AMERICAN ACADEMY OF NEUROLOGY

Part 2

Learn to think like a microbiologist

AAN 2024 Annual Meeting

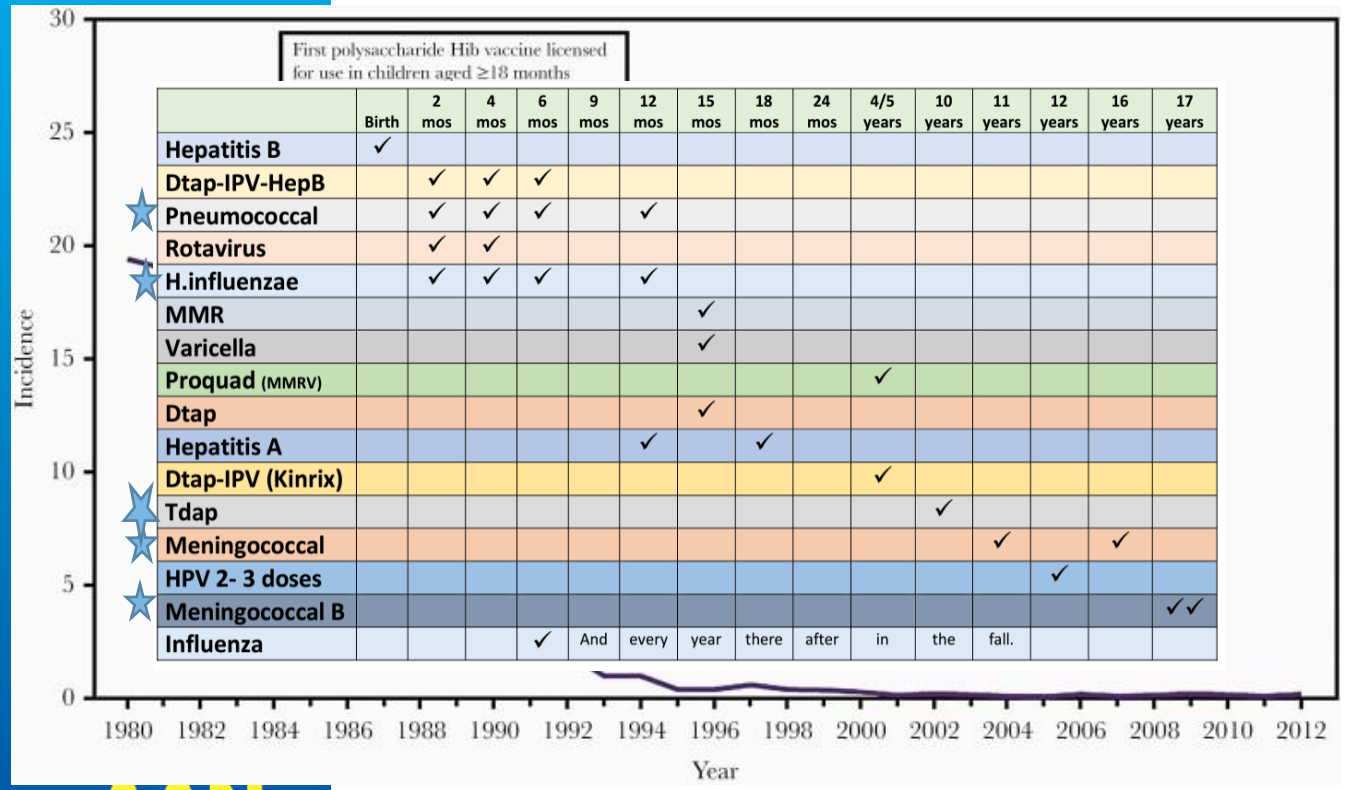
Objectives

- Brief Overview of Epidemiology of Meningitis/Encephalitis
  - Bacterial and Viral Etiologies
- Review Molecular Diagnostics and Accuracy of Infections of the Nervous System
  - Most Common {and Commonly Invoked} Bacterial Etiologies
  - Most Common Viral Etiologies

Disclosures

- None.

