct dehydration.

#### *Saccharomyces Cerevisiae*

*Saccharomyces cerevisiae* is a type of acospore-forming food yeast, also known as brewer's/baker's yeast. Yeasts are fungi that have a unicellular growth form and yield mucoid, bacteria-like colonies on laboratory media. Food yeasts, primarily *S. cerevisiae*, may cause allergic symptoms in occasional atopic persons but are rarely implicated with certainty (Middleton, 1998). Cases of vaginitis caused by *Saccharomyces cerevisiae* have been reported, and may be associated with baking

(Mandell, 2000). Guidelines on treatment of vaginitis, however, do not include a recommendation for PCR testing for *S. cerevisiae* in patients suspected of having none.

Two serum antibodies, anti-neutrophilic cytoplasmic antibodies (ANCA) and anti-*Saccharomyces cerevisiae* (ASCA) have been investigated as a technique to improve the efficiency and accuracy of diagnosing inflammatory bowel disease in order to potentially decrease the extent of the diagnostic work up or to avoid invasive diagnostic imaging. See [CPB 0249 - Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolic Assessment of Thiopurine Therapy \(./200\\_299/0249.html\)](#).

#### Sepsis Pathogen Panels

Sepsis is an acute syndrome that can often be fatal requiring early diagnosis and proper treatment. Blood culture (BC) is the gold standard for the identification of pathogens, however it has marked limitations, including that it is time-consuming and can only detect microbes that readily grow under culture conditions. Alternatively, molecular detection-based methodologies, such as polymerase chain reaction (PCR) testing, which is performed directly on positive blood culture samples, produce faster results but also have limitations such that a subculture (full culture) may still be required if the PCR panel does not include the causative pathogen.

Banerjee et al. (2015) performed a prospective randomized controlled trial evaluating outcomes associated with rapid multiplex PCR (rmPCR) detection of bacteria, fungi, and resistance genes directly from positive blood culture bottles (BCBs). A total of 617 patients with positive BCBs underwent stratified randomization into 3 arms: standard BCB processing (control, n = 207), rmPCR reported with templated comments (rmPCR, n = 198), or rmPCR reported with templated comments and real-time audit and feedback of antimicrobial orders by an antimicrobial stewardship team (rmPCR/AS, n = 212). The primary outcome was antimicrobial therapy duration. Secondary outcomes were time to antimicrobial de-escalation or escalation, length of stay (LOS), mortality, and cost. The authors found that time from BCB gram stain to microorganism identification was shorter in the intervention group (1.3 hours) vs control (22.3 hours) ( $p < .001$ ). Compared to the control group, both intervention groups had decreased broad-spectrum piperacillin-tazobactam (control 56 hours, rmPCR 44 hours, rmPCR/AS 45 hours;  $p = .01$ ) and increased narrow-spectrum  $\beta$ -lactam (control 42 hours, rmPCR 71 hours, rmPCR/AS 85 hours;  $p = .04$ ) use, and less treatment of contaminants (control 25%, rmPCR 11%, rmPCR/AS 8%;  $p = .015$ ). Time from gram stain to appropriate antimicrobial de-escalation or escalation was shortest in the rmPCR/AS group (de-escalation: rmPCR/AS 21 hours, control 34 hours, rmPCR 38 hours,  $p < .001$ ; escalation: rmPCR/AS 5 hours, control 24 hours, rmPCR 6 hours,  $p = .04$ ). Groups did not differ in mortality, LOS, or cost. The authors concluded that rmPCR reported with templated comments reduced treatment of contaminants and use of broad-spectrum antimicrobials, and that addition of antimicrobial stewardship enhanced antimicrobial de-escalation.

Box et al (2015) state that rapid diagnostics for bloodstream infections have been shown to improve outcomes. The authors conducted a multicenter, pre-post, quasi-experimental study to evaluate implementation of the Verigene BC-GP panel in combination with real-time support from the Antibiotic Stewardship Team (AST) in a community hospital system. The Verigene Gram-Positive Blood Culture Test (BC-GP) identifies 12 gram-positive organisms and 3 genetic markers of antibiotic resistance from positive blood culture media in 2.5 hours. The primary outcomes were average time to targeted antibiotic therapy and difference in antibiotic duration for contaminants. Secondary end points included hospital length of stay, mortality, pharmacy costs, and overall hospitalization costs. Adult patients with a gram-

positive bacteremia admitted in 2011 (pre-rapid testing) were compared with those admitted in 2014 (post-rapid testing). There were 103 patients in the preintervention group and 64 patients in the intervention group. The authors found that the optimized identification process, combined with AST intervention, improved mean time to targeted antibiotic therapy ( $p<0.001$ ) and decreased mean duration of antibiotic therapy for blood culture contaminants ( $p=0.03$ ). Median length of stay (9.1 vs 7.2 days,  $p=0.04$ ) and overall median hospitalization costs (\$17,530 vs \$10,290,  $p=0.04$ ) were lower in the intervention group. Mortality was similar between groups (9.1% vs 9.2%,  $p=0.98$ ). The authors concluded that rapid identification of gram-positive blood cultures with AST intervention decreased time to targeted antibiotic therapy, length of unnecessary antibiotic therapy for blood culture contaminants, length of stay, and overall hospital costs.

Cooper-Jones and Farrah (2017) state that the sensitivity and specificity of the FilmArray BCID panel are well established for organisms included in the panel; however, its primary limitation from a diagnostic standpoint is an inability to detect other pathogens not included in the panel. Used along with antimicrobial stewardship programs, the FA-BCID panel may improve patient outcomes by, for example, reducing the time it takes to receive appropriate antimicrobial therapy and shortening hospital stays. The authors identified 22 studies of the FA-BCID panel. Most studies compared the FA-BCID panel with the diagnostic gold standard (blood culture) for those suspected of sepsis. Two studies compared the FA-BCID panel with other rapid diagnostic tests, and one study used a combination MALDI-TOF MS and the FA-BCID panel and compared this with blood culture. The authors cite the larger study by Salimnia et al. (2016) who performed an eight-center, manufacturer-funded trial. Testing included 2,207 positive aerobic blood culture samples, 1,568 clinical and 639 seeded. Samples were tested fresh or were frozen for later testing within 8 h after the bottles were flagged as positive by an automated blood culture system. At least one organism was detected by the panel in 1,382 (88.1%) of the positive clinical specimens. The others contained primarily off-panel organisms. The panel reported multiple organisms in 81 (5.86%) positive clinical specimens. The unresolved blood culture identification sensitivity for all target detections exceeded 96%, except for *Klebsiella oxytoca* (92.2%), which achieved 98.3% sensitivity after resolution of an unavoidable phenotypic error. The sensitivity and specificity for vanA/B and blaKPC were 100%; those for mecA were 98.4 and 98.3%, respectively (Salimnia et al, 2016).

Tseng et al. (2018) state that although many studies have looked at its clinical and economic utility of rapid blood culture identification (BCID) in antimicrobial stewardship programs (AST), its comparative utility in gram-positive and gram-negative blood stream infections (BSIs) has not been as well characterized. The authors evaluated rapid PCR-based BCID panel on the management of gram-positive and gram-negative blood stream infections. The study was a quasi-experimental retrospective study at the Mayo Clinic in Phoenix, Arizona. All adult patients with positive blood cultures before BCID implementation (June 2015 to December 2015) and after BCID implementation (June 2016 to December 2016) were included. The outcomes of interest included time to first appropriate antibiotic escalation, time to first appropriate antibiotic de-escalation, time to organism identification, length of stay, infectious diseases consultation, discharge disposition, and in-hospital mortality. In total, 203 patients were included in this study. The authors found that there was a significant difference in the time to organism identification between the pre- and post-BCID cohorts (27.1 hours vs 3.3 hours,  $p < .0001$ ). BCID did not significantly reduce the time to first appropriate antimicrobial escalation or de-escalation for either gram-positive BSIs (GP-BSIs) or gram-negative BSIs (GN-BSIs). Providers were more likely to escalate antimicrobial therapy in GP-BSIs after gram stain and more likely to de-escalate therapy in GN-BSIs after susceptibilities. Although there were no significant differences in changes in antimicrobial therapy for organism identification by BCID vs traditional methods, more than one-quarter of providers (28.1%) made changes after organism

identification. There were no differences in hospital length of stay or in-hospital mortality comparing pre- vs post-BCID. The authors concluded that although BCID significantly reduced the time to identification for both GP-BSIs and GN-BSIs, BCID did not reduce the time to first appropriate antimicrobial escalation and de-escalation.

GenMark Diagnostics, Inc. ePlex Blood Culture Identification (BCID) [gram-positive (GP), gram-negative (GN) and fungal (FP)] Panels offers rapid identification of broad coverage of bacterial and fungal organisms, and includes antibiotic resistance genes including anaerobes and multidrug resistant organisms (MDRO), as well as common and emerging fungal pathogens, that can lead to sepsis. The ePlex is a qualitative nucleic acid multiplex in vitro diagnostic test used for simultaneous detection and identification of multiple potentially pathogenic bacterial or fungal organisms in a blood culture used to aid in the diagnosis of bloodstream infections when used in conjunction with other clinical information. The ePlex BCID panels are performed directly on blood culture samples identified as positive. "The results are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by the ePlex BCID-GN Panel may not be the definite cause of disease. Additional laboratory testing (e.g. sub-culturing of positive blood cultures for identification of organisms not detected by ePlex BCID-GN Panel and for susceptibility testing, differentiation of mixed growth, and association of antimicrobial resistance marker genes to a specific organism) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection" (FDA, 2019).

Huang et al. (2019) evaluated ePlex BCID panels for the detection of pathogens in bloodstream infections. The authors note that the ePlex (GenMark Diagnostics) blood culture identification (BCID) panels are fully automated PCR-based assays designed to identify Gram-positive and Gram-negative bacteria, fungi, and bacterial resistance genes within 1.5 hours from positive blood culture. The evaluation was performed prospectively on consecutive positive blood cultures (BacT/Alert aerobic SA and anaerobic SN bottles; bioMérieux, Marcy-l'Étoile, France) of adult patients sampled. In total, 216 positive blood culture episodes were evaluable, yielding 263 identification results. The sensitivity/positive predictive value for detection by the ePlex panels of targeted cultured isolates were 97% and 99% for the Gram-positive panel and 99% and 96% for the Gram-negative panel, resulting in overall agreement rates of 96% and 94% for the Gram-positive and Gram-negative panel, respectively. All 26 samples with targeted resistance results were correctly detected by the ePlex panels. The authors add that there are few pathogens currently not targeted by the panels, including possible emerging pathogens of clinical importance with increasing resistance to antimicrobials, questioning the versatility of the system for allowing adaptation of some of the panels. Further studies should be carried out in settings with higher prevalence of MDRO, and clinical indicators (outcome, duration of hospitalization, duration of antimicrobial therapy, etc.) and cost-benefit parameters should also be evaluated in different settings.

Zhang et al. (2020) state that the GenMark Dx ePlex investigational use only blood culture identification fungal pathogen panel (BCID-FP) rapidly detects 15 fungal targets simultaneously in blood culture samples positive for fungi by Gram staining. The authors conducted a multicenter clinical study to evaluate the performance of the BCID-FP. Blood culture samples collected at 10 United States sites and tested with BCID-FP at 4 sites were compared to the standard-of-care microbiological and biochemical techniques, fluorescence in situ hybridization using peptide nucleic acid probes (PNAFISH) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Discrepant results were analyzed by bi-

directional PCR/sequencing of residual blood culture samples. A total of 866 clinical samples, 120 retrospectively and 21 prospectively collected, along with 725 contrived samples were evaluated. Sensitivity and specificity of detection of Candida species (*C. albicans*, *C. auris*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. tropicalis*) ranged from 97.1 to 100% and 99.8 to 100%, respectively. For the other organism targets, sensitivity and specificity were as follows: 100% each for *Cryptococcus neoformans* and *C. gattii*, 98.6% and 100% for *Fusarium* spp., and 96.2% and 99.9% for *Rhodotorula* spp., respectively. In 4 of the 141 clinical samples, the BCID-FP panel correctly identified an additional Candida species, undetected by standard-of-care methods. The authors concluded that the BCID-FP panel offers a faster turnaround time for identification of fungal pathogens in positive blood cultures which may allow for earlier antifungal interventions and includes *C. auris*, a highly multidrug-resistant fungus.

BioFire offers the FilmArray Blood Culture Identification (BICD) Panel which can identify 24 targets, including gram-positive, gram-negative, and yeast pathogens, as well as 3 antimicrobial resistance genes associated with bloodstream infections, and the BICD2 test which identifies 43 targets simultaneously in 1 test with results in about 1 hour, detects pathogens and antimicrobial resistance genes directly from positive blood cultures. The 43 targets associated with bloodstream infections include gram-negative bacteria, gram-positive bacteria, yeast, and 10 antimicrobial resistance genes. The overall sensitivity is reported to be 99% and the specificity 99.8% (BioFire, 2020a).

An UpToDate review on "Detection of bacteremia: Blood cultures and other diagnostic tests" (Doern, 2020) state that "molecular methods such as nucleic acid amplification, matrix-assisted laser desorption ionization-time of flight mass spectrometry, nucleic acid sequencing, and peptide nucleic acid fluorescence in situ hybridization can be used for the rapid identification of organisms recovered from blood culture bottles. In some circumstances, molecular methods can be used for rapid identification directly in blood culture broth, obviating the need for subculture. In addition, molecular methods have the potential for the direct assessment of antimicrobial resistance determinants. Numerous studies have demonstrated the value of these rapid methods in the diagnosis and management of patients with bloodstream infections".

An UpToDate review on "Epidemiology, clinical presentation, and evaluation of parapneumonic effusion and empyema in children" (Janahi and Fakhoury, 2020) state that detection of specific pathogens in pleural fluid through PCR also can be used if the organism is not readily identified by culture or pneumococcal antigen detection. If a broad-range PCR is not available, specific testing should be done for *S. pneumoniae* (pneumococcus) and *S. pyogenes*. However, the degree to which PCR improves yield as compared with conventional culture techniques is not clear, because studies report highly varied sensitivities. Depending on the assay used, PCR results may not provide information about antibiotic resistance in the identified strain of *S. pneumoniae* or *S. aureus*. This is not a concern for group A streptococcus, because isolates are all susceptible to penicillin.

UpToDate on "Diagnostic evaluation of a pleural effusion in adults: Initial testing" (Heffner, 2020) does not mention PCR, NAAT testing on pleural fluid.

Franchetti et al. (2020) state that identification of the pathogens in pleural effusion has mainly relied on conventional bacterial culture or single species polymerase chain reaction (PCR), both with relatively low sensitivity. Thus, the authors investigated the efficacy of a commercially available multiplex bacterial PCR assay developed for pneumonia to identify the pathogens involved in pleural infection, particularly empyema. A prospective, monocentric, observational study including 194 patients with pleural effusion were evaluated based on imaging, laboratory

values, pleura ultrasound and results of thoracentesis including conventional microbiology studies during hospitalization. Multiplex bacterial PCR (Curetis Unyvero p55) was performed in batch and had no influence on therapeutic decisions. Overall, there were 51/197 cases with transudate and 146/197 with exudate. In 42% (n = 90/214) there was a clinical suspicion of parapneumonic effusion and the final clinical diagnosis of empyema was made in 29% (n = 61/214) of all cases. The most common microorganisms identified in the cases diagnosed with empyema were anaerobes followed by gram-positive cocci and gram-negative rods. The multiplex PCR assay identified more of the pathogens on the panel than the conventional methods ( $p = 0.008$ ). The authors concluded that the multiplex PCR-based assay had a higher sensitivity and specificity than conventional microbiology when only the pathogens on the pneumonia panel were taken into account. A dedicated pleural empyema multiplex PCR panel including anaerobes would be needed to cover most common pathogens involved in pleural infection. Amongst its limitations, one has to underline the fact that the molecular diagnostics were performed on frozen material which may have affected the outcome of the analysis. Cushwa and Medrano found differences in DNA extraction from blood, based on length of storage time and storage temperature. Thus, the sensitivity of the method may have been negatively impacted by the storage of the samples. The authors were unable to determine the clinical implications of the commercial multiplex PCR-based assay on the treatment and outcome of the patients. Thus, further studies are necessary to evaluate whether a molecular diagnostic technique can be helpful in optimizing treatment of pleural infections. The authors concluded that most causative organisms were not included in the pneumonia panel. Thus, a dedicated pleural empyema multiplex PCR including anaerobes is required in pleural infection. The multiplex bacterial PCR assay had a higher sensitivity and specificity than conventional microbiology to diagnose bacterial infection in pleural effusion when only pathogens included on the panel were taken into account.

Michos et al. (2016) discuss case reports on the use of FilmArray Blood Culture Identification (BCID) multiplex PCR system for pathogen detection from a child with septic arthritis that *Streptococcus pyogenes* was identified directly from synovial fluid, and a child with complicated pneumonia with pleural effusion that *Streptococcus pneumoniae* was identified from pleural fluid. Case #1 was a 6-year old female with history of sudden onset of fever, rash and a swollen right knee. Blood cultures taken on admission as well as synovial fluid cultures remained negative. The MRI of the right thigh and knee showed myositis with an extensive edema and multiple abscesses among muscular groups. An aliquot of the synovial fluid was tested with the FilmArray Blood Culture Identification System (BioFire Diagnostics, Inc., Salt Lake City, UT). The sample was processed in accordance with the manufacturer's instructions and there was a positive result for *Streptococcus pyogenes*. Antimicrobial treatment was modified and within three days of treatment, her fever declined and she slowly began to improve. The child was discharged after 21 days of treatment and on 1 and 2-month follow-up she was doing very well. Case #2 was a 2-year-old male who presented with a 4-day history of fever, cough and progressive dyspnea for 2 days. The FilmArray BCID test is a multiplexed PCR-based diagnostic test approved for use with positive blood culture material. This assay has been shown to have good sensitivity and specificity in blood samples from adults and children. Gram stain of pleural fluid showed no organisms and blood and pleural fluid cultures were sterile. *Streptococcus pneumoniae* was detected from the pleural effusion by FilmArray BCID, and serotype 3 was identified by polymerase chain reaction. The antimicrobial therapy was modified to teicoplanin and ceftriaxone and was continued for 14 days. On the 5th hospital day, the child became better, ultrasound of the chest showed only residual fluid and chest tubes were removed. The boy was discharged home after 15 days. These cases describe the off-label use of the FilmArray BCID multiplex test to provide diagnostic information for pathogen detection in synovial and pleural fluid. In conclusion, FilmArray BCID test could support the rapid diagnosis of a variety of infectious pathogens, from direct testing of clinical specimens in pediatric

patients, however, that will need to be confirmed in larger prospective studies. Rapid identification of bacterial pathogens could benefit patient care, facilitate better use of antibiotics and antimicrobial stewardship when used timely as part of a routine microbiology service.