Public Health Perspective



Vaccines

- H. influenzae vaccine introduced in USA 1985
- Meningococcal – polysaccharide vaccine (1970s), conjugate meningococcal vaccine (2005), Group B meningococcal (2014)
- Pneumococcal vaccine 14 strains (1977), 23 strains in (1983 PPSV23), 7 strains in (2000 PCV7) and 13 stain in (2010 PCV13)

QR Codes for Slide-Specific References

Gilsdorf JR. J Infect Dis. 2021;224(12 Suppl 2):S321-S330.

Global Burden of Meningitis, Encephalitis

	Absolute numbers (thousands)		Age-standardised rate (per 100 000 people)	
	2016	Percentage change, 1990–2016	2016	Percentage change, 1990–2016
All neurological disorders				
Deaths	9039 (8772 to 9364)	39% (34 to 44)	144 (140 to 149)	−28% (−30 to −26)
DALYs	276 143 (246 544 to 307 994)	15% (9 to 21)	3968 (3557 to 4396)	−27% (−31 to −24)
Tetanus				
Deaths	37 (22 to 47)	−89% (−91 to −86)	1 (0 to 1)	−91% (−93 to −88)
DALYs	2367 (1446 to 3063)	−90% (−93 to −88)	34 (20 to 43)	−91% (−93 to −89)
Incidence	90 (51 to 121)	−89% (−92 to −86)	1 (1 to 2)	−91% (−93 to −88)
Meningitis				
Deaths	318 (265 to 409)	−21% (−36 to 9)	5 (4 to 6)	−36% (−47 to −12)
DALYs	21 866 (18 205 to 28 281)	−28% (−42 to 3)	306 (254 to 398)	−36% (−48 to −10)
Incidence	2821 (2464 to 3310)	13% (10 to 16)	39 (35 to 46)	−4% (−7 to −1)
Encephalitis				
Deaths	103 (84 to 138)	−2% (−36 to 70)	1 (1 to 2)	−27% (−51 to 21)
DALYs	6704 (5469 to 8574)	−15% (−44 to 41)	93 (76 to 118)	−32% (−54 to 10)
Incidence	6534 (5957 to 7165)	29% (26 to 33)	90 (82 to 98)	−5% (−6 to −4)
Stroke				

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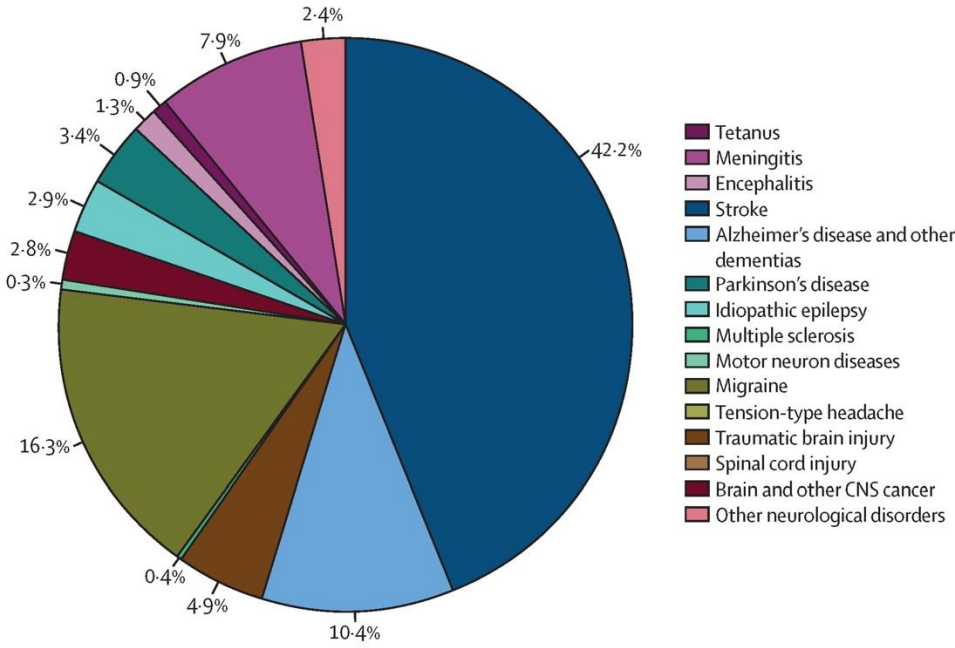
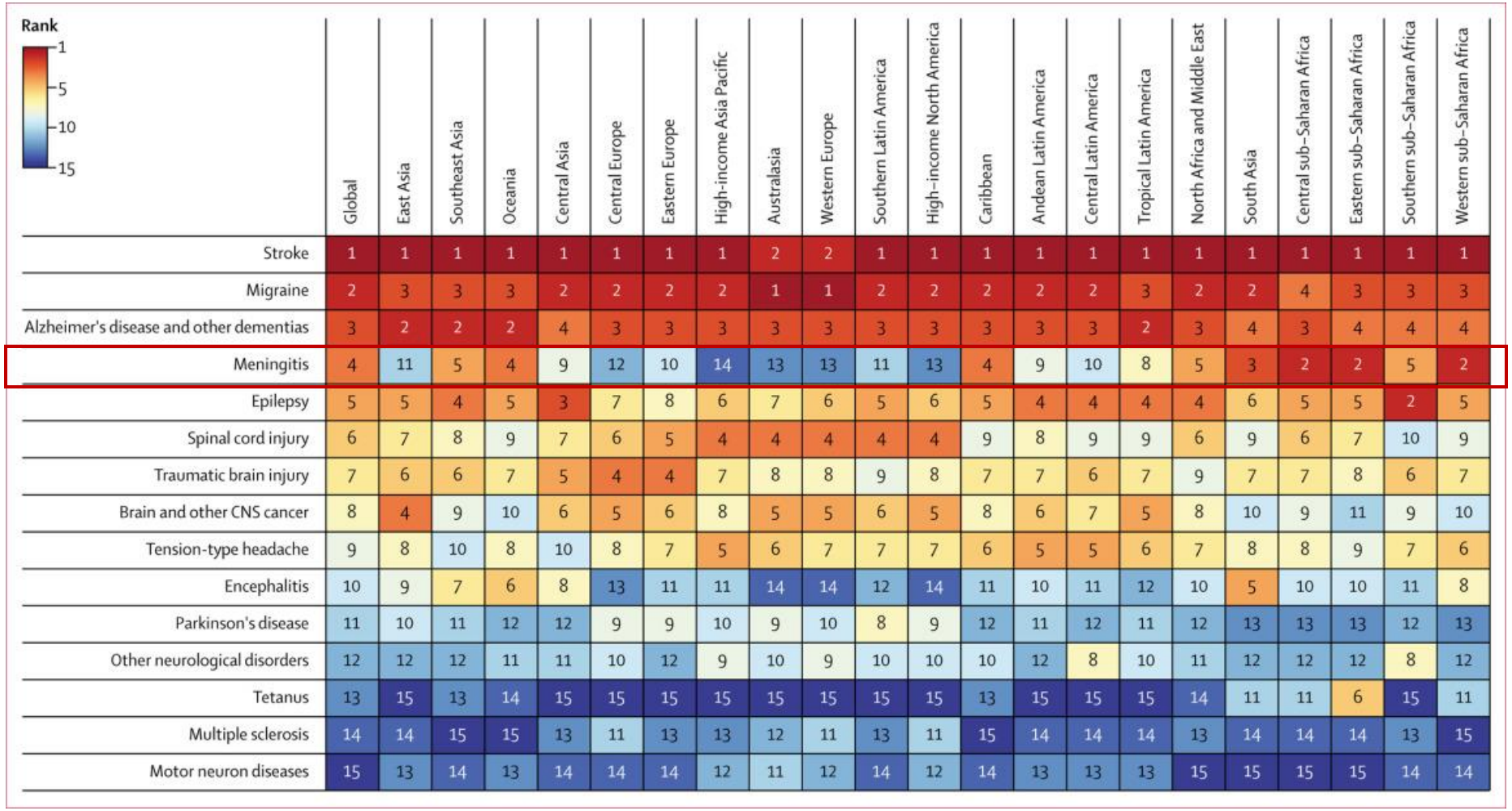