UpToDate on "Spontaneous bacterial peritonitis in adults: Diagnosis" (Runyon, 2020) states that after paracentesis for spontaneous bacterial peritonitis (SBP), blood culture bottles are inoculated with ascitic fluid. Culturing ascitic fluid as if it were blood (with immediate bedside inoculation of ascitic fluid into blood culture bottles) has been shown to increase the culture-positivity of the ascitic fluid. In a study of patients with an ascitic fluid PMN count  $\geq 250$  cells/mm<sup>3</sup> (in the absence of prior antibiotic treatment, pancreatitis, tuberculous peritonitis, or malignancy-related ascites), immediate inoculation increased the sensitivity of cultures from about 50 to 77 percent (with delayed inoculation) to 80 to 100 percent. The volume of fluid cultured also has a dramatic impact on the sensitivity of ascitic fluid cultures. In one report, for example, inoculation of 10 or 20 mL of fluid into 100 mL blood culture bottles led to a much higher culture-positivity rate than a 1 mL inoculum (93 versus 53 percent). A diagnosis of SBP is made if the polymorphonuclear cell (PMN, also referred to as neutrophils) count in the ascitic fluid is  $\geq 250$  cells/mm<sup>3</sup>, culture results are positive, and secondary causes of peritonitis are excluded. However, the author does not mention the utility of NAAT, or PCR testing directly from the blood culture bottles containing ascitic fluid.

Yang and colleagues (2019) evaluated the clinical value of bacterial detection using multiplex real-time PCR assays in peritoneal dialysis-associated peritonitis (PDAP). Seventy peritoneal dialysate specimens were collected and traditional bacterial culture and universal primer RT-PCR detection of the bacterial were used. The positive rate of traditional culture method was 65.71% (46/70) and that of universal primer RT-PCR was 81.42% (57/70). For 6 clinical commonly pathogenic bacteria, multiplex, and monoplex RT-PCR all detected 38 positive ones within the 57 specimens that were detected positive by universal primer RT-PCR. The results of the 2 methods were completely identical. Detecting bacteria by universal primer PCR and Monoplex RT-PCR needs 4–5 and 6–9 h, respectively, while multiplex RT-PCR needs less than 3 h. The authors concluded that their results demonstrated that the multiplex RT-PCR can detect several kinds of bacteria simultaneously and it is also more practical and convenient than monoplex RT-PCR. Compared with traditional culture method, RT-PCR assay is more sensitive and rapid.

Bruns et al. (2016) conducted a prospective multicenter study to investigate whether identification of bactDNA from blood or ascitic fluid (AF) by multiplex polymerase chain reaction (PCR) is associated with increased 90-day mortality in 218 patients with cirrhosis and signs of infection. BactDNA in either compartment was detected in 134 (61%) patients, comprising 54 with bactDNA in blood and AF, 48 with AF bactDNA only, and 32 with blood bactDNA only. BactDNA was associated with spontaneous bacterial peritonitis and blood stream infections (SBP/BSI), acute-on-chronic liver failure (ACLF), encephalopathy and markers of inflammation. The prevalence of bactDNA in patients with proven SBP/BSI (36/49; 73%) was similar to that in patients with sterile ACLF (37/52; 71%). Actuarial 90-day survival was 56  $\pm$  5% in the absence of bactDNA in both compartments and did not differ if bactDNA was detected in blood only (63  $\pm$  9%), AF only (63  $\pm$  7%), or in blood and AF (52  $\pm$  7%). Predictors of 90-day mortality were SBP (HR = 3.10; 95% CI: 1.90-5.06), BSI (HR = 4.94; 95% CI: 2.71-9.02), and ACLF (HR = 2.20; 95% CI: 1.44-3.35). The detection of resistance genes in blood or AF in the absence of SBP/BSI (n = 11) was associated with poor 1-year survival (HR = 2.35; 95% CI: 1.03-5.35). The authors concluded that BactDNA in sterile body fluids did not indicate increased mortality in cirrhotic patients with suspected infection. Using multiplex PCR for risk stratification cannot be recommended in these patients.

Hardick et al. (2013) state that traditional diagnostics for spontaneous bacterial peritonitis (SBP) rely on culture techniques for proper diagnosis, although recent reports suggest that the presence of bacterial DNA in peritoneal fluid in patients with cirrhosis and ascites is an indicator of SBP. Nucleic acid amplification tests (NAATs) have come to the forefront of infectious disease diagnostic development due to their higher sensitivity and specificity, culture growth independence, and rapid time to result compared to those of conventional culture methods. NAATs that target the bacterium-specific 16S rRNA gene can offer many advantages. The authors evaluated a previously published broad-range PCR (16S PCR) coupled with high-resolution melt analysis (HRMA) and compared with standard culture techniques for diagnosis of SBP in 106 peritoneal fluid samples from patients with suspected SBP. 16S PCR identified 21/106 (19.8%) ascitic fluid samples as positive for eubacterial DNA, compared to 17 (16%) samples that were positive by culture. The overall sensitivity for detecting the presence of eubacterial DNA in an ascitic fluid sample and the specificity of the 16S PCR compared to that of standard microbiological culture techniques were 100% (17/17) and 91.5% (85/89), respectively. Overall, HRMA concordance with species identification was 70.6% (12/17), although the 5 samples that were discordant at the species level were SBP resulting from a polymicrobial infection, and species-level identification for polymicrobial infections is outside the capability of HRMA. Both the broad-range 16S PCR and HRMA analysis provide useful diagnostic adjunctive assays for clinicians in detecting and identifying pathogens responsible for SBP. One of the current limitations of PCR-HRMA is its inability to resolve polymicrobial infections. Although HRMA did correctly identify all culture-positive samples with polymicrobial infections based on multiple dominant peaks in the derived melting curve, the ability to resolve the curve to identify the individual organisms involved is still lacking. For investigation of suspected cases of polymicrobial infections by HRMA, more costly multiplex sequencing technologies, such as Pyrosequencing, would need to be incorporated to definitively resolve the composition of species involved. Ideally, future studies would include a large, sequential, prospective analysis where ascitic samples, blood counts, and more complete clinical information, including PMN counts, were obtained from the patients. Additionally, future studies would also include a direct comparison with another well-validated molecular method of bacterial DNA detection and identification, as well as standard microbiological culture diagnostics. Overall, 16S PCR coupled with HRMA could prove to be useful diagnostic adjunctive assays when determining suspected cases of SBP, particularly given the short total time for the assays, at 4 h, and the high sensitivity, specificity, and ability to perform species-level identification of bacterial pathogens.

#### Sporotrichosis

Sporotrichosis can be confirmed by obtaining a swab or a biopsy of a freshly opened skin nodule and submits it for fungal culture (Kauffman, 2010). Serologic testing and PCR assay show promise for accurate and specific diagnosis but are available only in research laboratories (AAP, 2009).

#### *Staphylococcus Saprophyticus*

*Staphylococcus saprophyticus* is a coagulase-negative species of *Staphylococcus* that is associated with urinary tract infection in adolescent girls and young adult women, often after sexual intercourse. Persons with urinary tract infections may be treated empirically based upon evidence of infection from dipstick analysis of urine and urine microscopy. Urine culture may be indicated in high-risk persons or in persons who have failed to respond to empiric treatment. The role of PCR testing in *S. saprophyticus* has not been established.

#### Streptococcal Infections (Including Screening for Hemolytic Streptococcus in Pregnancy)

The AAP (2009) stated that presumptive diagnosis of group B Streptococcal infection can be made by identifying gram-positive cocci in body fluids that typically are sterile (such as cerebrospinal, pleural, or joint fluid). Cultures of blood, other typically sterile body fluids, or a suppurative focus are necessary to establish the diagnosis. Serotype identification is available in reference laboratories. The AAP (2009) stated that commercially available real-time PCR tests for group B streptococci in vaginal swab specimens have high sensitivity and specificity, but data are limited regarding their usefulness for women with unknown colonization status at admission for delivery.

Group B beta-hemolytic streptococcus colonizes 20 % of pregnant women. Intrapartum fetal colonization leads to invasive disease in 1 to 2 infants of every 1,000 births in the U.S., and has a mortality of approximately 6 %. Several protocols using intra-partum chemoprophylaxis have been devised to improve management of the disease, but confusion continues about details and implementation. The 2002 CDC's recommendations on the prevention of perinatal Group B streptococcal (GBS) disease recommended that cultured-based screening for vaginal and rectal GBS colonization of all pregnant women at 35 to 37 weeks' gestation. These guidelines further stated that, although a PCR test is under development, "further studies are needed to determine whether this type of test can be adapted for use outside the research setting."

Updated guidelines from the CDC (2010) stated that, despite the availability of PCR and other nucleic acid amplification tests (NAAT) for GBS, "utility of such assays in the intrapartum setting remains limited." The guidelines explain that, although a highly sensitive and specific test with rapid turnaround time could be used to assess intra-partum GBS colonization and therefore obviate the need for antenatal screening, "data on currently available assays do not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status on admission for labor." The CDC guidelines explain that the additional time required for enrichment of samples (which is necessary to increase the sensitivity of NAATs to acceptable levels) makes it not feasible for intrapartum testing, and the sensitivity of assays in the absence of enrichment is not adequate in comparison to culture. In addition, the CDC cited concerns regarding real-world turnaround time, test complexity, availability of testing at all times, staffing requirements, and costs. The CDC guidelines stated that, in settings that can perform NAAT, such tests might prove useful for the limited circumstance of a woman at term with unknown colonization status and no other risk factors. The CDC guidelines explained that even optimal NAAT would have drawbacks in the intra-partum setting, including a delay in administration of antibiotics while waiting for the result, and no anti-microbial susceptibility testing for penicillin-allergic women.

Current guidelines from the American College of Obstetricians and Gynecologists (2002) on prevention of early-onset group B streptococcal disease in newborns have concluded: "Current rapid tests for the detection of GBS colonization at the time of labor or rupture of membranes do not have sufficient sensitivity and specificity to eliminate the need for culture-based prenatal screening."

An assessment of the evidence for PCR testing and other rapid diagnostic tests for GBS colonization reached similar conclusions about the poor quality of available evidence (Honest et al, 2006). The investigators found that the quality of available studies of PCR and other rapid diagnostic testing for GBS was generally poor, and that "[b]efore implementation in practice, a robust technology assessment of their accuracy, acceptability, and cost-effectiveness is required."

The AAP (2012) stated that group A streptococcal pharyngitis can be confirmed by culture on sheep blood agar, and latex agglutination, fluorescent antibody, coagglutination, or precipitation techniques performed on colonies growing on the

agar plate can differentiate group A from other β-hemolytic streptococci. The AAP (2012) noted that several rapid diagnostic tests for group A streptococcal pharyngitis are available using techniques such as optical immunoassay and chemiluminescent DNA probes. The specificities of these tests generally are high, but the reported sensitivities vary considerably. Because of the very high specificity of these rapid tests, a positive test result generally does not require throat culture confirmation. However, because of their variable sensitivity, when a patient suspected on clinical grounds of having group A streptococcal pharyngitis has a negative rapid streptococcal test, a throat culture should be obtained to ensure that the patient does not have group A streptococcal infection. Current published evidence-based guidelines from the Infectious Diseases Society of America make similar recommendations for rapid diagnostic tests and throat culture for diagnosis of group A streptococcal pharyngitis (Shulman, et al., 2012). There is no recommendation for the use of PCR testing in the diagnosis of group A streptococcal pharyngitis.

Guidelines from the Institute for Clinical Systems Improvement (Snellman, et al., 2013) state that a study indicates the utility of a real-time polymerase chain reaction assay as a replacement for both rapid antigen testing and culture. According to the guidelines, this recommendation is based upon low quality evidence, citing a 2003 study by Uhl, et al.. The guidelines note that a polymerase chain reaction (PCR) method requires a minimum of 30 to 60 minutes to perform the test, and in order to be used efficiently, it would require batch testing. The ICSI guidelines state that, when PCR testing is used, a backup plated culture is not necessary.

In order to evaluate the performance of a PCR test for group A streptococcus, Anderson, et al. (2013) collected a total of 796 pharyngeal swabs at three separate clinical centers. Each specimen was analyzed using the illumigene group A strep DNA amplification assay (Meridian Bioscience Inc., Cincinnati, OH). To confirm GAS identification, the results were compared to those from direct and extracted culture methods using Gram staining and a GAS-specific latex agglutination test. Discrepant results were resolved using an alternative PCR test. The prevalence of culture-detected GAS in this study was 12.8% (102/796 specimens). The illumigene assay detected GAS in 74/74 direct culture-positive specimens (100% sensitivity) and 100/102 extracted culture-positive specimens (98.0% sensitivity). GAS was detected by the illumigene assay in an additional 42 specimens that were direct culture negative (94.2% specificity) and 16 specimens that were extracted culture negative (97.7% specificity). Compared to culture, the positive predictive value of the illumigene group A strep DNA amplification assay was 63/8% (54-72) and the negative predictive value was 100% (99-100). Analysis of discrepant results using an alternative PCR assay detected GAS nucleic acid in 13/16 (81.3%) false-positive specimens and 1/2 false-negative specimens. Using the alternative PCR test as the gold standard for discrepant results, the authors reported a final sensitivity of 99.0% and a specificity of 99.6% for the detection of GAS in pharyngeal swabs using the illumigene assay. Limitations of this study included the use of another PCR test as the gold standard for evaluating discrepant results; it is now known whether the alternative PCR assay shares the same limitations as the illumigene DNA amplification assay. It is not known whether the additional specimens detected by DNA amplification were more likely than culture to represent a group A strep carrier state in a patient with a concurrent viral pharyngitis. In addition, unlike culture and similar to rapid detection assays, DNA amplification assays cannot distinguish viable from nonviable organisms. In addition, culture is still needed to detect other causes of pharyngitis.

Unlike most rapid detection tests for group A streptococcal pharyngitis, group A strep DNA amplification assays are not point of care tests and results are not available during the initial office visit. Although DNA amplification assays take 30

minutes to one hour, the total amount of time for results would depend upon the time to transport the specimen to a reference laboratory and the timing of the batch runs during the day.

Cultures or rapid diagnostic tests may be useful in other group A streptococcus infections, such as pyoderma. According to the AAP (2012), cultures of impetiginous lesions are not indicated routinely, because lesions often yield both streptococci and staphylococci, and determination of the primary pathogen may not be possible. In suspected invasive group A streptococcus infections, cultures of blood and focal sites of possible infection are indicated. In necrotizing fasciitis, magnetic resonance imaging can be helpful for confirming the anatomic diagnosis.

According to the AAP (2009), microscopic examination of fluids that ordinarily are sterile can yield presumptive evidence of infections by enterococci and non-group A or group B streptococcus. Diagnosis is established by culture and serogrouping of the isolate, using group specific antisera. Identification of the Enterococcus species may be useful to predict anti-microbial susceptibility. In some circumstances, biochemical testing may be necessary to accurately identify the organism. Anti-microbial susceptibility testing of enterococci isolated from sterile sites is important to determine ampicillin and vancomycin hydrochloride susceptibility as well as gentamicin sulfate susceptibility to assess potential of gentamicin for synergy with ampicillin.

Updated guidelines on management of perinatal group B streptococcal infection from the CDC (Verani et al, 2010) state that, despite the availability of nucleic acid amplification tests (NAAT) such as PCR for group B streptococcal infection, "the utility of such assays in the intrapartum setting remains limited." The guidelines stated: "Although a highly sensitive and specific test with rapid turnaround time could be used to assess intrapartum GBS [group B streptococcal] colonization and therefore obviate the need for antenatal screening, data on currently available assays do not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status on admission for labor."

The CDC guidelines expressed concern about the amount of time for enrichment of samples that is necessary for PCR testing (Verani et al, 2010). The guidelines explained that the additional time required for enrichment of samples makes it not feasible for intra-partum testing, and the sensitivity of assays in the absence of enrichment is not adequate in comparison to culture. The guidelines also noted concerns regarding real-world turnaround time, test complexity, availability of testing at all times, staffing requirements, and costs.

The guidelines stated: "In settings that can perform NAAT, such tests might prove useful for the limited circumstance of a woman at term with unknown colonization status and no other risk factors" (Verani et al, 2010). The guidelines noted, however, that even optimal nucleic acid amplification tests would have drawbacks in the intra-partum setting, including a delay in administration of antibiotics while waiting for the result, and no anti-microbial susceptibility testing for penicillin-allergic women.

The CDC guidelines commented that other rapid tests in addition to PCR and other NAAT have been developed to detect group B streptococcal infection rapidly from non-enriched samples, including optical immunoassays and enzyme immunoassays. The guidelines concluded, however, that none of these rapid tests is sufficiently sensitive when used on a direct specimen to detect group B streptococcal colonization reliably in the intra-partum setting (Verani et al, 2010).

Guidelines from the American Academy of Pediatrics (AAP, 2011) indicate that PCR testing of broth enrichment as an option for antepartum screening for GBS. ACOG guidelines (2011) also mention this option. The AAP guidelines indicated however,

that this use of PCR is a "C" recommendation, meaning that there is "insufficient evidence for efficacy, or efficacy does not outweigh possible adverse consequences."