Feigin, V. et al. (2019). *Lancet Neurology*, *18*(5), 459–480.;
Feigin, V. L., et al. (2020). *The Lancet Neurology*, *19*(3), 255–26

Meningitis: Global Disparity



	Global	East Asia	Southeast Asia	Oceania	Central Asia	Central Europe	Eastern Europe	High-income Asia Pacific	Australasia	Western Europe	Southern Latin America	High-income North America	Caribbean	Andean Latin America	Central Latin America	Tropical Latin America	North Africa and Middle East	South Asia	Central sub-Saharan Africa	Eastern sub-Saharan Africa	Southern sub-Saharan Africa	Western sub-Saharan Africa
Stroke	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1	1
Migraine	2	3	3	3	2	2	2	2	1	1	2	2	2	2	2	3	2	2	4	3	3	3
Alzheimer's disease and other dementias	3	2	2	2	4	3	3	3	3	3	3	3	3	3	3	2	3	4	3	4	4	4
Meningitis	4	11	5	4	9	12	10	14	13	13	11	13	4	9	10	8	5	3	2	2	5	2
Epilepsy	5	5	4	5	3	7	8	6	7	6	5	6	5	4	4	4	4	6	5	5	2	5
Spinal cord injury	6	7	8	9	7	6	5	4	4	4	4	4	9	8	9	9	6	9	6	7	10	9
Traumatic brain injury	7	6	6	7	5	4	4	7	8	8	9	8	7	7	6	7	9	7	7	8	6	7
Brain and other CNS cancer	8	4	9	10	6	5	6	8	5	5	6	5	8	6	7	5	8	10	9	11	9	10
Tension-type headache	9	8	10	8	10	8	7	5	6	7	7	7	6	5	5	6	7	8	8	9	7	6
Encephalitis	10	9	7	6	8	13	11	11	14	14	12	14	11	10	11	12	10	5	10	10	11	8
Parkinson's disease	11	10	11	12	12	9	9	10	9	10	8	9	12	11	12	11	12	13	13	13	12	13
Other neurological disorders	12	12	12	11	11	10	12	9	10	9	10	10	10	12	8	10	11	12	12	12	8	12
Tetanus	13	15	13	14	15	15	15	15	15	15	15	15	13	15	15	15	14	11	11	6	15	11
Multiple sclerosis	14	14	15	15	13	11	13	13	12	11	13	11	15	14	14	14	13	14	14	14	13	15
Motor neuron diseases	15	13	14	13	14	14	14	12	11	12	14	12	14	13	13	13	15	15	15	15	14	14

Feigin, V. et al. (2019). *The Lancet Neurology*, *18*(5), 459–480.;

Incidence, Bacterial Meningitis

Table 1. Incidence of Bacterial Meningitis in the United States, 1998–2007, Stratified According to Age Group, Race, and Pathogen.*