In June 2019, the American College of Obstetricians and Gynecologists (ACOG) changed the universal screening recommendations for the prevention of group B streptococcal (GBS) early-onset disease in newborns. ACOG now recommends performing universal GBS screening between 36 0/7 and 37 6/7 weeks of gestation. The rationale for the timing change is based on two factors 1) the use of antibiotic prophylaxis is recommended as a default for women with unknown GBS screening test results who give birth before 37 0/7 weeks of gestation and 2) this new recommended timing for screening provides a 5-week window for valid culture results that include births that occur up to the gestational age of at least 41 0/7 weeks. In the United States, 1.9% of women give birth between 35 0/7 and 35 6/7 weeks gestation versus 6.7% who give birth at 41 0/7 weeks of gestation or more. This change is also likely to reduce the reported incidence of discrepant antepartum culture results and colonization status at the time of birth. In clinical situations in which a pregnant woman at term does not give birth within this 5-week screening accuracy window, and whose original GBS screening culture was negative, ACOG states that repeat GBS screening is reasonable and may help guide management beyond 41 0/7 weeks of gestation.

#### Syphilis

Syphilis, a chronic infection with clinical manifestations occurring in distinct stages, is caused by the spirochete *Treponema pallidum*. Diagnostic studies for syphilis include a targeted clinical history and physical examination, serologic tests, investigations of sexual contacts and, if appropriate, darkfield microscopic examination of fluids from lesions, CSF tests, and radiologic examination (CDC, 2002; Beer and Berkow, 1999).

According to the AAP, the non-treponemal antibody tests (VDRL, RPR, and ART) are useful for screening; the treponemal tests (FTA-ABS and MHA-TP) are used to establish a presumptive diagnosis (AAP, 2006). Quantitative non-treponemal antibody tests are used to assess the adequacy of therapy and to detect re-infection and relapse. Most current guidelines indicate no specific role for PCR testing in the screening or diagnosis of syphilis (AAP, 2006; CDC, 2002; AAP, 2009). The Association for Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases (2002) suggested that either the direct fluorescent antibody test or the PCR test may be useful for use for oral or other lesions where contamination with commensal treponemes is likely.

Two classes of serologic tests for syphilis (STS) aid in diagnosing syphilis and other related treponemal diseases:

- Screening, non-treponemal tests using lipoprotein antigens detect syphilitic reagins and include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagent (RPR) tests.
- Specific treponemal tests detect anti-treponemal antibodies and include fluorescent treponemal antibody absorption (FTA-ABS) test, microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP), and *Treponema pallidum* hemagglutination assay (TPHA).

The VDRL test is a flocculation test for syphilis in which reagent antibody in the patient's serum reacts visibly with cardiolipin, the antigen. Reactive and weakly reactive VDRL tests are considered positive for *T. pallidum*, and should be confirmed by one of the more specific treponemal tests, and the reactive tests should be quantitated by serial dilution.

Before treatment (except in infections of less than 1 yr), CSF examination is recommended to exclude neurosyphilis. The cell count and differential and total protein are usually measured, and VDRL or other nonspecific (reagin) serologic tests performed. Treponemal tests of CSF are not helpful.

#### T-Cell Lymphomas: Gene Rearrangements

T-cell receptor gene rearrangement helps distinguish between benign lymphadenopathy and malignant lymphoma. This test is specifically used to detect clonal gene rearrangements in the T-cell receptor beta-chain constant region. The presence of a monoclonal gene rearrangement usually, but not always, reflects the presence of a T-lymphocytic neoplasm while polyclonal gene rearrangement patterns are found in benign reactive conditions.

The evaluation of lymph nodes, bone marrows, and other tissues for the presence of lymphoma usually involves a multiparameter approach. The obligatory first step when evaluating a tissue for suspected lymphoma is to examine the tissue microscopically for morphology. In many cases, morphologic examination is sufficient to establish a diagnosis of malignant lymphoma. There is, however, a significant proportion of cases in which additional studies are needed in order to establish a definitive diagnosis. Those additional studies include immunoperoxidase staining of the tissue sections, flow cytometric evaluation of fresh cells from the specimen, and molecular analysis. Molecular analysis includes such modalities as cytogenetics (including FISH) and PCR. All of these special studies are intended to provide some evidence that can help to distinguish between benign lymphadenopathy and malignant lymphoma. In addition, the special tests can sometimes help to establish both the lineage and the presence of prognostically significant subtypes of malignant lymphoma.

#### Toxoplasma Gondii

According to the CDC, *Toxoplasma gondii* infection is generally asymptomatic, although 20 % of infected persons may develop cervical lymphadenopathy and/or a flu-like illness. However, immunocompromised patients may develop central nervous system (CNS) disease, myocarditis, or pneumonitis. Toxoplasmosis is caused by accidental ingestion of contaminated substances (e.g., soil contaminated with cat feces, on fruits and vegetables, raw or partly cooked meat [especially pork, lamb, or venison]).

Diagnosis may be made by isolating parasites from blood or other body fluids, and observing them in patient specimens via microscopy or histology. Detection of organisms is rare, serology (reference laboratory needed) can be a useful adjunct in diagnosing toxoplasmosis. However, IgM antibodies may persist for 6 to 18 months and thus may not necessarily indicate recent infection.

For congenital infection, diagnosis may be made by isolation of *T. gondii* from placenta, umbilical cord, or infant blood. PCR of white blood cells, CSF or amniotic fluid, or IgM and IgA serology, performed by a reference laboratory.

Asymptomatic healthy, but infected, persons do not require treatment. Spiramycin or pyrimethamine plus sulfadiazine may be used for immunocompromised persons, in specific cases. Pyrimethamine plus sulfadiazine (with or without steroids) may be given for ocular disease when indicated. Folinic acid is given with pyrimethamine plus sulfadiazine to counteract bone marrow suppression.

#### Trichomoniasis

Trichomoniasis is the term for infection of the vagina or male genital tract with *Trichomonas vaginalis* (Beers and Berkow, 1999). *T. vaginalis* is a flagellated protozoan found in the GU tract of both men and women. The organism is more

common in women, affecting about 20 % during the reproductive years and causing vaginitis, urethritis, and possibly cystitis. *T. vaginalis* is more difficult to detect in men; probably causes prostatitis and cystitis; and may account for 5 to 10 % of all cases of male urethritis in some areas. Asymptomatic infected men often infect their sex partners. The infection may co-exist with gonorrhea and other sexually transmitted diseases.

Diagnosis of vaginal trichomoniasis (*T. vaginalis*) is usually performed by microscopy of vaginal secretions, but this method has a sensitivity of only about 60 % to 70 % (CDC, 2002). According to the CDC, culture is the most sensitive commercially available method of diagnosis. The AGM-MSSVD (2002) states that up to 95 % of female cases can be diagnosed by culture; 60 to 80 % of male cases can be diagnosed by urethral culture or culture of first-void urine, and that sampling both simultaneously will significantly increase the diagnostic rate. According to the American Academy of Pediatrics (AAP, 2006), culture of the organism and antibody tests using an enzyme immunoassay and immunofluorescence techniques for demonstration of the organism are more sensitive than wet-mount preparations but generally are not required for diagnosis. A rapid antigen detection assay for Trichomonas and Candidiasis has a sensitivity of 86 % and a specificity of 99 % for *T. vaginalis* (WHO, 1999). A commercially available, rapid, automated hybridization assay is available that uses DNA probes to directly detect Candida, Trichomonas and Gardnerella in vaginal swab samples (WHO, 1999). This assay has a sensitivity of 88 to 91 % and a specificity of 100 % (WHO, 1999) for *T. vaginalis*. PCR assay for trichomonas is somewhat more sensitive (93 %) than antigen detection or hybridization assay for *T. vaginalis* but less sensitive than culture or microscopy (WHO, 1999). In addition, the PCR assay is less specific (96 %) than microscopy, culture, antigen or hybridization assay, with the latter methods having sensitivities of 99 to 100 % (WHO, 1999).

The diagnosis of *T. vaginalis* in women is discussed in the section on vaginal discharge. In women, an immediate diagnosis of trichomoniasis can usually be made by examining vaginal secretions under microscopy (wet mount). The lashing movements of the flagella and striking motility of the oval-shaped organisms are readily observed. Cultures and antibody tests using an enzyme immunoassay and immunofluorescence techniques for demonstration of the organism are more sensitive and specific than direct examination, but according to the AAP, are generally not required for the diagnosis. Trichomoniasis is also commonly diagnosed on a Papanicolaou smear.

*T. vaginalis* may sometimes cause non-gonococcal urethritis in men. According to the CDC (2002), diagnostic procedures for *T. vaginalis* reserved for situations in which these infection is suspected (e.g., contact with trichomoniasis and genital lesions suggestive of genital herpes) or when non-gonococcal urethritis is not responsive to therapy. In men, an immediate diagnosis of *T. vaginalis* can be made by examining a wet mount of urethral secretions and by culture. Examining the centrifuged sediment of urine and prostatic secretions may also be helpful.

The CDC guidelines indicated no role for PCR testing in the diagnosis of *T. vaginalis* (CDC, 2002; CDC, 2006). The AAP (2006) noted that an FDA-licensed PCR test for *T. vaginalis* is not available in the United States but may be available as a research diagnostic test or from commercial laboratories. Other guidelines indicated no role for PCR testing for this indication (ACOG, 2006; BASHH, 2007; AAP, 2009).

Guidelines from the CDC (Workowski et al, 2010) stated that an FDA-cleared PCR assay for detection of gonorrhea and chlamydial infection (Roche Amplicor) has been modified for *T. vaginalis* detection in vaginal or endocervical swabs and in urine from women and men; sensitivity ranges from 88 % to 97 % and specificity from 98 % to 99 %. *T. vaginalis* RNA can also be detected by transcription-mediated amplification (TMA) (Gen-Probe APTIMA). The CDC guidelines stated that,

in men, wet preparation is not a sensitive test. Culture testing of urethral swab, urine, or semen is one diagnostic option; however, PCR or TMA have superior sensitivity for *T. vaginalis* diagnosis in men.

Lee et al (2012) evaluated the usefulness of PCR for diagnosis of *T. vaginalis* infection among male patients with chronic recurrent prostatitis and urethritis. Between June 2001 and December 2003, a total of 33 patients visited the Department of Urology, Hanyang University Guri Hospital and were examined for *T. vaginalis* infection by PCR and culture in TYM medium. For the PCR, these researchers used primers based on a repetitive sequence cloned from *T. vaginalis* (TV-E650). Voided bladder urine (VB1 and VB3) was sampled from 33 men with symptoms of lower urinary tract infection (urethral charge, residual urine sensation, and frequency). Culture failed to detect any *T. vaginalis* infection whereas PCR identified 7 cases of trichomoniasis (21.2 %); 5 of the 7 cases had been diagnosed with prostatitis and 2 with urethritis. PCR for the 5 prostatitis cases yielded a positive 330 bp band from both VB1 and VB3, whereas positive results were only obtained from VB1 for the 2 urethritis patients. The authors concluded that they showed that the PCR method could detect *T. vaginalis* when there was only 1 *T. vaginalis* cell per PCR mixture. They stated that these findings strongly support the usefulness of PCR on urine samples for detecting *T. vaginalis* in chronic prostatitis and urethritis patients.

Muzny and Schwebke (2013) noted that *T. vaginalis* is the most common curable sexually transmitted infection worldwide. *T vaginalis* infections in women can range from asymptomatic to acute inflammatory vaginitis. In men, this infection is typically asymptomatic but is increasingly being recognized as a cause of non-gonococcal urethritis. Diagnosis of *T vaginalis* has traditionally been made by direct microscopic examination of a wet mount of vaginal fluid or through the use of culture. The recent commercial availability of nucleic acid amplification tests for the detection of *T vaginalis* has seen these replace culture as the gold standard for diagnosis. Nitroimidazoles (i.e., metronidazole and tinidazole) are the mainstay of therapy. In the case of treatment failure due to drug resistance or in the case of a severe nitroimidazole allergy, alternative intra-vaginal therapies exist, although their effectiveness has not been evaluated systematically. Novel systemic agents other than nitroimidazoles for the treatment of *T vaginalis* are needed, and efforts to promote and support anti-microbial drug development in this setting are necessary.

Furthermore, an UpToDate review on "Trichomoniasis" (Sobel, 2014) states that "Men -- The most reliable methods for diagnosis of trichomonas urethritis in the male are by culture or a nucleic acid amplification test (i.e., PCR or transcription-mediated amplification [TMA]) of first fraction urine or a urethral swab specimen, but these tests are not widely available. Saline microscopy of a urethral swab specimen has low sensitivity and is not recommended".

#### Trichosporonosis

Trichosporon species are fungal soil inhabitants and common colonizers of human skin and GI tracts. *Trichosporon beigelii* causes the superficial dermatomycosis known as white piedra, a distal infection of the hair shaft. *T beigelii* can also cause onychomycosis, otomycosis, or superficial skin infections. This organism is also associated with summer-type hypersensitivity pneumonitis, a type of hypersensitivity pneumonitis commonly found in Japan.

Trichosporon has been implicated in severe, disseminated infections (trichosporonosis) associated with several immunocompromised states, particularly hematologic malignancies.

No role has been established for PCR for diagnosing trichosporonosis. According to the AAP (2009), trichosporon infection is diagnosed by blood culture and histopathological examination of tissue. The diagnosis of trichosporonosis is usually confirmed by a positive blood culture result obtained in the evaluation of a febrile (usually neutropenic) patient (Hale, 2002). The urine may be the first body fluid to grow Trichosporon in culture in the setting of disseminated disease, and it should not be presumed to be a contaminant or colonizer in the high-risk host (i.e., in the setting of neutropenic fever).

#### Tularemia

Growth of *F. tularensis* in culture is the definitive means of confirming the diagnosis of tularemia (CDC, 2011). Some clinical laboratories can identify presumptively *F. tularensis* in ulcer exudate or aspirate material by direct fluorescent antibody or PCR assays (AAP, 2009; CDC, 2011). Suspect growth on culture may be identified presumptively by direct fluorescent antibody or PCR. The diagnosis of tularemia can also be established serologically by demonstrating a 4-fold change in specific antibody titers between acute and convalescent sera.

#### uBiome (SmartGut, SmartJane, SmartFlu, and Explorer)

In 2016, uBiome launched the SmartGut microbiome test which detects beneficial and pathogenic microorganisms associated with conditions affecting the gut (e.g., constipation, diarrhea, bloating, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis and Crohn's disease, obesity and diabetes). The sequencing-based clinical microbiome screening test determines the relative abundance of microbial groups in an individual's stool. SmartGut results include a microbiome diversity score which compares the individual's microbiome to those from healthy participants. SmartGut includes "unique", healthy microbiome ranges which are supplied from a large microbiome research database. This is a physician-ordered test, for adults aged 18 years and older, that can be administered at home; however, per uBiome, SmartGut is not a diagnostic test (uBiome, 2018).

A SUMSearch2 on "uBiome and SmartGut" did not retrieve any results. A PubMed search found no clinical outcome studies for uBiome SmartGut.

uBiome offers the SmartJane DNA sequencing test which simultaneously genotypes 14 high-risk HPV strains, 5 low-risk HPV strains, 4 common STIs (chlamydia, gonorrhea, syphilis, and mycoplasma genitalium), and microbial risk factors for HPV infection, bacterial vaginosis, cervicitis, idiopathic infertility, pelvic inflammatory disease, bacterial vaginosis, and aerobic vaginitis, via a home test vaginal swab sample. Results include comparison levels of 23 microorganisms in the vaginal microbiome to a healthy reference range. SmartJane is ordered by a healthcare provider; however, the test is not intended to screen for cancer or replace traditional Pap smears or well woman visits (uBiome, 2018).

A SUMSearch2 and PubMed search on "uBiome and SmartJane" did not retrieve any clinical outcome studies.

UpToDate search on "uBiome" and "SmartJane" did not retrieve any results.

uBiome offers the Explorer home-test kit to individuals interested in monitoring their microbiome as often as they choose to see how their microbes change over time. The Explorer allows individuals to swab five sites: gut, mouth, nose, genitals and skin and submit for DNA sequencing analysis. Individuals will receive an evaluation of their flora which provides a microbiome diversity score that compares their microbiomes to healthy participants from a large microbiome research dataset. Individuals can order their own test without a healthcare provider order (uBiome, 2018).

A SUMSearch2 on "uBiome and Explorer" did not retrieve any results. A PubMed search on "ubiome and Explorer" did not retrieve any clinical outcome studies.

uBiome offers the SmartFlu at-home cold and flu screening test which analyzes more than 20 microbiomes from a nasal swab to help identify viruses and bacteria associated with the flu, common cold and upper respiratory bacterial infection. A SUMSearch2 and PubMed search on "SmartFlu" did not retrieve any clinical outcome studies.

#### Ureaplasma Parvum

The AAP (2015) noted that "Several rapid, sensitive real-time polymerase chain reaction assays for detection of *U urealyticum* and *U parvum* have been developed. Many of these assays have greater sensitivity than culture, but they are not widely available outside of reference laboratories".

An UpToDate (Baum, 2016) chapter on mycoplasma hominis and ureaplasma urealyticum infections states that "The options for diagnosing infections caused by *M. hominis* and *Ureaplasma* spp are limited due to the shortcomings of both culture and PCR-based techniques. When available, specimens should be sent for both culture and PCR. Given the difficulty in obtaining a diagnosis, patients are often treated empirically."

Hunjak et al (2014) determined the incidence of *Ureaplasma urealyticum* and *Ureaplasma parvum* (UP) in symptomatic and asymptomatic women of reproductive age and estimated antibiotic susceptibility of ureaplasma isolates. This study included 424 ureaplasma-positive women of 1,370 tested women who visited gynecological practices during 2010. Cervico-vaginal or urethral swab specimens from each patient were obtained for cultivation and molecular typing by RT-PCR. *Ureaplasma* spp. was identified by cultivation in 424 (34.4 %) cases, of which 79.0 % were from women with symptoms and 21.0 % from women without symptoms. Among ureaplasma-positive women, 121 (28.5 %) were pregnant. Genotyping was successful in 244 strains, and the majority of samples were identified as UP (92.6 %). Among genotyped isolates, there were 79.5 % from symptomatic and 20.5 % from asymptomatic women; 29.9 % from pregnant and 70.1 % from non-pregnant women. There was no difference in the incidence of ureaplasma type regarding symptoms. Antibiotic susceptibility of 424 ureaplasma isolates identified by cultivation showed that all strains were susceptible to doxycycline, josamycin, erythromycin, tetracycline, clarithromycin and pristinamycin, but there was lower susceptibility to quinolone antibiotics, i.e., 42.9 and 24.5 % isolates were susceptible to ofloxacin and ciprofloxacin, respectively. The authors concluded that the findings of this study showed that UP was the most frequent isolated ureaplasma species (92.6 %). Regarding antibiotic susceptibility, quinolones are not the best choice for the treatment of ureaplasma infections, while macrolides and tetracyclines are still effective.

An eMedicine review on "Ureaplasma infection" (Waites, October 22, 2015) stated that "Molecular techniques such as the PCR assay are available from research or reference laboratories using published methods or their own internally developed protocols. Molecular techniques such as PCR are not required when culture is available for *M hominis* and *Ureaplasma* species, although it should be acknowledged that PCR assays may be inherently more sensitive for detection of small numbers of organisms in clinical material. Thus far, no PCR assays are approved by the FDA or sold commercially for these organisms. Therefore, the availability of molecular testing is quite limited. Fastidious slow-growing mycoplasmal species, such as *M genitalium* and *M fermentans*, may cause clinically significant illnesses in the respiratory tract, urogenital tract, or other sites. Their presence can be reliably detected only by molecular techniques such as the PCR assay. Seeking molecular techniques for diagnostic purposes is not usually

practical because of the difficulty in their detection and the fact that their role in human disease is not well established. A few research laboratories in the United States are capable of testing for the presence of *M genitalium* and *M fermentans* via PCR". The work-up discusses culturing of both *M hominis* and *U urealyticum*; it does not mention *U parvum*.

Xu and colleagues (2016) discussed the PCR-hybridization assay that they developed for high-throughput simultaneous detection and differentiation of *Ureaplasma urealyticum* and *Ureaplasma parvum* using 1 set of primers and 2 specific DNA probes based on urease gene nucleotide sequence differences. First, *U. urealyticum* and *U. parvum* DNA samples were specifically amplified using 1 set of biotin-labeled primers. Furthermore, amine-modified DNA probes, which can specifically react with *U. urealyticum* or *U. parvum* DNA, were covalently immobilized to a DNA-BIND plate surface. The plate was then incubated with the PCR products to facilitate sequence-specific DNA binding. Horseradish peroxidase-streptavidin conjugation and a colorimetric assay were used. Based on the results, the PCR-hybridization assay developed by these researchers can specifically differentiate *U. urealyticum* and *U. parvum* with high sensitivity (95 %) compared with cultivation (72.5 %). The authors concluded that the findings of this study demonstrates a new method for high-throughput simultaneous differentiation and detection of *U. urealyticum* and *U. parvum* with high sensitivity. They stated that based on these observations, the PCR-hybridization assay developed in this study is ideal for detecting and discriminating *U. urealyticum* and *U. parvum* in clinical applications.

Kyndel et al (2016) determined if *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* are more common in pre-menopausal women with urethral pain syndrome than in asymptomatic controls. These investigator used a case-control study design to compare the prevalence of *M. genitalium*, *U. urealyticum*, and *U. parvum* using PCR analysis in urine. Urethral pain syndrome was defined as localized urethral pain with or without accompanying lower urinary tract symptoms (LUTS) during the past month or longer and at least 1 negative urine culture.

Among the 28 cases, 46 % carried *Ureaplasma* species compared with 64 % of the 92 controls overall ( $p = 0.09$ ). There were no significant differences in the prevalence of *U. parvum* and *U. urealyticum* among controls than in patients with urethral pain syndrome ( $p = 0.35$  and  $p = 0.33$ , respectively). Co-colonization with *U. parvum* and *urealyticum* was infrequent, and there was only 1 case of *M. genitalium* colonization, which occurred among the controls. The symptomatic profile of *Ureaplasma* carriers with urethral pain syndrome was heterogeneous with no clear pattern and did not differ significantly compared with patients negative for *Ureaplasma*. The authors concluded that they found no evidence to support the notion that *M. genitalium*, *U. parvum*, and *U. urealyticum* are more prevalent in women with urethral pain syndrome than in women without LUTS.

Moi et al (2016) stated that a non-syndromic approach to treatment of people with non-gonococcal urethritis (NGU) requires identification of pathogens and understanding of the role of those pathogens in causing disease. The most commonly detected and isolated micro-organisms in the male urethral tract are bacteria belonging to the family of Mycoplasmataceae, in particular *Ureaplasma urealyticum* and *Ureaplasma parvum*. To better understand the role of these *Ureaplasma* species in NGU, these researchers performed a prospective analysis of male patients voluntarily attending a drop in sexually transmitted infections (STI) clinic in Oslo. Of 362 male patients who were tested for NGU using microscopy of urethral smears, these investigators found the following sexually transmissible micro-organisms: 16 % *Chlamydia trachomatis*, 5 % *Mycoplasma genitalium*, 14 % *U. urealyticum*, 14 % *U. parvum* and 5 % *Mycoplasma hominis*. They found a high concordance in detecting in turn *U. urealyticum* and *U. parvum* using 16s rRNA gene and *ureD* gene as targets for nucleic acid amplification testing (NAAT). While there was a strong association between microscopic signs of NGU and C.

trachomatis infection, association of M. genitalium and U. urealyticum infections in turn were found only in patients with severe NGU (greater than 30 polymorphonuclear leucocytes, PMNL/high powered fields, HPF). U. parvum was found to colonize a high percentage of patients with no or mild signs of NGU (0 to 9 PMNL/HPF). The authors concluded that urethral inflammatory response to ureaplasmas is less severe than to C. trachomatis and M. genitalium in most patients and that testing and treatment of ureaplasma-positive patients should only be considered when other STIs have been ruled out.

Kasprzykowska et al (2014) stated that genital ureaplasmas are considered opportunistic pathogens of human genitourinary tract involved in adverse pregnancy sequelae and infertility. While association of Ureaplasma urealyticum with urogenital tract infections is well-established, the role of Ureaplasma parvum in these infections is still insufficient. In this preliminary study, these researchers compared how often cervico-vaginal colonization with U. parvum is associated with the presence of these microorganisms in the upper genitourinary tract of fertile and infertile women. They used PCR assay to determine the prevalence of U. parvum and U. urealyticum in pairs of specimens, i.e., vaginal swabs and Douglas' pouch fluid samples from consecutive 40 women with no symptoms of genital tract infection. In total, 19 (47.5 %) of the 40 samples were positive for ureaplasmas. U. parvum was simultaneously detected in pairs of samples in 5 (55.5 %) of the 9 (47.4 %) women positive in PCR assay. As many as 5 (18.5 %) of the 27 infertile women and 1 (7.7 %) of the 13 fertile women showed infection of the upper genital tract with U. parvum. The authors concluded that the findings of this preliminary study demonstrated that colonization of the lower genital tract with U. parvum can produce asymptomatic infection of the upper reproductive system in women.

These findings also implied that U. parvum may be present in the upper genital tract at the time of conception and might be involved in adverse pregnancy outcomes.

Marovt et al (2015) stated that there is mounting evidence stating that Ureaplasma urealyticum causes non-gonococcal urethritis in males, whereas Ureaplasma parvum does not seem to be of clinical significance. However, the clinical role of U. parvum and U. urealyticum in lower urogenital tract infections in females remains unclear. These researchers determined the frequency of U. parvum and U. urealyticum among 145 Ureaplasma spp. culture-positive women with symptoms of lower urogenital tract infection ( $n = 75$ ) and those without ( $n = 70$ ), and ascertained possible associations between the detection of U. parvum and U. urealyticum with selected characteristics. Endo-cervical, urethral, and vaginal swabs, and first voided urine were obtained. Polymerase chain reaction (PCR) was performed to differentiate ureaplasmas. No significant association between the detection of U. parvum or U. urealyticum and symptom status was found. Significantly more women aged 25 years and younger were infected with U. urealyticum (23.4 %) compared to those aged above 25 years (9.2 %) [odds ratio (OR) 3.0 (1.1; 8.1);  $p = 0.03$ ] and significantly less women aged 25 years and younger (83.5 %) were infected with U. parvum compared to those aged above 25 years (95.5 %) [OR 0.2 (0.1; 0.9);  $p = 0.03$ ]. The detection of Chlamydia trachomatis was significantly associated to both U. parvum and U. urealyticum ( $p = 0.021$ ), and to U. parvum alone with borderline significance ( $p = 0.063$ ). The authors concluded that although neither U. parvum nor U. urealyticum seem to cause symptoms in females, their role in the female urogenital tract remains unknown, taking into account their ubiquity, possible augmentation of the urogenital microenvironment, and ascending capability to the sterile upper reproductive tract.

#### Urinary Tract Infection

Wojno, et al. (2020) reported on a retrospective record review of 582 consecutive elderly patients presenting with symptoms of lower urinary tract infection (UTI) was conducted. All patients had traditional urine cultures and PCR molecular testing run

in parallel. A total of 582 patients (mean age 77; range 60-95) with symptoms of lower UTI had both urine cultures and diagnostic PCR between March and July 2018. PCR detected uropathogens in 326 patients (56%, 326/582), while urine culture detected pathogens in 217 patients (37%, 217/582). PCR and culture agreed in 74% of cases (431/582): both were positive in 34% of cases (196/582) and both were negative in 40% of cases (235/582). However, PCR and culture disagreed in 26% of cases (151/582): PCR was positive while culture was negative in 22% of cases (130/582), and culture was positive while PCR was negative in 4% of cases (21/582). Polymicrobial infections were reported in 175 patients (30%, 175/582), with PCR reporting 166 and culture reporting 39. Further, polymicrobial infections were identified in 67 patients (12%, 67/582) in which culture results were negative. Agreement between PCR and urine culture for positive cultures was 90%, exceeding the noninferiority threshold of 85% (95% conflict of interest 85.7%-93.6%). The investigators concluded that multiplex PCR is noninferior to urine culture for detection and identification of bacteria. The investigators stated that further investigation may show that the accuracy and speed of PCR to diagnose UTI can significantly improve patient outcomes.

An accompanying editorial (Werneburg, 2020) observed that, in 22% of cases, multiplex-PCR identified organisms where standard culture was negative. The editorialist noted that bacteria uniquely detected by PCR in this study included a number of genera that were also detected in the urinary tract of healthy patients without urinary tract infection in another report. Such findings highlight the importance of further investigation into the urinary microbiome as well as the uropathogenic capacity of noncultureable and other poorly-studied bacteria, and whether their presence in urine should guide therapy

Vollstedt, et al. (2020) collected urine specimens from 3,124 patients with symptoms of urinary tract infection. Of these, multiplex polymerase chain reaction testing detected bacteria in 61.1% (1910) of specimens. Pooled Antibiotic Susceptibility Testing results were available for 70.8% (1352) of these positive specimens. Of these positive specimens, 43.9% (594) were monomicrobial, while 56.1% (758) were polymicrobial. The odds of resistance to ampicillin ( $p = 0.005$ ), amoxicillin/clavulanate ( $p = 0.008$ ), five different cephalosporins, vancomycin ( $p = <0.0001$ ), and tetracycline ( $p = 0.010$ ) increased with each additional species present in a polymicrobial specimen. In contrast, the odds of resistance to piperacillin/tazobactam decreased by 75% for each additional species present (95% CI 0.61, 0.94,  $p = 0.010$ ). For one or more antibiotics tested, thirteen pairs of bacterial species exhibited statistically significant interactions compared with the expected resistance rate obtained with the Highest Single Agent Principle and Union Principle.

Daly, et al. (2020) reported on a retrospective study of existing data from 66,383 patients seen for possible urinary tract infection by house-call primary care providers. Patients were divided into two cohorts. One cohort of patients was treated based upon the results from standard urine cultures (SUC). The other cohort was treated in accordance with results from an assay combining Multiplex-Polymerase Chain Reaction (M-PCR) and Pooled Antibiotic Susceptibility Testing (P-AST) of urine specimens. The total number of emergency department visits and hospitalizations were compared between the two cohorts.

The investigators found that the use of the combined M-PCR/P-AST was associated with a 13.7% decrease in hospital admissions and/or emergency department utilization when compared to the use of SUC testing (3.27% vs. 3.79%;  $p = 0.003$ ). The investigators concluded that these findings suggest that use of a combined M-PCR/P-AST assay in outpatient management of suspected urinary tract infection may improve patient outcomes and reduce emergency department and hospital

utilization. The investigators noted that randomized studies are underway to investigate further the role that M-PCR/P-AST may aid in the management of UTI in the elderly population.

The National Institute for Health and Care Excellence's guideline on "Urinary tract infections in adults" (NICE, 2015) did not mention PCR testing.

The Urogenital Pathogen Test (UPX) (Lab Genomics, LLC) is a real-time PCR test that allows for simultaneous analyses of 18 urinary pathogens, along with 19 antibiotic resistance targets, with final results reported within 48 to 72 hours. The developer reports that the test has an overall 97% sensitivity and 99% specificity.

The Urogenital Pathogen with Rx Panel (UPX) identifies infectious agents in a urine sample via nucleic acid (DNA or RNA) multiplex amplified probe technique. Analyses for genitourinary pathogens include identification of 21 bacterial and fungal organisms and identification of 21 associated antibiotic-resistance genes.

The Qclear UTI panel (Lifescan Labs, Thermo Fisher Scientific) identifies genitourinary pathogens from a urine sample via nucleic acid (DNA or RNA) semiquantitative identification. DNA from 16 bacterial organisms and 1 fugal organism is analyzed with the multiplex amplified probe technique via quantitative PCR.

The Qclear UTI - Reflex ABR test (Lifescan Labs, Thermo Fisher Scientific) identifies genitourinary pathogens and antibiotic-resistance gene detection from a urine sample via multiplex amplified probe technique. The results are reported as an antimicrobial stewardship risk score.

#### Vaginal Discharge

The 3 diseases most frequently associated with vaginal discharge are trichomoniasis (caused by *T. vaginalis*), bacterial vaginosis (caused by a replacement of the normal vaginal flora by an overgrowth of anaerobic microorganisms, mycoplasmas, and *Gardnerella vaginalis*), and candidiasis (usually caused by *Candida albicans*). *C. trachomatis* or *N. gonorrhoeae* can sometimes cause vaginal discharge, but can be distinguished in that the vaginal discharge is accompanied by a mucopurulent cervicitis.

Current Centers for Disease Control and Prevention Guidelines on management of diseases characterized by vaginal discharge (CDC, 2002) do not indicate any role for PCR tests in the assessment of vaginal discharge unless the sexually transmitted diseases *C. trachomatis* or *N. gonorrhoeae* are suspected based on history of sexual activity and presence of mucopurulent cervicitis. Otherwise, the cause of vaginal infection can be diagnosed by pH and microscopic examination of the discharge.

#### Varicella Zoster Virus

Primary varicella zoster virus (VZV) infection results in chickenpox, manifested by a generalized, pruritic, vesicular rash and mild fever and systemic symptoms (AAP, 2009). Most cases of varicella in the United States occur in children younger than 10 years of age. Immunity generally is lifelong. Immunocompromised persons with primary (varicella) or recurrent (zoster) infection are at increased risk of severe disease.

The virus establishes latency in the dorsal root ganglia during primary infection. Re-activation results in herpes zoster ("shingles"), which are grouped vesicular lesions appearing in a dermatomal distribution, sometimes accompanied by pain localized to the area. Zoster occasionally can become disseminated in immunocompromised patients, with lesions appearing outside the primary dermatomes and with visceral complications.

Varicella virus can be isolated from scrapings of vesicle base during the first 3 to 4 days of the eruption but rarely from other sites, including respiratory tract secretions. A significant increase in serum varicella IgG antibody by any standard serologic assay can retrospectively confirm a diagnosis. According to the AAP (2009), these antibody tests are reliable for determining immune status in healthy hosts after natural infection but are not necessarily reliable in immunocompromised. Many commercially available tests are not sufficiently sensitive to demonstrate a vaccine-induced antibody response.

According to the AAP (2009), rapid diagnostic tests (PCR, direct fluorescent antibody) are the methods of choice of diagnosing varicella virus infection. Varicella virus infection can be diagnosed using PCR testing of body fluid or tissue. The advantages of PCR testing over other methods is that it is very sensitive and can distinguish wild-type strains from vaccine virus.

Varicella and zoster may be treated with intravenous or oral acyclovir, valacyclovir, famciclovir, and foscarnet. The decision to use therapy and the duration and route of therapy should be determined by specific host factors, extent of infection, and initial response to therapy. Oral acyclovir is not recommended for routine use in otherwise healthy children with varicella, because it results in only a modest reduction in symptoms. Oral acyclovir should be considered for otherwise healthy persons at increased risk of moderate-to-severe varicella. Intravenous therapy is recommended for immunocompromised patients.

In 2012 the American Academy of Pediatrics Red Book recommendations stated that vesicular fluid or a scab can be used to identify VZV using a PCR test and to distinguish between wild-type and vaccine-strain VZV, which may especially be desirable and informative in immunized children who develop herpes zoster. The Red Book recommendations specify PCR assay currently is the diagnostic method of choice. Viral culture and DFA assay are less sensitive than PCR assay and do not distinguish vaccine strain from wild-type viruses (Red Book Online, 2012).