Characteristic	1998–1999	2000–2001	2002–2003	2004–2005	2006–2007	Percent Change, 2006–2007 vs. 1998–1999 (95% CI)
	no. of cases per 100,000 population (95% CI)					
Age group						
<2 Mo	73.46 (56.45 to 94.35)	88.28 (69.69 to 109.95)	56.59 (42.13 to 74.45)	77.27 (60.58 to 96.90)	80.69 (63.53 to 101.42)	10 (1 to 20)
2–23 Mo	14.20 (11.85 to 16.91)	11.49 (9.45 to 13.92)	6.56 (5.06 to 8.38)	6.95 (5.47 to 8.89)	6.91 (5.30 to 8.77)	–51 (–55 to –48)
2–10 Yr	1.55 (1.20 to 1.96)	1.48 (1.16 to 1.88)	0.94 (0.68 to 1.27)	1.07 (0.79 to 1.43)	0.56 (0.36 to 0.82)	–64 (–68 to –59)
11–17 Yr	1.03 (0.71 to 1.43)	0.87 (0.60 to 1.22)	0.62 (0.39 to 0.94)	0.56 (0.34 to 0.86)	0.43 (0.25 to 0.71)	–58 (–64 to –51)
18–34 Yr	0.99 (0.79 to 1.22)	0.86 (0.68 to 1.07)	0.70 (0.54 to 0.89)	0.76 (0.59 to 0.97)	0.66 (0.50 to 0.86)	–33 (–38 to –27)
35–49 Yr	1.23 (1.01 to 1.48)	1.30 (1.08 to 1.55)	1.08 (0.89 to 1.31)	0.91 (0.74 to 1.13)	0.95 (0.76 to 1.16)	–23 (–29 to –17)
50–64 Yr	2.15 (1.75 to 2.57)	1.83 (1.49 to 2.21)	2.09 (1.75 to 2.48)	1.79 (1.49 to 2.14)	1.73 (1.44 to 2.06)	–19 (–25 to –14)
≥65 Yr	2.64 (2.13 to 3.16)	2.20 (1.76 to 2.72)	2.21 (1.78 to 2.71)	1.51 (1.16 to 1.94)	1.92 (1.53 to 2.38)	–27 (–32 to –22)
All ages	2.00 (1.85 to 2.15)	1.82 (1.69 to 1.97)	1.49 (1.38 to 1.62)	1.41 (1.30 to 1.54)	1.38 (1.27 to 1.50)	–31 (–33 to –29)
Race†						
White	1.71 (1.55 to 1.87)	1.58 (1.43 to 1.73)	1.28 (1.15 to 1.42)	1.27 (1.14 to 1.41)	1.28 (1.14 to 1.40)	–25 (–28 to –23)
Black	4.07 (3.57 to 4.62)	3.85 (3.40 to 4.35)	3.12 (2.72 to 3.57)	2.62 (2.28 to 3.03)	2.41 (2.13 to 2.84)	–41 (–44 to –37)
Other	1.55 (0.98 to 2.23)	0.68 (0.37 to 1.18)	0.76 (0.44 to 1.25)	0.67 (0.39 to 1.14)	0.46 (0.25 to 0.86)	–70 (–75 to –64)
Pathogen						
Streptococcus pneumoniae	1.09 (0.98 to 1.20)	1.03 (0.93 to 1.13)	0.93 (0.83 to 1.03)	0.76 (0.68 to 0.85)	0.81 (0.72 to 0.90)	–26 (–29 to –23)
Neisseria meningitidis	0.44 (0.37 to 0.51)	0.37 (0.31 to 0.44)	0.23 (0.19 to 0.29)	0.22 (0.17 to 0.27)	0.19 (0.14 to 0.24)	–58 (–61 to –54)
Group B streptococcus	0.24 (0.20 to 0.30)	0.30 (0.25 to 0.36)	0.21 (0.17 to 0.26)	0.27 (0.22 to 0.32)	0.25 (0.21 to 0.31)	4 (–3 to 12)
Haemophilus influenzae	0.12 (0.09 to 0.17)	0.10 (0.07 to 0.14)	0.10 (0.07 to 0.13)	0.10 (0.07 to 0.14)	0.08 (0.05 to 0.11)	–35 (–42 to –27)
Listeria monocytogenes	0.10 (0.08 to 0.16)	0.03 (0.01 to 0.05)	0.03 (0.01 to 0.05)	0.05 (0.04 to 0.10)	0.05 (0.03 to 0.08)	–46 (–53 to –39)

* CI denotes confidence interval.
† Race was obtained from medical records. "Other" includes American Indian or Alaska Native, Asian or Pacific Islander, or other race. Within a site and age group, cases with missing data for race were assumed to have a distribution of race similar to that among cases with available data.

Thigpen et al. 2011. N Engl J Med; 364:2016-2025

Case Fatality, Bacterial Meningitis

Table 3. Characteristics of Patients with Bacterial Meningitis Identified by the Emerging Infections Programs Network, 2003–2007.

Characteristic	*Neisseria meningitidis*	*Haemophilus influenzae*	Group B Streptococcus	*Listeria monocytogenes*	*Streptococcus pneumoniae*	All
	percent of patients					
Pediatric patients	**N=107**	**N=42**	**N=222**	**N=13**	**N=203**	**N=587**
Male sex	62.6	61.9	49.5	46.2	54.7	54.5
Race*						
White	69.2	71.4	47.3	30.8	59.1	56.7
Black	17.8	16.7	42.3	38.5	25.6	30.2
Other	2.8	9.5	3.2	0.0	3.0	3.4
Underlying medical condition†						
Immunocompromising condition	1.3	6.5	0.0		6.9	3.0
Chronic condition	8.8	9.7	3.6		9.1	6.7
Prematurity only	1.3	3.2	11.9		2.3	5.9
None	88.8	80.6	84.5		81.7	84.4
Case fatality rate						
All pediatric patients	3.8	0	7.3	7.7	9.4	6.9
Pediatric patients <2 yr	2.5	0	7.5	0	7.7	6.3
Adult patients	**N=125**	**N=69**	**N=80**	**N=44**	**N=765**	**N=1083**
Male sex	48.8	46.4	40.0	56.8	49.9	49.1
Race*						
White	52.8	62.3	45.0	70.5	54.5	54.8
Black	20.8	24.6	33.8	11.4	29.8	28.0
Other	4.8	2.9	2.5	6.8	2.0	2.6
Underlying medical condition or risk group†						
Immunocompromising condition	11.3	15.0	22.7		25.0	22.5
Chronic condition	18.6	36.7	36.4		35.1	32.7
Smoking	14.4	8.3	7.6		8.4	8.7
Age ≥65 yr only	2.1	8.3	4.5		7.0	7.4
None	53.6	31.7	28.8		24.5	28.7
Case fatality rate						
All adult patients	10.4	7.2	20.8	20.5	17.5	16.4
Adult patients ≥50 yr	9.1	5.1	30.0	24.2	18.3	18.0

* Race was obtained from medical records. "Other" includes American Indian or Alaska Native, Asian or Pacific Islander, and other race. Data on race were not available for some patients; therefore the percentages do not sum to 100%.

† For underlying medical conditions, immunocompromising conditions include multiple myeloma, sickle cell disease, asplenia, organ transplantation, immunoglobulin deficiency, immunosuppressive therapy, human immunodeficiency virus or the acquired immunodeficiency syndrome (HIV–AIDS), leukemia, Hodgkin's disease, lupus, the nephrotic syndrome, and chronic kidney disease. Chronic conditions include asthma or chronic obstructive pulmonary disease, diabetes, cirrhosis, alcohol abuse, atherosclerotic cardiovascular disease, congestive heart failure, burns, cerebrospinal fluid leak, injection-drug use, and cerebrovascular accident (as well as presence of hydrocephalus or ventriculoperitoneal shunt in children). Some conditions were added for study during the surveillance period; not all were identified a priori. Patients with more than one condition were counted for only one, according to the following hierarchy: immunocompromising condition, chronic condition, smoker only (if adult), and prematurity or age of 65 years or older only. Data from the New York site are not included, since cases of HIV–AIDS are not reported at that site. Data for patients with bacterial meningitis from *L. monocytogenes* infection are also not reported, since FoodNet does not consistently record underlying medical conditions for these patients.