#### Whipple's Disease

Whipple's disease (WD) is a syndrome with a wide variety of clinical manifestations (Pitcher and Fry, 2000). Blood cultures of affected patients are negative. The diagnosis of WD is usually made by upper endoscopy showing whitish or yellowish plaques distributed on a friable mucosa (Mandell, 2000). The diagnosis usually can be established on histopathological examination of tissue obtained from biopsy of the small bowel if characteristic PAS-positive material is present in the lesions. However, electron microscopy or PCR to detect Whipple bacilli (*Tropheryma whippelii*, formerly *T. whippelii*) is now recommended in every newly identified patient, and is mandatory in doubtful cases. Thus, a PCR should now be routinely performed on small bowel tissue and additionally (in patients with long-standing systemic involvement or with suspected CNS manifestation) on CSF. Biopsies from abdominal or peripheral lymph nodes or from other organs may yield diagnostic material as well.

A number of antibiotic regimens have been used for the treatment of Whipple's disease with variable success. In addition to specific antibiotic therapy, various symptomatic treatments and supportive measures may be required, depending upon the clinical manifestations of the disease.

#### Whooping Cough (Pertussis)

Pertussis begins with mild upper respiratory tract symptoms similar to the common cold (catarrhal stage) and progresses to cough and then usually to paroxysms of cough (paroxysmal stage) characterized by inspiratory whoop and commonly

followed by vomiting. Pertussis is caused by a fastidious, gram-negative, pleomorphic bacillus, *Bordetella pertussis*. *Bordatella parapertussis* is another cause of prolonged cough illness.

Culture still is considered the "gold standard" for laboratory diagnosis of pertussis (CDC, 2017). Polymerase chain reaction is being used increasingly for detection of *B. pertussis* because of its improved sensitivity and more rapid result (AAP, 2009). However, the PCR test lacks sensitivity in previously immunized people, and unacceptably high rates of false-positive results are reported from some laboratories. The AAP (2009) stated that no FDA-licensed PCR test is available, and there are no widely accepted standardized protocols, reagents, or reporting formats. The sensitivity of direct fluorescent antibody is low and is not recommended for laboratory confirmation of pertussis.

According to the Centers for Disease Control and Prevention (CDC) (2017), "disease presentation can vary with age and history of previous exposure or vaccination. Young infants may present to a clinic or hospital with apnea and no other disease symptoms. Adults and adolescents with some immunity may exhibit only mild symptoms or have the typical prolonged paroxysmal cough. In all persons, cough can continue for months."

Clinical case definition for pertussis (CDC, 2017):

In the absence of a more likely diagnosis, a cough illness lasting greater than or equal to 2 weeks with *at least one* of the following signs or symptoms:

- Paroxysms of coughing; or
- Inspiratory "whoop"; or
- Posttussive vomiting; or
- Apnea (with or without cyanosis) (for infants less than 1 year of age only).

Probable case classification:

- Meets the clinical case definition, is not lab confirmed, and is not epidemiologically linked to a lab-confirmed case; or
- For infants less than 1 year of age only: acute cough illness of any duration with at least one of the following:
  - Paroxysms of coughing; or
  - Inspiratory "whoop"; or
  - Posttussive vomiting; or
  - Apnea (with or without cyanosis); or
  - PCR positive or contact with a lab-confirmed case of pertussis.

Confirmed:

- Acute cough illness of any duration with isolation of *B. pertussis* from a clinical specimen; or
- Meets the clinical case definition AND is PCR positive for pertussis; or
- Meets the clinical case definition AND had contact with a laboratory-confirmed case of pertussis. To confirm a case by epidemiologic linkage, the case must be directly linked (i.e., by a first-generation contact) to a laboratory-confirmed case by either culture or PCR.

Laboratory criteria for diagnosis (CDC, 2017):

- Isolation of *B. pertussis* from a clinical specimen
- Positive PCR assay for pertussis

The timing of PCR testing for pertussis can significantly impact the accuracy of the diagnosis. CDC (2017) states that PCR has optimal sensitivity during the first 3 weeks of coughing symptoms, as bacterial DNA is still present in the nasopharynx. After the fourth week of pertussis symptoms, the amount of bacterial DNA rapidly diminishes, increasing the risk of obtaining false-negative results.

"While PCR is increasingly used as the sole diagnostic test for pertussis, CDC recommends that PCR be used in conjunction with culture when feasible, rather than as an alternative test. Even when a laboratory has validated its PCR method, culturing for *B. pertussis* should continue; this is especially important to confirm the circulation of *B. pertussis* when an outbreak is suspected (CDC, 2017).

PCR testing is also recommended for individuals with confirmed exposure and who have a cough illness occurring less than 21 days. For persons with cough greater than or equal to 21 days, the CDC does not recommend laboratory testing since PCR and culture are only sensitive during the first 2 to 3 weeks when bacterial DNA is still present in the nasopharynx (CDC, 2017).

#### Wound Infection

Gentili et al (2012) stated that the impact of poly-microbial bacterial infection on chronic wounds has been studied extensively, but standard bacteriological analysis is not always sensitive enough. Molecular approaches represent a promising alternative to the standard bacteriological analysis. This work aimed to assess the usefulness of a panbacterial quantitative real-time PCR reaction to quantitate the total bacterial load in chronic wounds treated with Cutimed™ Sorbact™, a novel therapeutic approach based on hydrophobic binding of bacteria to a membrane. The results obtained by panbacterial real-time PCR on conserved sequences of the bacterial 16S gene showed that the bacterial burden significantly decreased in 10 out of 15 healing chronic wounds, and did not change in 5 out of 5 non-healing chronic wounds. On the contrary, classical culture for *S. aureus* and *P. aeruginosa*, and real-time PCR for *bacteroides* and *fusobacterium* did not show any correlation with the clinical outcome. This study also showed that quantification of chronic wounds by panbacterial real-time PCR is to be performed on biopsies and not on swabs. The authors concluded that these results showed that panbacterial real-time PCR is a promising and quick method of determining the total bacterial load in chronic wounds, and suggested that it might be an important biomarker for the prognosis of chronic wounds under treatment.

Also, and UpToDate review on "Clinical assessment of wounds" (Armstrong and Meyr, 2014) does not mention the use of PCR testing as a management tool.

The Lesion Infection (Wound) (Lab Genomics LLC, Thermo Fisher Scientific) panel is a multiplex PCR test that analyzes surgical wound pathogens (34 microorganisms and identification of 21 associated antibiotic-resistance genes) obtained from a swab that is detected by nucleic acid (DNA and RNA) process.

#### Miscellaneous Indications

An UpToDate review on "Polymerase chain reaction" (Klanderman, 2012) does not mention the use of PCR for listeria, salmonella, and vibrio species; and an UpToDate review on "Infection with less common *Campylobacter* species and related bacteria" (Skirrow, 2012) does not mention the use of PCR.

An UpToDate review on "Epidemiology and prevention and control of vancomycin-resistant enterococci" (Anderson, 2012) states that "PCR techniques for identification of vanA and vanB genes from stool/rectal samples have been developed but their impact on surveillance for VRE has yet to be determined". Furthermore, the AAP Committee on Infectious Diseases has no recommendation for use of PCR testing for enterococci (2009). There is some mention by the CDC about

PCR being an option for detecting clonal spread in a hospital. However, that would be for hospitalized patients on a hospital claim; and there is no recommendation for PCR for standard clinical management of outpatients.

Xenotropic murine leukemia virus-related virus (XMRV) is a gamma-retrovirus that was first described in 2006. Initial reports linked the virus to prostate cancer, and later to chronic fatigue syndrome (CFS), but these were followed by a large number of studies in which no association was found. It has not been established that XMRV can infect humans, nor has it been demonstrated that XMRV is associated with or causes human disease. Numerous researchers have suggested that XMRV detection may result from contamination of clinical specimens and laboratory reagents with mouse retroviruses or related nucleic acids.

Pakneshan et al (2013) states that BRAF represents one of the most frequently mutated protein kinase genes in human tumors. The mutation is commonly tested in pathology practice. BRAF mutation is seen in melanoma, papillary thyroid carcinoma (including papillary thyroid carcinoma arising from ovarian teratoma), ovarian serous tumors, colorectal carcinoma, gliomas, hepato-biliary carcinomas and hairy-cell leukemia (HCL). In these cancers, various genetic aberrations of the BRAF proto-oncogene, such as different point mutations and chromosomal rearrangements, have been reported. The most common mutation, BRAF V600E, can be detected by DNA sequencing and immunohistochemistry on formalin fixed, paraffin embedded tumor tissue. Detection of BRAF V600E mutation has the potential for clinical use as a diagnostic and prognostic marker. In addition, a great deal of research effort has been spent in strategies inhibiting its activity. Indeed, recent clinical trials involving BRAF selective inhibitors exhibited promising response rates in metastatic melanoma patients. Clinical trials are underway for other cancers. However, cutaneous side effects of treatment have been reported and therapeutic response to cancer is short-lived due to the emergence of several resistance mechanisms. In this review, the authors gave an update on the clinical pathological relevance of BRAF mutation in cancer. It is hoped that the review will enhance the direction of future research and assist in more effective use of the knowledge of BRAF mutation in clinical practice.

An UpToDate review on "Clinical features and diagnosis of hairy cell leukemia" (Tallman and Aster, 2013) states that "The pathogenesis of HCL is largely unknown. However, studies indicate that most cases are associated with a V600E activating mutation in the serine/threonine kinase BRAF (an isoform of RAF), implicating BRAF signaling in HCL. Response to BRAF inhibitor therapy has been described in a patient with refractory HCL, in line with the idea that oncogenic BRAF signaling enhances HCL proliferation and survival .... While numerous genetic abnormalities have been described, none has been incorporated into the diagnostic criteria for HCL yet. As described above, initial studies suggest that the vast majority of cases demonstrate BRAF mutations. Further study is needed to define the sensitivity and specificity of these mutations for HCL before testing for BRAF mutations becomes a routine part of diagnosis .... It also appears that HCL-v lacks BRAF mutations, though further study is needed to confirm this finding".

An UpToDate review on "Treatment of enterococcal infections" (Murray, 2013) does not mention the use of PCR.

Wei et al (2013) noted that Hantaan viruses cause 2 severe diseases lacking efficient treatment, yet no effective prophylactic vaccines are available. Continued exploration of alternative anti-viral agents to treat hantavirus-related syndromes remains compulsory. The fluorescence-based quantitative real-time PCR (qPCR) has become the touchstone for target gene quantification. In the present study, standard curves for Hantaan virus (HTNV), mouse, and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were generated by serial 10-fold dilutions of the constructed recombinant plasmid pGEM-T/HTNV, pGEM-T/mouse-GAPDH, and

pGEM-T/human-GAPDH, respectively. Comparisons between the indirect immunofluorescence assay and qPCR assay in the detection of HTNV-infected Vero E6 cells showed improved detection limit and sensitivity of latter method. To characterize the inhibitory effect of several conventional antivirals (arbidol and ribavirin) and unconventional anti-virals (indomethacin and curcumin) on HTNV, the levels of viral RNAs were measured for 4 days post-treatment of HTNV-infected Vero E6 cells and 18 days post-inoculation of HTNV-infected suckling mice. The authors noted that these findings validated that HTNV was sensitive to ribavirin and arbidol treatment, while indomethacin and curcumin may also be therapeutically effective in treating HTNV infection. They concluded that the establishment and application of qPCR may be a useful tool for the evaluation of potential anti-virals for Hantaan virus infection in-vitro and in-vivo.

Mohamed et al (2013) described the design and evaluation of a rapid and robust quantitative real-time PCR (QRT-PCR) assay able to detect a wide range of hantaviruses. Primers with the potential to detect different hantaviruses were designed from conserved regions of different hantavirus L segments, as identified from multiple sequence alignments. By using SYBR-green-based QRT-PCR 100-1000 target molecules of in-vitro produced RNA and less than 100 copies of hantavirus RNA from different hantavirus clades and regions of the world were detected. When using the assay on clinical samples from patients with acute HFRS, Puumala hantavirus (PUUV) RNA was confirmed in all previously positive samples. Notably, the broad reacting L-segment QRT-PCR also detected viral RNA in HFRS patient samples, previously negative by a QRT-PCR targeting the S segment of PUUV. The authors concluded that this novel assay provides a powerful tool for diagnosis of hantaviruses from different clades and regions and may also be useful in surveys with the purpose of finding new hantaviruses in rodent or insectivore species.

UpToDate reviews on "Clinical features and diagnosis of chronic fatigue syndrome" (Gluckman, 2013), "Clinical manifestations and pathogenesis of human parvovirus B19 infection" (Jordan, 2013), and "Epidemiology and diagnosis of hantavirus infections" (Hjelle, 2013) do not mention the use of quantitative PCR.

An UpToDate review on "Polymerase chain reaction" (Raby, 2013) does not mention the use of PCR for giardia lamblia, vibrio cholera and yersinia enterocolitica.

An UpToDate review on "Epidemiology, clinical manifestations, and diagnosis of giardiasis" (Leder and Weller, 2013) states that "Research tools include serology, culture and polymerase chain reaction (PCR) techniques. Serologic tests are not of value in the diagnosis of acute giardiasis. IgG and IgM antibodies persist after infection so may be useful in epidemiologic studies. Studies utilizing quantitative PCR are facilitating increased understanding of the relationship between infection and clinical disease. In one study using PCR to detect Giardia in stool samples, parasite concentrations as low as 10 parasites/100 microL could be detected. This study also showed 100 percent correlation between PCR, microscopy and ELISA in patients with symptoms suggestive of giardiasis. PCR could also be a valuable tool for screening of water supplies".

An UpToDate review on "Infection with less common Campylobacter species and related bacteria" (Allos, 2013) states that "Studies using PCR assays identified the organism more frequently in the stool samples of children recently diagnosed with Crohn's than in samples from controls. *Campylobacter concisus* is a part of oral flora; it may contribute to the development of periodontal disease. Additionally, the organism has been identified in a brain abscess in a patient with chronic sinusitis". There is no mentioning of vibrio cholerae and yersinia enterocolitica.

The American College of Medical Genetics and Genomics' practice guideline on "MTHFR polymorphism testing" (Hickey et al, 2013) states that "MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous thromboembolism. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia".

Wikipedia states that "In a recent study, samples of lesions on the skin, eyes, and lung from 182 patients with presumed herpes simplex or herpes zoster were tested with quantitative PCR or with viral culture. In this comparison, viral culture detected VZV with only a 14.3 % sensitivity, although the test was highly specific (specificity = 100 %). By comparison, quantitative PCR resulted in 100 % sensitivity and specificity. Overall testing for herpes simplex and herpes zoster using PCR showed a 60.4 % improvement over viral culture.

Furthermore, the CDC's Manual for the Surveillance of Vaccine-Preventable Diseases notes that real-time PCR [also known as quantitative PCR] has been designed that distinguishes vaccine strain from wild-type [varicella]; results rapidly available (within 3 hours). (Last updated April 1, 2014).

#### Acanthamoeba in Corneal Ulceration

In a retrospective, cross-sectional study, Ikeda et al (2012) examined the diagnostic value of real-time PCR for detecting Acanthamoeba in eyes diagnosed with Acanthamoeba keratitis (AK) by conventional tests. These researchers also determined the pre-operative prognosis-determining factors in eyes with AK. A total of 104 eyes of 103 patients who were diagnosed with AK or with bacterial or bacteria-associated keratitis (BK) by conventional tests were included in this study. A total of 29 eyes with AK and 75 eyes with BK were evaluated for Acanthamoeba and bacterial DNA by real-time PCR. The Acanthamoeba copy numbers, bacterial load, and clinical parameters in the patients with AK were assessed for those significantly associated with poor outcome, that is, final visual acuity (VA) of less than 20/50 or requiring keratoplasty, by logistic regression analysis. Main outcome measures were Acanthamoeba DNA copy number, bacterial DNA copy number, and odds ratio (OR) for poor prognosis. The detection of amoebic DNA was 50 times more sensitive by real-time PCR than by conventional cyst counting. The Acanthamoeba copy numbers at the 1st visit (mean of  $4.7 \times 10^5 \pm 3.2 \times 10^5$  copies) were significantly correlated with the AK stage, and both were significant risk factors for a poor outcome. The Acanthamoeba DNA copy numbers at the 1st visit and AK stage had a significantly high risk for poor outcome (OR of Acanthamoeba DNA copy per logarithm of copy numbers: 3.48, 95 % confidence interval [CI]: 1.04 to 111.63,  $p < 0.05$ ; OR of AK stage: 2.8 per stage increase, 95 % CI: 1.07 to 7.30,  $p < 0.05$ , after adjustment of age). In the AK cases with poor outcome, the amoebic DNA was not reduced by more than 90 % after 1 month of treatment. The weak amoebic reduction was significantly associated with advanced AK stages or previous use of steroids. Bacterial 16S rDNA was detected in 53.6 % of the eyes with AK, but it was not associated with any risk for refractoriness. The authors concluded that real-time PCR was effective in detecting and managing AK. The Acanthamoeba copy number as well as AK stage at the 1st visit were significantly associated with poor outcome.

Goh et al (2018) noted that AK is an uncommon but serious corneal infection, in which delayed diagnosis carries a poor prognosis. Conventional culture requires a long incubation period and has low sensitivity; PCR and in-vivo confocal microscopy

(IVCM) are available alternative diagnostic modalities that have increasing clinical utility. These researchers compared confocal microscopy, PCR, and corneal scrape culture in the early diagnosis of AK. They reviewed the case notes of patients with a differential diagnosis of AK between June 2016 and February 2017 at the Bristol Eye Hospital, United Kingdom. Clinical features at presentation, and results of IVCM, PCR, and corneal scrape cultures were analyzed. A total of 25 case records were reviewed; AK was diagnosed in 14 patients (15 eyes). Based on the definition of "definite AK", the diagnostic sensitivities of IVCM, PCR, and corneal scrape cultures were 100 % [95 % CI: 63.1 % to 100 %], 71.4 % (95 % CI: 41.9 % to 91.6 %) and 33.3 % (95 % CI: 9.9 % to 65.1 %), respectively. The 3 methods showed a specificity of 100 % and a positive predictive value (PPV) of 100 %. Using a reference standard of only positive corneal cultures, IVCM, and PCR had a sensitivity of 100 % (95 % CI: 29.2 % to 100 %) and 75 % (95 % CI: 19.4 % to 99.4 %), respectively. The authors concluded that all 3 diagnostic tests were highly specific, and a positive test result was highly predictive of disease presence. IVCM was both highly sensitive and specific when performed by an experienced operator; PCR was a useful adjunct in the diagnosis of AK because of its wider availability compared with IVCM, and it may be used in combination with IVCM for microbiologic confirmation.