Thigpen et al. 2011. N Engl J Med; 364:2016-2025

Incidence, Viral

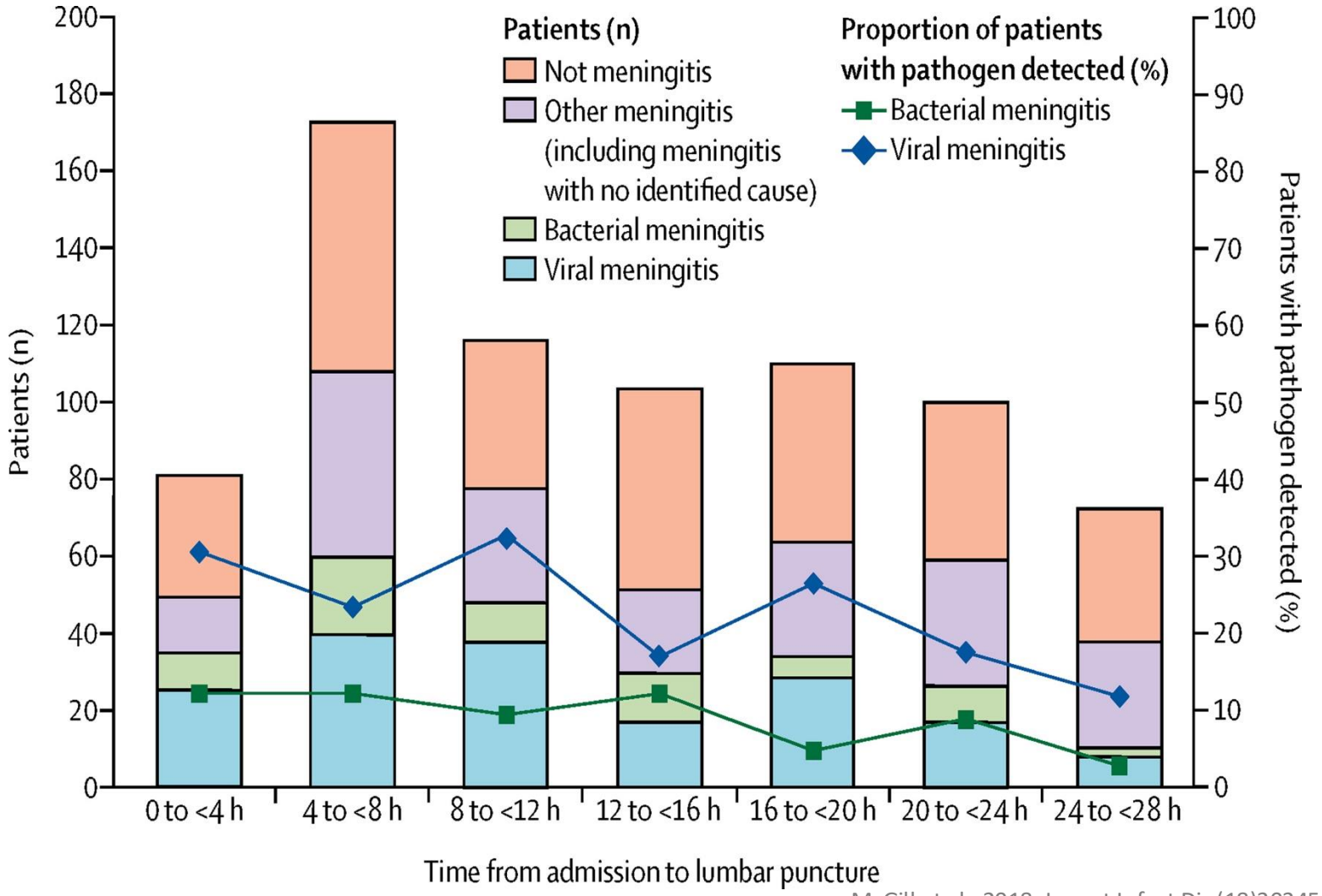
	All meningitis				Bacterial meningitis				Viral meningitis						Unknown cause	
	All patients (n=1117)	Not meningitis (n=454)	All meningitis (n=637)	p value*	All bacterial meningitis (n=99)	Pneumococcal meningitis (n=53)	Meningococcal meningitis (n=28)	p value	All viral meningitis (n=231)	Enteroviral meningitis (n=127)	HSV meningitis (n=55)	VZV meningitis (n=43)	p value†	p value‡	Purulent meningitis (n=41)	Lymphocytic meningitis (n=199)
(Continued from previous page)																
Positive Brudzinski's sign	30/184 (16%)	11/72 (15%)	18/108 (17%)	0·839	4/12 (33%)	2/6 (33%)	1/3 (33%)	1	10/41 (24%)	5/26 (19%)	5/10 (50%)	0/4 (0)	0·123	0·712	0/11 (0)	3/34 (9%)
Glasgow Coma Scale	15 (15–15)	15 (15–15)	15 (15–15)	0·807	14 (10–15)	11 (9–14)	15 (14–15)	<0·001	15 (15–15)	15 (15–15)	15 (15–15)	15 (15–15)	0·25	<0·001	15 (15–15)	15 (15–15)
White blood cell count (×10³ cells per L)	9·4 (7·1–12·9)	9·3 (6·8–12·9)	9·45 (7·4–13·0)	0·252	16·4 (12·5–21·9)	16·9 (13·7–21·5)	17·8 (11·1–24·4)	0·74	8·8 (7·1–10·6)	8·8 (6·9–10·6)	9·4 (7·9–12·0)	8·6 (6·4–10·3)	0·07	<0·001	9·6 (7·9–13·9)	8·9 (7·1–11·8)