Megha et al (2020) stated that AK is a blinding condition reported from both developed and developing countries. Limited knowledge on the clinical characteristics of AK and scarce laboratory diagnostic facilities in such countries poses difficulties in the accurate diagnosis. These researchers described the epidemiological and clinical characteristics as well as management of AK in a tertiary care hospital in North India. All clinically suspicious cases of AK presenting to the authors' center were screened for Acanthamoeba. All patients diagnosed as Acanthamoeba on microscopic examination, culture and PCR were given Polyhexamethylene biguanide (PHMB) eye drops 0.02 % half hourly for 1 week, then hourly for 1 week and then gradually tapered according to the response. Out of 300 consecutive patients evaluated, Acanthamoeba was detected in 11 (3.6 %) patients. A history of trauma was elicited in majority of the patients, 6 (55 %). The most common complaints were eye pain, redness and watering in all of the patients, diminution of vision (8, 72.7 %), photophobia (7, 63.6 %) and foreign body sensation (2, 18.2 %). Complete healing with vascularization and scarring was observed in 7 patients (63.6 %) patients whereas progression to perforation of corneal ulcer and corneal melt was observed in 3 (27.3 %) cases and these patients underwent therapeutic keratoplasty later; 1 patient did not come for follow-up examination. The authors concluded that the most common risk factor for the occurrence of AK is trauma followed by contact lens use.

Furthermore, an UpToDate review on "Complications of contact lenses" (Soong et al, 2021) states that "The diagnosis of infectious keratitis is confirmed by slit lamp examination that demonstrates a corneal epithelial defect with an underlying stromal infiltrate, with or without hypopyon. Occasionally, infectious keratitis with large, fulminant ulcers may be visible without magnification. Most contact lens-associated infectious keratitis is bacterial, but certain features suggest fungal or protozoal (e.g., Acanthamoeba) pathogens ... Obtaining corneal cultures is usually not necessary for initial treatment of small peripheral ulcers. However, cultures should be obtained if the infection is particularly severe (central infiltrate, large infiltrate > 2 mm, associated with stromal melting, or multifocal), if it does not respond to initial empiric antibacterial therapy, if the patient has a history of corneal surgery, or if a nonbacterial infectious keratitis is suspected ... Specimens should be submitted for Gram stain and bacterial and fungal cultures; special media are required when nontuberculous mycobacterial or amoebic infections are suspected. Immunofluorescent stains may be helpful, but cultures are the top priority when there is limited sample. Culture and Gram stain can be important in microbial identification of unusual bacteria, drug-resistant bacteria (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA]), or nonbacterial organisms such as fungi

and Acanthamoeba. Chronic or atypical corneal infections may eventually require deeper lamellar corneal excisional biopsies for identification. Other specialized testing (e.g., confocal microscopy or polymerase chain reaction assays) may be warranted for nonbacterial infections”.

#### Bordetella Holmesii for Diagnosis of Whooping Cough

An UpToDate review on “Pertussis infection in adolescents and adults: Clinical manifestations and diagnosis” (Cornia and Lipsky, 2021) states that “PCR is used for diagnosis of pertussis in most cases reported to the National Notifiable Diseases Surveillance System in the United States; culture and direct fluorescent antibody testing were the main diagnostic tests employed in the early 1990s. PCR testing is more sensitive than culture as it may detect small numbers of viable and nonviable organisms; specificity is high. Primers from four chromosomal regions are employed: repeated insertion sequences (such as IS481, which is also present in lesser quantities in *Bordetella holmesii* and *Bordetella bronchiseptica*), the pertussis toxin gene (PT), the PT promoter region, and the adenylate cyclase gene (not specific to *B. pertussis*)”.

#### Cutibacterium Acnes (formerly Proionibacterium Acnes) for Diagnosis of Corneal Ulcers

An UpToDate review on “Corneal abrasions and corneal foreign bodies: Clinical manifestations and diagnosis” (Jacobs, 2021) does not mention PCR testing of *Cutibacterium acnes* (formerly *proionibacterium acnes*) as a diagnostic tool.

#### Differential Diagnosis of Onychauxis versus Onychogryphosis

An UpToDate review on “Overview of nail disorders” (Rich, 2021) does not mention PCR as a management option.

#### Drainage from Otitis Externa

In a retrospective, case review, Gruber et al (2015) described a subset of necrotizing otitis externa (NOE) patients with a refractory disease and negative cultures. In these cases, these researchers decided to use a polymerase chain reaction (PCR) assay from surgically obtained tissue under sterile conditions to improve pathogen detection sensitivity. A total of 19 consecutive patients diagnosed with NOE between January 2008 and January 2014 were included in this trial; 3 patients of this cohort presented a culture-negative disease. Main outcome measures were positive detection of pathogens using a PCR assay in cases with a complicated course of NOE and clinical resolution of the disease after targeted therapy according to PCR results. Surgical samples were obtained under sterile conditions from 3 patients with negative cultures and a refractory disease course of NOE. PCR assays were performed using pan-bacteria and pan-fungi protocols. In all 3 samples, a positive result for a fungal pathogen was recorded and followed by successful empirical targeted therapy. The authors concluded that patients who presented with a refractory culture-negative NOE should be suspected as suffering from a fungal disease. The PCR assay may be an important laboratory adjunct in detecting pathogens responsible for NOE and can aid to promote therapy and disease resolution.

Furthermore, UpToDate reviews on “External otitis: Pathogenesis, clinical features, and diagnosis” (Goguen, 2021), “Evaluation of otorrhea (ear discharge) in children” (Strother and Sadow, 2021) and “Evaluation of earache in children” (Greenes, 2021) do not mention PCR testing as a management tool.

In a prospective, observational study, Louie et al (2008) tested a multiplex real-time polymerase chain reaction (rt-PCR) method for simultaneous detection of multiple organisms in bloodstream infections. A total of 200 adult (greater than 18 years) patients from the emergency room (ER), intensive care units (ICUs), and general medicine wards at risk of a bloodstream infection and who manifested signs of systemic inflammatory response syndrome (SIRS). Whole blood samples for PCR testing were collected at the same time as blood culture (BC); PCR results were compared to blood and other culture results. PCR detected potentially significant bacteria and fungi in 45 cases compared to 37 by BC; PCR detected the methicillin resistance (*mecA*) gene in all 3 culture-confirmed methicillin-resistant *Staphylococcus aureus* cases. More than 68 % of PCR results were confirmed by blood, urine, and catheter culture. Independent clinical arbitrators could not rule out the potential clinical significance of organism(s) detected by PCR, but not by BC. PCR did not detect *Enterococcus faecalis* in 5 BC-confirmed cases. On average, 7 patient samples could be tested simultaneously with the PCR method in 6.54 +/- .27 hours. The authors concluded that multiplex PCR detected potentially significant bacteria and fungi that were not found by BC. BC found organisms that were not detected by PCR. These researchers stated that despite limitations of both BC and PCR methods, PCR could serve as an adjunct to current culture methods to facilitate early detection of bloodstream infections. Early detection of microorganisms has the potential to facilitate evidence-based treatment decisions, anti-microbial selection, and adequacy of anti-microbial therapy.

#### Parvovirus for Mast Cell Activation Syndrome

The American Academy of Allergy, Asthma & Immunology's webpage on "Mast Cell Activation Syndrome (MCAS)" (2021) states that " Since symptoms of anaphylaxis can be similar to symptoms caused by other conditions that do not involve mast cells, diagnostic criteria assure that mast cell activation is responsible for the episode. These criteria require the presence of anaphylactic symptoms, the elevation of mast cell mediators during symptoms and the resolution of symptoms with appropriate treatment(s). Once these criteria are met, further testing should rule out primary clonal mast cell disorders that can also cause these symptoms. The patient's blood should be tested for mutation of mast cell growth receptor KIT, called KIT D816V. If positive, it indicates a clonal mast cell disorder. A negative blood test for KIT D816V is helpful but not 100 % accurate, so one of several scoring systems should be used, to follow symptoms and lab results to determine if the presentation is consistent with a clonal mast cell disorder. If so, a bone marrow biopsy and aspirate is indicated. The biopsy offers a high level of ability (sensitivity) to find KIT D816V mutation and allows examining bone marrow mast cells for their shape and abnormal cell surface markers. If the bone marrow biopsy is negative for abnormal and clonal mast cells, it establishes the diagnosis of idiopathic mast cell activation syndrome". [Mast Cell Activation Syndrome \(MCAS\)](https://www.aaaai.org/conditions-and-treatments/related-conditions/mcas) (<https://www.aaaai.org/conditions-and-treatments/related-conditions/mcas>)

Furthermore, UpToDate reviews on "Mast cell disorders: An overview" (Akin, 2021), and "Mastocytosis (cutaneous and systemic): Evaluation and diagnosis in adults" (Castells and Akin , 2021) do not mention testing for parvovirus as a management option.

#### PCR Panels in Podiatry for Detecting Multiple Bacteria and Fungi and Antibiotic Resistance Testing

The majority of the available evidence centers on the use of PCR assays for detecting antibiotic resistance in *Mycobacterium tuberculosis*.

Chang et al (2010) stated that multidrug-resistant tuberculosis has emerged as a global health threat. Given poor treatment outcomes of fluoroquinolone-resistant multidrug-resistant tuberculosis, there is a pressing need for rapid drug susceptibility testing of multidrug-resistant *Mycobacterium tuberculosis* against

fluoroquinolones. This review aimed at evaluating these rapid assays. PubMed and OvidSP were used to search Medline and Embase for publications in English regarding rapid assays that tested ofloxacin, levofloxacin or moxifloxacin. Studies were included only in the concurrent presence of sensitivity and specificity data. Summary estimates of sensitivity and specificity were generated by the bi-variate random effects model when there were at least 3 sets of data under the same assay category that tested the same fluoroquinolone with reference to a standard test. Of 108 articles identified, 24 articles were included in a meta-analysis of rapid assays that tested ofloxacin in culture isolates. Overall, rapid genotypic assays targeting gyrA only were significantly less specific (96 % versus 99 %) and non-significantly less sensitive (88 % versus 94 %) than rapid phenotypic assays. To test for the presence or absence of ofloxacin resistance to a certainty threshold of 90 %, the required pre-test prevalence ranges of ofloxacin resistance for genotypic assays targeting gyrA only were 29 % to 47 % overall, 36 % to 55 % for PCR-DNA sequencing, and 23 % to 44 % for others. Corresponding ranges were 7 % to 65 % for phenotypic assays overall and 3 % to 75 % for mycobacteria growth indicator tube (MGIT). The authors concluded that assuming that the mean pre-test prevalence of fluoroquinolone resistance in culture isolates of multidrug-resistant M. tuberculosis was approximately 20 %, rapid genotypic assays other than PCR-DNA sequencing, targeting gyrA only, can reliably screen for ofloxacin resistance.

Xu et al (2010) noted that the reference standard methods for drug susceptibility testing of *Mycobacterium tuberculosis*, such as culture on Lowenstein-Jensen or Middlebrook 7H10/11 medium, are very slow to give results; and due to the emergence of multidrug-resistant *M. tuberculosis* and extensively drug-resistant *M. tuberculosis*, there is an urgent demand for new, rapid, and accurate drug susceptibility testing methods. PCR-single-strand conformational polymorphism (PCR-SSCP) analysis has been proposed as a rapid method for the detection of resistance to rifampin, but its accuracy has not been systematically evaluated. These investigators performed a systematic review and meta-analysis to evaluate the accuracy of PCR-SSCP analysis for the detection of rifampin-resistant tuberculosis. They searched the Medline, Embase, Web of Science, BIOSIS, and LILACS databases and contacted authors if additional information was required. A total of 10 studies met inclusion criteria for rifampin resistance detection. These researchers applied the summary receiver operating characteristic (SROC) curve to perform the meta-analysis and to summarize diagnostic accuracy. The sensitivity of PCR-SSCP analysis for the rapid detection of rifampin-resistant tuberculosis was 0.79 (95 % confidence interval [CI]: 0.75 to 0.82), the specificity was 0.96 (95 % CI: 0.94 to 0.98), the positive likelihood ratio was 16.10 (95 % CI: 5.87 to 44.13), the negative likelihood ratio was 0.20 (95 % CI: 0.10 to 0.40), and the diagnostic odds ratio (DOR) was 100.93 (95 % CI: 31.95 to 318.83). The authors concluded that PCR-SSCP analysis is a sensitive and specific test for the rapid detection of rifampin-resistant *M. tuberculosis*. Moreover, they stated that additional studies in countries with a high prevalence of multidrug-resistant *M. tuberculosis* and also cost-effectiveness analysis are needed to obtain a complete picture on the utility of this method for rapid drug resistance detection in *M. tuberculosis*.

Flandrois et al (2014) noted that tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*. It remains a major health threat, killing over 1 million people every year worldwide. An early antibiotic therapy is the basis of the treatment, and the emergence and spread of multi-drug and extensively drug-resistant mutant strains raise significant challenges. As these bacteria grow very slowly, drug resistance mutations are currently detected using molecular biology techniques. Resistance mutations are identified by sequencing the resistance-linked genes followed by a comparison with the literature data. The only online database is the TB Drug Resistance Mutation database (TBDReAM database); however, it requires mutation detection before use, and its interrogation is complex due to its loose syntax and grammar. The MUBII-TB-DB database is a simple, highly structured text-based database that contains a set of *Mycobacterium tuberculosis*

mutations (DNA and proteins) occurring at 7 loci: rpoB, pncA, katG; mabA(fabG1)-inhA, gyrA, gyrB, and rrs. Resistance mutation data were extracted after the systematic review of Medline referenced publications before March 2013. MUBII analyzed the query sequence obtained by PCR-sequencing using 2 parallel strategies: a BLAST search against a set of previously reconstructed mutated sequences, and the alignment of the query sequences (DNA and its protein translation) with the wild-type sequences. The post-treatment includes the extraction of the aligned sequences together with their descriptors (position and nature of mutations). The whole procedure is performed using the internet. The results were graphs (alignments) and text (description of the mutation, therapeutic significance). The system is quick and easy to use, even for technicians without bioinformatics training. The authors concluded that MUBII-TB-DB is a structured database of the mutations occurring at 7 loci of major therapeutic value in tuberculosis management. Moreover, the system provides interpretation of the mutations in biological and therapeutic terms and can evolve by the addition of newly described mutations. Its goal is to provide easy and comprehensive access through a client-server model over the Web to an up-to-date database of mutations that lead to the resistance of *M. tuberculosis* to antibiotics.

Theron et al (2014) noted that accurate and rapid tests for tuberculosis (TB) drug resistance are critical for improving patient care and decreasing the transmission of drug-resistant TB. Genotype MTBDRsl (MTBDRsl) is the only commercially-available molecular test for detecting resistance in TB to the fluoroquinolones (FQs; ofloxacin, moxifloxacin and levofloxacin) and the 2nd-line injectable drugs (SLIDs; amikacin, kanamycin and capreomycin), which are used to treat patients with multidrug-resistant (MDR)-TB. In a Cochrane review, these investigators obtained summary estimates of the diagnostic accuracy of MTBDRsl for FQ resistance, SLID resistance and extensively drug-resistant TB (XDR-TB; defined as MDR-TB plus resistance to a FQ and a SLID) when performed indirectly (i.e., on culture isolates confirmed as TB positive), and directly (i.e., on smear-positive sputum specimens). To compare summary estimates of the diagnostic accuracy of MTBDRsl for FQ resistance, SLID resistance and XDR-TB by type of testing (indirect versus direct testing). The populations of interest were adults with drug-susceptible TB or drug-resistant TB. The settings of interest were intermediate and central laboratories. These investigators searched the following databases without any language restriction up to January 30, 2014: Cochrane Infectious Diseases Group Specialized Register; Medline; Embase; ISI Web of Knowledge; MEDION; LILACS; BIOSIS; SCOPUS; the metaRegister of Controlled Trials; the search portal of the World Health Organization (WHO) International Clinical Trials Registry Platform; and ProQuest Dissertations & Theses A&I. They included all studies that determined MTBDRsl accuracy against a defined reference standard (culture-based drug susceptibility testing (DST), genetic testing or both). They also included cross-sectional and diagnostic case-control studies; and excluded unpublished data and conference proceedings. For each study, 2 review authors independently extracted data using a standardized form and assessed study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. These researchers performed meta-analyses to estimate the pooled sensitivity and specificity of MTBDRsl for FQ resistance, SLID resistance, and XDR-TB. They explored the influence of different reference standards, and performed the majority of analyses using a bivariate random-effects model against culture-based DST as the reference standard.

These researchers included 21 unique studies: 14 studies reported the accuracy of MTBDRsl when done directly, 5 studies when done indirectly and 2 studies that did both. Of the 21 studies, 15 studies (71 %) were cross-sectional and 11 studies (58 %) were located in low-income or middle-income countries. All studies but 2 were written in English; 9 (43 %) of the 21 included studies had a high risk of bias for patient selection. At least half of the studies had low risk of bias for the other QUADAS-2 domains. As a test for FQ resistance measured against culture-based DST, the pooled sensitivity of MTBDRsl when performed indirectly was 83.1 % (95 %

CI: 78.7 % to 86.7 %) and the pooled specificity was 97.7 % (95 % CI: 94.3 % to 99.1 %), respectively (16 studies, 1,766 participants; 610 confirmed cases of FQ-resistant TB; moderate quality evidence). When performed directly, the pooled sensitivity was 85.1 % (95 % CI: 71.9 % to 92.7 %) and the pooled specificity was 98.2 % (95 % CI: 96.8 % to 99.0 %), respectively (7 studies, 1,033 participants; 230 confirmed cases of FQ-resistant TB; moderate quality evidence). For indirect testing for FQ resistance, 4 (0.2 %) of 1,766 MTBDRsl results were indeterminate, whereas for direct testing 20 (1.9 %) of 1,033 were MTBDRsl indeterminate ( $p < 0.001$ ). As a test for SLID resistance measured against culture-based DST, the pooled sensitivity of MTBDRsl when performed indirectly was 76.9 % (95 % CI: 61.1 % to 87.6 %) and the pooled specificity was 99.5 % (95 % CI: 97.1 % to 99.9 %), respectively (14 studies, 1,637 participants; 414 confirmed cases of SLID-resistant TB; moderate quality evidence). For amikacin resistance, the pooled sensitivity and specificity were 87.9 % (95 % CI: 82.1 % to 92.0 %) and 99.5 % (95 % CI: 97.5 % to 99.9 %), respectively. For kanamycin resistance, the pooled sensitivity and specificity were 66.9 % (95 % CI: 44.1 % to 83.8 %) and 98.6 % (95 % CI: 96.1 % to 99.5 %), respectively. For capreomycin resistance, the pooled sensitivity and specificity were 79.5 % (95 % CI: 58.3 % to 91.4 %) and 95.8 % (95 % CI: 93.4 % to 97.3 %), respectively. When performed directly, the pooled sensitivity for SLID resistance was 94.4 % (95 % CI: 25.2 % to 99.9 %) and the pooled specificity was 98.2 % (95 % CI: 88.9 % to 99.7 %), respectively (6 studies, 947 participants; 207 confirmed cases of SLID-resistant TB, 740 SLID susceptible cases of TB; very low quality evidence). For indirect testing for SLID resistance, 3 (0.4 %) of 774 MTBDRsl results were indeterminate, whereas for direct testing 53 (6.1 %) of 873 were MTBDRsl indeterminate ( $p < 0.001$ ). As a test for XDR-TB measured against culture-based DST, the pooled sensitivity of MTBDRsl when performed indirectly was 70.9 % (95 % CI: 42.9 % to 88.8 %) and the pooled specificity was 98.8 % (95 % CI: 96.1 % to 99.6 %), respectively (8 studies, 880 participants; 173 confirmed cases of XDR-TB; low quality evidence).

The authors concluded that in adults with TB, a positive MTBDRsl result for FQ resistance, SLID resistance, or XDR-TB can be treated with confidence. However, MTBDRsl did not detect approximately 1 in 5 cases of FQ-resistant TB; and did not detect approximately 1 in 4 cases of SLID-resistant TB. Of the 3 SLIDs, MTBDRsl has the poorest sensitivity for kanamycin resistance. MTBDRsl will miss between 1 in 4 and 1 in 3 cases of XDR-TB. The diagnostic accuracy of MTBDRsl was similar when done using either culture isolates or smear-positive sputum. As the location of the resistance causing mutations can vary on a strain-by-strain basis, further research is needed on test accuracy in different settings and, if genetic sequencing is used as a reference standard, it should examine all resistance-determining regions. These researchers stated that given the confidence one can have in a positive result, and the ability of the test to provide results within a matter of days, MTBDRsl may be used as an initial test for 2nd-line drug resistance. However, when the test reported a negative result, clinicians may still wish to carry out conventional testing.

Mao and colleagues (2015) stated that the genotype MTBDRsl is a new-generation PCR-based line-probe assay for rapid identification of the resistance to the 2nd-line anti-tuberculosis drugs with a single strip. In a meta-analysis, these researchers evaluated the performance of Genotype MTBDRsl in detecting drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol in comparison with the phenotypic drug susceptibility test. They searched PubMed, Embase and the Cochrane Library and calculated the sensitivity, the specificity, the positive likelihood ratio (PLR), negative likelihood ratio (NLR), DOR, corresponding 95 % CI, and the SROC area under the curves (AUC), and tested heterogeneity in accuracy estimates with the Spearman correlation coefficient and Chi-square. The summarized sensitivity (95 % CI), specificity (95 % CI), and AUC (standard error) were 0.869 (0.847 to 0.890), 0.973 (0.965 to 0.979) and 0.9690 (0.0188) for fluoroquinolones, 0.868 (0.829 to 0.900), 0.998 (0.994 to 0.999) and 0.9944

(0.0050) for amikacin, 0.879 (0.838 to 0.914), 0.970 (0.958 to 0.978) and 0.9791 (0.0120) for capreomycin, 0.501 (0.461 to 0.541), 0.991 (0.983 to 0.996) and 0.9814 (0.0114) for kanamycin and 0.686 (0.663 to 0.709), 0.871 (0.852 to 0.888) and 0.7349 (0.0639) for ethambutol, respectively. The authors concluded that the genotype MTBDRsl demonstrated excellent accuracy for detecting drug resistance to fluoroquinolones, amikacin, and capreomycin, but it may not be an appropriate choice for detection of kanamycin and ethambutol.

Brossier et al (2016) stated that detecting resistance to fluoroquinolones (FQ) and 2nd-line injectable drugs (amikacin [AMK], kanamycin [KAN], and capreomycin [CAP]) is crucial given the worldwide increase in the incidence of extensively drug-resistant tuberculosis (XDR-TB). A new version of the GenoType MTBDRsl test (v2.0) has been developed to improve the detection of resistance to FQ (involving gyrA and gyrB mutations) and to 2nd-line injectable drugs (involving rrs and eis promoter mutations) in *Mycobacterium tuberculosis*. A collection of 127 multidrug-resistant (MDR) *M. tuberculosis* complex strains was tested using the 1st (v1) and 2nd (v2.0) versions of the MTBDRsl test, as well as DNA sequencing. The specificities in resistance detection of v1 and v2.0 were similar throughout, whereas the levels of sensitivity of v2.0 were superior for FQ (94.8 % versus 89.6 %) and KAN (90.5 % versus 59.5 %) but similar for AMK (91.3 %) and CAP (83.0 %). The sensitivity and specificity of v2.0 were superior to those of v1 for the detection of pre-XDR strains (83.3 % versus 75.0 % and 88.6 % versus 67.1 %, respectively), whereas the sensitivity of v2.0 was superior to that of v1 only for the detection of XDR strains (83.0 % versus 49.1 %). The authors concluded that MTBDRsl v2.0 is superior to MTBDRsl v1 and efficiently detects the most common mutations involved in resistance to FQ and aminoglycosides/CAP. However, due to mutations not recognized by v2.0 or to the presence of resistance mechanisms not yet characterized (particularly mechanisms related to mono-resistance to aminoglycosides or CAP), the results for wild-type strains obtained with MTBDRsl v2.0 should be confirmed by further DNA sequencing and phenotypic drug susceptibility testing.

These investigators stated that the performance of molecular tests in the detection of resistance has markedly improved in recent years, and substantial further improvements are unlikely to be made in the future. These tests, with their inherent complexity as a drawback, are now facing the “glass ceiling” of the ability of existing drug susceptibility testing (DST) methods, the generally accepted gold standards, to properly classify strains as susceptible or resistant. Indeed, some mutations implicated in low levels of resistance that have an impact on patient outcome cannot be detected adequately with phenotypic methods.

Shahmohammadi and co-workers (2016) evaluated the prevalence and antibiotic susceptibility patterns among MRSA and methicillin-sensitive *S. aureus* (MSSA) isolates collected from 4 hospitals in Iran. A total of 183 isolates of *S. aureus* were collected from various clinical specimens of 4 hospitals in Iran. The isolates were identified by using the conventional biochemical tests; 3 methods:

- oxacillin agar disk diffusion,
- oxacillin agar screening, and
- PCR were applied to determine susceptibility to oxacillin.

The conventional disk agar diffusion test was used to evaluate the antibiotic sensitivity of our isolates against 15 antibiotics, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Of 183 isolates, 77 isolates (42.1 %) were found to be MRSA, by the PCR method. The highest antibiotic resistance was found to be against penicillin, co-trimoxazole, erythromycin, and tetracycline respectively. All isolates were susceptible to vancomycin, according to the results of disk agar diffusion. Among other antibiotics, teicoplanin (84 %) and fusidic acid (80.5 %) were more active against MRSA isolates. For the different methods

evaluated, the sensitivities and specificities were as follows: for disk agar diffusion (84.9 % and 95.9 %) and for agar screening test with oxacillin concentrations of 0.6 µg/ml (70.8 % and 97.4 %), 4 µg/ml (96.1 % and 97.2 %) and 6 µg/ml (96 % and 96.3 %), respectively. The authors concluded that the results of this study showed that 47 % of *S. aureus* isolates were MRSA. Overall, in this research study, resistance to all test anti-microbial agents in MRSA isolates were higher than that of MSSA isolates. They stated that these findings also revealed that 85 % of mecA-positive isolates and 15 % of mecA-negative isolates were resistant to methicillin; while 96 % of mecA-negative isolates were sensitive to methicillin. Meanwhile 4 % of mecA-positive isolates were also sensitive to methicillin.

Gardee et al (2017) stated that early detection of resistance to 2nd-line anti-tuberculosis drugs is important for the management of multidrug-resistant tuberculosis (MDR-TB). The GenoType MTBDRsl version 2.0 (VER 2.0) line probe assay has been re-designed for molecular detection of resistance-conferring mutations of fluoroquinolones (FLQ) (*gyrA* and *gyrB* genes) and 2nd-line injectable drugs (SLID) (*rrs* and *eis* genes). This study evaluated the diagnostic performance of the GenoType MTBDRsl VER 2.0 assay for the detection of 2nd-line drug resistance compared with phenotypic DST, using the Bactec MGIT 960 system on *Mycobacterium tuberculosis* complex isolates from South Africa. A total of 268 repository isolates collected between 2012 and 2014, which were rifampin mono-resistant or MDR based on DST, were selected. MTBDRsl VER 2.0 testing was performed on these isolates and the results analyzed. The MTBDRsl VER 2.0 sensitivity and specificity indices for culture isolates were the following: FLQ, 100 % (95 % CI: 95.8 to 100 %) and 98.9 % (95 % CI, 96.1 to 99.9 %); SLID, 89.2% (95 % CI: 79.1 to 95.6 %) and 98.5 % (95 % CI: 95.7 to 99.7 %). The sensitivity and specificity observed for individual SLID were the following: amikacin, 93.8 % (95 % CI: 79.2 to 99.2 %) and 98.5 % (95 % CI: 95.5 to 99.7 %); kanamycin, 89.2 % (95 % CI: 79.1 to 95.6 %) and 98.5 % (95 % CI: 95.5 to 99.7 %); and capreomycin, 86.2 % (95 % CI: 68.3 to 96.1 %) and 95.9 % (95 % CI: 92.2 to 98.2 %). An inter-operator reproducibility of 100 % and an overall inter-laboratory performance of 93 % to 96 % were found. The authors concluded that the overall improvement in sensitivity and specificity with excellent reproducibility made the GenoType MTBDRsl VER 2.0 a highly suitable tool for rapid screening of clinical isolates for 2nd-line drug resistance for use in high-burden TB/HIV settings.

These investigators stated that "In a press release by the WHO in May 2016, the use of the GenoType MTBDRsl VER 2.0 assay as "an initial test, instead of phenotypic culture-based DST" to detect FLQ and SLID resistance in confirmed RIF-R and MDR patients was recommended. Appropriately trained laboratory staff, quality assurance, and availability of laboratory infrastructure are requisite recommendations of the WHO to implement use of this assay. Our study provides support and evidence for these recommendations and the implementation of the assay in South Africa".

An UpToDate review on "Overview of antibacterial susceptibility testing" (Turbett and Pierce, 2021) states that "Genotypic Methods -- Although phenotypic methods remain the cornerstone of antimicrobial susceptibility testing in clinical microbiology laboratories, molecular assays that test for specific resistance genes have been increasingly incorporated into routine use. Techniques commonly used to detect bacterial nucleic acid sequences conferring antibiotic resistance include polymerase chain reaction (PCR) and DNA hybridization. A number of such genotypic assays have been cleared by the United States Food and Drug Administration (FDA), in some cases as screening tools for the identification of multidrug-resistant bacteria in hospital settings, and in others for diagnostic purposes".

Cox et al (2015) stated that *Gardnerella vaginalis* is a Gram-variable anaerobic bacterium present in 100 % of women with BV, which is a complex polymicrobial condition with no single causative agent. The current laboratory detection method for BV relies on a Gram-stain Nugent score to estimate the quantity of different bacterial morphotypes in the vaginal micro flora. While the Nugent score can distinguish between women with and without BV, a significant proportion are categorized as intermediate, which fails to differentiate a normal from an abnormal vaginal micro flora. A singleplex *G. vaginalis* TaqMan real-time quantitative PCR (qPCR) assay was developed and compared with the "gold standard" Nugent score. Detection and quantification of *G. vaginalis* was performed on vaginal specimens with positive, negative and intermediate Nugent scores. The *G. vaginalis* qPCR assay demonstrated high analytical specificity against a broad microbial panel and analytical sensitivity down to  $3.1 \times 10(4)$  copies ml(-1). There was a significantly higher *G. vaginalis* load in women with BV compared with intermediate and non-BV women ( $p$  value =  $5.1 \times 10(-14)$ ). All Nugent scores in keeping with BV had qPCR loads of greater than or equal to  $10(7)$  copies ml(-1). Among the 24 undefined women (11.8 %) in the study with an intermediate flora, 14 (58.3 %) had a *G. vaginalis* load of greater than or equal to  $10(7)$  copies ml(-1). The authors concluded that a threshold of  $10^7$  copies ml(-1) had positive and negative predictive values of 57.1 and 100 % for BV; the high qPCR loads among the intermediate Nugent scores suggested the need for a new approach in classifying BV and the potential for qPCR to play a role.

Jespers et al (2016) stated that a next-generation diagnostic tool for BV, consisting of quantitative and/or qualitative molecular criteria, has not yet been identified. The optimal diagnostic tool should not only diagnose BV in diverse populations, but should also detect early signs of transition to dysbiosis. These researchers evaluated a tool based on log<sub>10</sub>-transformed qPCR data for *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, *Lactobacillus vaginalis*, *Lactobacillus* genus, *Atopobium vaginae* and *Gardnerella vaginalis* in vaginal specimens of 426 African women to detect dysbiosis and predict transition to dysbiosis. *G. vaginalis* ( $p = 0.204$ ) and *A. vaginae* ( $p = 0.001$ ) were more commonly present in women who evolved to an intermediate (Nugent 4 to 6) or BV score (Nugent 7 to 10) compared to women who continued to have a normal Nugent score. The combination of *G. vaginalis*, *A. vaginae* and *Lactobacillus* genus counts performed best for diagnostic accuracy for BV -- sensitivity 93.4 % and specificity 83.6 %; and for predictive accuracy for BV -- sensitivity 79 % and specificity 52 %. *L. crispatus* combinations did not perform well. The authors concluded that a triple -- *G. vaginalis*-*A. vaginae*-*Lactobacillus* genus -- qPCR tool holds promise for research in sub-Saharan Africa or when developed as a next-generation clinical diagnostic modality for BV, ideally engineered as a rapid assay.

Lamont et al (2020) noted that the "bacterial vaginosis syndrome" has significant adverse effects for women and babies, including pre-term birth and increased risk of acquisition of sexually transmitted infections and HIV. Currently, the gold standard for diagnosis is Gram stain microscopy of vaginal secretions, which is not readily available, is somewhat subjective, and does not differentiate between the likely different subtypes of vaginal dysbioses that may have different etiologies, microbiology, responses to antibiotics, and phenotypic outcomes. With new information from molecular-based, cultivation-independent studies, there is increasing interest in the use of molecular techniques for the diagnosis of bacterial vaginosis. These investigators reviewed the evidence on and the rationale behind the use of molecular techniques for the diagnosis of bacterial vaginosis. They found a number of commercially available molecular diagnostic tests, a few of which have Food and Drug Administration (FDA) and/or Conformité Européenne in-vitro diagnostic (CE-IVD) approval, and they compared their performance with respect to sensitivities and specificities. Molecular-based tests have the advantage of objectivity, quantification, detection of fastidious organisms, and validity for self-obtained vaginal swabs. The performance of the molecular tests against standard

microscopy is impressive, but further education of users on interpretation is needed. Bacterial vaginosis is the major cause of vaginal dysbiosis and should be recognized for the threat it is to women's genital tract health. Quantitative assessment of microbial abundance, the diversity of other organisms present, specific primers for gene sequence regions, and clades and biovars of target microbes should be recognized and incorporated into future molecular diagnostic tests to better differentiate between vaginal eubiosis and dysbiosis.

In this review, *atopobium vaginae* and *megasphaera* species are not listed in the quantitative PCR analysis of organisms for the prediction of bacterial vaginosis; and quantitative PCR has a sensitivity, specificity and positive predictive value of 44 %, 100 % and 8 %, respectively for lactobacilli  $>10^8$ .

Furthermore, an UpToDate review on "Approach to females with symptoms of vaginitis" (Sobel, 2021) states that "Microscopic examination of normal vaginal discharge reveals a predominance of squamous epithelial cells, rare polymorphonuclear leukocytes (PMNs), and *Lactobacillus* species morphotype ... If microscopy is not available, commercial diagnostic testing methods (e.g., rapid antigen, NAATs, and polymerase chain reaction [PCR]) are used for confirming the clinical suspicion of BV, vaginal candidiasis, or trichomonas vaginitis". Moreover, this UTD review does not mention *atopobium vaginae* and *megasphaera* species.

Stool H. Pylori Detection for Evaluation of Dysphagia (e.g., the PylorDx and the PyloriAR/Stool H. Pylori Antibiotic Resistance Panel (NGS)).

UpToDate reviews on "Approach to the evaluation of dysphagia in adults" (Fass, 2021), "Evaluation of dysphagia in children" (Woodward, 2021) and "Oropharyngeal dysphagia: Clinical features, diagnosis, and management" (Lembo, 2021) do not mention PCR testing of *H pylori* as a management tool.

#### Candida Auris

The StatPearls review on "Candida auris" (Sikora et al, 2023) does not mention PCR testing as an evaluation method for suspected *Candida auris*.

#### COVID-19 Testing in Immunocompromised Patients

Donato et al (2021) noted that point-of-care (POC) tests are in high demand in order to facilitate rapid care decisions for patients suspected of SARS-CoV-2. These researchers carried out a clinical validation study of the Cue Health POC nucleic acid amplification test (NAAT) using the Cue lower nasal swab, compared to a reference NAAT using standard nasopharyngeal swab, in 292 symptomatic and asymptomatic outpatients for SARS-CoV-2 detection in a community drive through collection setting. Positive percent agreement between Cue COVID-19 and reference SARS-CoV-2 test was 91.7 % (22 of 24); or 95.7 % (22 of 23) when one patient with no tie-breaker method was excluded. Negative percent agreement was 98.4 % (239 of 243), and there were 25 (8.6 %) invalid or canceled results. The authors concluded that the Cue COVID-19 test showed very good positive and negative percent agreement with central laboratory tests, and will be useful in settings where accurate POC testing is needed to facilitate management of patients suspected of COVID-19.

The authors stated that this study had several drawbacks. First, the study site was a community-based collection center where patients with a clinical order for SARS-CoV-2 testing were recruited. While the location was chosen based on a relatively high rate (approximately 10 %) of positive test results, this approach limited the number of positive cases compared to studies including more positives from residual lab samples. Second, at the time this study was conducted, the Cue COVID-19 test had not yet received FDA Emergency Use Authorization (EUA) for testing residual or banked specimens; thus, a prospective comparison was the only

means to examine the performance of the device. Third, while sample collection, a crucial step in infectious disease testing, was carried out by nursing staff as is typical in the POC settings; to prevent backlogs in clinical specimen collection, the Cue test was carried out by laboratory staff in the POC environment. Third, this trial did not have a method for resolving all discrepant results observed between the Cue and reference NAAT; thus, an incorrect reference method result cannot be ruled out. Fourth, it was also not possible to conduct a formal limit of detection study due to the design of the assay at that time. Fifth, patients with invalid/canceled results were not able to be retested as directed by the Cue instructions for use because study participants left the facility before POC testing was completed.

A Delphi white paper on “Recommendations for COVID-19 testing practices in immunocompromised patients” (Grimley et al, 2023) noted that “This Delphi panel highlights that there is consensus among clinicians with extensive experience treating IC patients that there are gaps in current COVID-19 testing methods and that accurate at-home testing could greatly benefit IC patients. Allowing IC patients access to more accurate at-home molecular diagnostic tests would be beneficial to patients as it provides an at-home testing option with high sensitivity to detect COVID-19 early in the disease course. This understanding of the current gaps in patient care and opportunities to improve COVID-19 testing in IC patients with NAATs, such as Cue’s COVID-19 OTC Test, could help optimize patient outcomes and potentially reduce the impact of severe COVID-19 for IC patients as well as the economic burden on the healthcare system. This white paper also noted that “The Cue COVID-19 Test for Home and Over the Counter (OTC) Use has not been FDA cleared or approved, but it has been authorized by the FDA under an Emergency Use Authorization (EUA). This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens”.

Rebbapragada et al (2023) stated that the COVID-19 pandemic highlighted the critical need for rapid and accurate molecular diagnostic testing. The Cue COVID-19 POC Test (Cue POCT) is a NAAT, authorized by Health Canada and FDA as a POCT for SARS-CoV-2 detection. Cue POCT was deployed at a network of clinics in Ontario, Canada with n = 13,848 patrons tested between July 17, 2021 and January 31, 2022. The clinical performance and operational experience with Cue POCT were examined for this testing population composed mostly of asymptomatic individuals (93.7 %). A head-to-head prospective clinical verification was performed between July 17 and October 4 for all POCT service clients (n = 3,037) with paired COVID-19 testing by Cue and RT-PCR. Prospective verification showed a clinical sensitivity of 100 % and clinical specificity of 99.4 % for Cue COVID-19 POCT. The lack of false negatives as well as low false positive rate (0.64 %), underscored the high accuracy (99.4 %) of Cue POCT to provide rapid PCR quality results. Low error rates (cancellation rate of 0 % and invalid rate of 0.63 %) with the current software version were additionally noted. Taken together, these findings highlighted the value of accurate molecular COVID-19 POCT in a distributed service delivery model to rapidly detect cases in the community with the potential to curb transmission in high-exposure settings (i.e., in-flight, congregate workplace, and social events). The insights gleaned from this operational implementation are readily transferable to future POCT diagnostic services.

These investigators stated that while the Cue COVID-19 test has a claim for home use and “over the counter” sale in the U.S. under the FDA emergency use authorization, this study was unable to examine the performance with specimens self-collected by individuals without formal training; Health Canada authorization only permitted collection by trained healthcare workers. In contrast to healthcare professionals, untrained individuals may have a learning curve to perform reliable self-swabbing which could in turn impact down-stream test performance. However, Cue provides an in-app video, step-by-step visual mobile app instructions, and printed instruction sheets to ensure appropriate sampling methods, and there is

ample data with other systems to show self-swabbing is reliable, safe, and comparable to healthcare worker collection. Furthermore, the detection of variants was not confirmed in clinical specimens by viral culture and sequencing; however, testing of contrived specimens confirmed that the Cue COVID-19 POCT was able to detect wild type and 5 variant strains including, B.1.1.7-Alpha, B.1.351-Beta, B.1.617-Delta, P.1-Gamma, and B.1.1.529-Omicron. Other drawback of this trial included the lack of direct comparison to antigen testing, detailed cost-benefit, and health outcomes analysis. However, when factoring the full operational costs of RT-PCR testing (specimen collection, transportation, laboratory personnel, facilities, reagents, and equipment) and serial antigen testing on consecutive days, the cost of Cue COVID-19 testing provides an attractive value proposition. In addition, Cue POCT did not incur many of the logistical costs or operational burdens experienced by lab RT-PCR; delays in lab results compromise early initiation of treatment and associated health outcome benefits that incur complex health system costs that are harder to enumerate.

Katzman et al (2023) stated that in early 2020, the World Health Organization (WHO) prioritized research toward the development of rapid COVID-19 tests as one of the most important actions in successfully addressing the public health crisis. At the time of writing, the Visby COVID-19 PCR test is 1 of 8 rapid POC NAATs that have been authorized by the FDA for emergency use. These tests include: Visby Medical COVID-19 POC test, Lucira COVID-19 All-In-One Test Kit, BioFire Respiratory Panel 2.1-EZ, Roche cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test on the cobas Liat System, Cue COVID-19 test, Abbott ID NOW COVID-19, Mesa Biotech Accula SARS-CoV-2 test, and Cepheid Xpert Xpress SARS-CoV-2 test. The Visby Medical COVID-19 POC test was granted EUA for POC use on February 8, 2021 for qualitative detection of SARS-CoV-2 nucleic acid via its single-use, fully disposable POC device. These researchers examined the performance characteristics of the test (Visby Medical COVID-19 POC) using residual specimens submitted to a central laboratory for RT-qPCR testing. These investigators noted that only 2.0 % of specimens initially tested on the Visby device were invalid and required repeat testing in accordance with the manufacturer's instructions for use. Upon repeat testing, both samples agreed with the results obtained from the reference method. The rate of invalid results obtained on the Visby POC device was substantially lower than that of the invalid rates reported for similar molecular POC devices (e.g., the Cue COVID-19 rapid test (8.6 %; Donato et al, 2021) and the Abbott ID NOW test (8.6 %). The authors concluded that this trial showed that the Visby COVID-19 test has many desirable POC test characteristics -- it is highly concordant with the central laboratory-based RT-qPCR reference method, has a low rate of invalid results that require repeat testing, can detect lower viral loads than some other rapid tests, and provides a qualitative assessment of COVID-19 status within minutes. Combined with its portability and ease of use, this device may be an attractive alternative to central laboratory RT-qPCR testing, specifically in settings staffed by non-lab personnel where rapid results at the POC are needed for timely patient care.

The authors stated that this study had several drawbacks. First, this small, single-center study included a random sampling of residual non-frozen clinical specimens previously tested on the reference methods. Due to limited access to other sample types at the time of the study (e.g., nasal and mid-turbinate), only NP swabs were included in this evaluation. Second, comparisons between the test methods were not carried out via prospective recruitment of patients. Patient data regarding the presence/absence of symptoms at the time of presentation was not known as this information could not be obtained due to the nature of the IRB approval. Third, information regarding the time of presentation as it related to the onset of symptoms in symptomatic individuals could not be captured. The limit of detection (LoD) experiment performed was significantly abbreviated; thus, it was possible that these researchers would have verified a lower value had a full, formal LoD experiment been performed. These researchers stated that further investigations

are needed to probe positive and negative percent agreement when symptomatic and asymptomatic patients are prospectively tested by both Visby and reference NAAT methods.

Furthermore, an UpToDate review on "COVID-19: Diagnosis" (Caliendo and Hanson, 2024) does not mention Cue Health point-of-care COVID-19 test / home NAAT (nucleic acid amplification tests) test as a management tool.

#### JC Polyomavirus for Progressive Multifocal Leukoencephalopathy Before Starting Rituximab

Berger et al (2018) examined the observed risk of progressive multifocal leukoencephalopathy (PML) in patients treated with the anti-CD20 monoclonal antibody rituximab in the regulatory authority-approved autoimmune indications rheumatoid arthritis (RA), granulomatosis with polyangiitis (GPA), and microscopic polyangiitis (MPA). This was a cumulative analysis of confirmed PML cases in patients receiving rituximab for RA or GPA/MPA from both spontaneous reports and clinical trial sources, as captured in the manufacturer global company safety and clinical databases. Overall reporting rates were calculated and patient case details were summarized. As of November 17, 2015, there were 9 confirmed PML cases among patients who had received rituximab for RA, and 2 for GPA. Corresponding estimated reporting rates were 2.56 per 100,000 patients with RA (estimated exposure ≈ 351,396 patients) and less than 1 per 10,000 patients with GPA/MPA (estimated exposure 40,000 to 50,000 patients). In all cases, patients had 1 or more potential risk factor for PML independent of rituximab treatment. In the RA population, the estimated reporting rate of PML generally remained stable and low since 2009 despite increasing rituximab exposure. There was no pattern of latency from time of rituximab initiation to PML development and no association of PML with the number of rituximab courses. Global post-marketing safety and clinical trial data showed that the occurrence of PML is very rare among rituximab-treated patients with RA or GPA/MPA and has remained stable over time.

Furthermore, the American College of Rheumatology (ACR)'s medication guide (2024) has no recommendations for JCV testing before starting rituximab therapy.

#### Oral Swab for Mycoplasma Hominis and Ureaplasma Urealyticum for Screening of Oral Infection

An UpToDate review on "Oral lesions" (Lodi, 2024) states that "The diagnosis of herpetic infection is generally clinical. Available confirmatory investigations include HSV DNA detection by polymerase chain reaction (PCR), rapid direct immunofluorescence assays (DFAs), serologic tests showing immunoglobulin G (IgG)- and immunoglobulin M (IgM)-specific antibody responses, and presence of multinucleated giant cells on a Tzanck smear (these are also seen with varicella-zoster virus infection)". This UTD review does not mention the use of PCR for screening.

#### PCR Pharyngitis Panel for Diagnosis Stomatitis and Otitis Media

UpToDate reviews on "Acute otitis media in adults" (Limb et al, 2024), "Acute otitis media in children: Clinical manifestations and diagnosis" (Wald, 2024), "Herpetic gingivostomatitis in young children" (Keels and Clements, 2024), and "Oral toxicity associated with systemic anticancer therapy" (Negrin and Treister, 2024) do not mention PCR pharyngitis panel as a management option.

#### Pneumocystis Jirovecii

Pneumocystis pneumonia (PCP) is a rare, serious lung infection caused by the fungus *Pneumocystis jirovecii*. Most individuals with PCP are immunocompromised due to a medical condition (e.g., cancer, chronic lung diseases, HIV, inflammatory diseases or autoimmune diseases, and solid organ or stem cell transplant), or from

medication (e.g., corticosteroids). Testing for PCP is carried out by taking a sputum sample from the patient's lungs. Sometimes, a small sample of lung tissue (a biopsy) or a blood sample is used to diagnose PCP. Samples are analyzed in clinical laboratories; and 1 method that can be used is PCR that detects PCP DNA.

#### Pruritis Ani

An UpToDate review on "Approach to the patient with anal pruritus" (Breen and Bleday, 2024) does not mention PCR testing for candida as a management option.

#### Rectal Swab Testing for Identification of Antibiotic Resistant Bacteria Before Prostate Biopsy

Liss et al (2019) conducted pilot testing regarding implementation of a point-of-care (POC) qPCR-based test (EST200) targeting bacterial clonal groups representing the majority of sepsis-causing Escherichia coli (*E. coli*) before prostate biopsy to determine antibiotic selection. After institutional review board (IRB) approval, these researchers obtained rectal swabs to compare real-time qPCR analysis on a Rotor-Gene Q instrument (Qiagen, Hilden, Germany) to standard culture on ciprofloxacin infused (10 mg/L) MacConkey agar and susceptibility testing. Techniques were compared by an area under the receiver operative curve (AUC). A total of 140 men participated in the study, 102 pre-biopsy cultures were used to guide prophylaxis. These investigators did not meet their accrual for the randomized portion of the clinical study; however, they did randomize 38 men without pre-biopsy cultures to physician choice of antibiotic versus PCR-based approach. Regarding predicting fluoroquinolone resistant (FQR) at biopsy, pre-biopsy cultures had an AUC of 0.91 (95 % CI: 0.84 to 1.00,  $p > 0.001$ ) and PCR had an AUC of 0.71 (95 % CI: 0.58 to 0.84,  $p = 0.005$ ) (AUC comparison;  $Z = 2.31$ ,  $p = 0.02$ ). PCR correctly identified 4 of 5 FQR specimens. The PCR test attained an AUC of 0.79 (95 % CI: 0.56 to 1.00,  $p = 0.044$ ) for detection of total FQR on the day of the biopsy. Risk-based techniques may over-compensate with additional antibiotics (21 % versus 0 %,  $p = 0.10$ ). The authors concluded that EST200 was a rapid PCR-based microbial detection system that has moderate ability to detect total FQR at the time of biopsy. Moreover, these researchers stated that this study was under-powered, yet provide opportunities to improve the POC PCR method, such as tabletop testing in less than 20 mins and included additional antibacterial resistant genes.

The authors stated that the drawbacks of this study included the small sample size that did not reach the recruitment objective; and thus under-powered, which limited the data analysis and interpretation of the results. Once the standard of care became rectal culture after a significant infection, it became difficult to enroll men who did not already have a rectal swab noting presence or absence of FQR organisms. These investigators did not have equipoise to randomized men with known colonization of FQR. They could not move sites as the PCR lab was set up within walking distance of only the VA urology clinic. These researchers have identified the significant barriers in conducting a POC test for prostate biopsy infection and currently are planning a large, follow-up study after PCR test improvements identified in this trial. The authors noted that they did not build the PCR to detect common FQR mutations and focused on sequence type due to previous data and they were expanding the PCR test.

Liss et al (2022) stated that FQR *E. coli* causes trans-rectal prostate biopsy infections. In a prospective, observational study, these investigators identified fluoroquinolones resistance by the incorporation of genetic profiling to influence antibiotic selection for trans-rectal prostate biopsy and whether the addition of this genetic testing could improve the prediction of FQR detection at the time of biopsy. Rectal swabs were collected within 30 days of an upcoming prostate biopsy. These swabs were sent for phenotypic and genotypic assessment to predict FQR on the day of the biopsy. Phenotype: Specimens were inoculated onto MacConkey agar containing ciprofloxacin using standard culture techniques to determine FQR

status. Genotype: These investigators compared cultures to PCR sequence typing (E. coli- ST131/H30/ST69) and bacterial plasmids (gyrA, qnrQ, and qnrS). The presence of FQR on this testing was compared to the 2nd rectal swab collected just before biopsy (2 hours after ciprofloxacin prophylaxis), which served as the gold standard for FQR. Overall, the FQR rate was 23.6 %. The bacterial plasmids (qnr) were present in 54.1 % of samples, and multidrug-resistant E. coli ST131 was present in 12.5 % of samples. In comparison, phenotypic assessment using rectal culture had a better prediction for the presence of FQR as compared to genotypic testing (AUC = 0.85 in phenotype arm versus AUC = 0.45 in genotype arm). The authors concluded that they detected a high prevalence of FQR genes in the rectum; however, the addition of PCR-based genotyping did not improve the prediction of culture-based FQR at the time of biopsy.

#### Repeat Mpox Testing to Confirm Resolution of the Lesion

An UpToDate review on "Treatment and prevention of mpox (monkeypox)" (Isaacs et al, 2024) states that "If new lesions are appearing or lesions are progressing after 14 days of tecovirimat, a repeat swab of a lesion should be obtained. If PCR testing is negative for orthopoxvirus, other causes of persistent lesions should be suspected, such as bacterial superinfection". However, this UTD review does not provide any information to support repeat mpox testing to confirm resolution of infection.

Moreover, the WHO's interim rapid response guidance on "Clinical Management and Infection Prevention Control for Monkeypox" (2022) did not mention lesions should be tested to ensure that the infection was cured.

#### Respiratory Virus for Children with Febrile Seizure

An UpToDate review on "Clinical features and evaluation of febrile seizures" (Millichap, 2024) does not mention PCR testing as a management tool.

#### Toxoplasmosis in Uveitis

UpToDate reviews on "Uveitis: Etiology, clinical manifestations, and diagnosis" (Papaliodis, 2024) and "Toxoplasmosis: Ocular disease" (Garweg and Petersen, 2024) do not mention PCR as a management tool.

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