C-reactive protein (mg/L)	50 (22–122)	55 (28–121)	43 (19–123)	0·034	164 (67–261)	169 (69–263)	184 (111–295)	0·34	20 (15–38)	20 (16–39)	11 (10–28)	26 (19–76)	0·02	<0·001	38 (15–148)	31 (18–82)
C-reactive protein <10 mg/L	453/1047 (41%)	163/428 (38%)	278/596 (47%)	0·006	6/93 (6%)	5/49 (10%)	0/27 (0)	0·15	122/210 (55%)	42/119 (35%)	44/53 (83%)	37/41 (90%)	<0·001	<0·001	10/38 (26%)	105/183 (57%)
CSF opening pressure (cm CSF)	20 (15–26)	18 (15–21)	22 (16–28)	1	30 (21–40)	36 (26–40)	30 (18–35)	0·07	21 (16–27)	21 (15–26)	22 (20–29)	25 (16–30)	0·34	<0·001	24 (21–30)	20 (15–25)
CSF leucocyte count (×10⁶ cells per L)	77 (5–306)	NA	155 (44–450)	<0·001	1800 (377–4850)	2180 (668–4340)	2000 (480–7175)	0·81	188 (67–355)	118 (44–218)	374 (225–718)	249 (106–450)	<0·001	<0·001	133 (29–730)	102 (34–255)
CSF neutrophil percentage	5 (0–37)	NA	10 (0–47)	<0·001	90 (66–95)	90 (68–96)	90 (79–98)	0·62	5 (0–14)	8 (2–22)	1 (0–10)	0 (0–10)	<0·001	<0·001	80 (60–90)	4 (0–10)
CSF protein (g/L)	0·53 (0·32–0·98)	0·32 (0·25–0·45)	0·81 (0·53–1·38)	<0·001	4·00 (2·00–6·68)	5·63 (3·10–8·12)	3·00 (1·17–6·67)	0·03	0·76 (0·54–1·12)	0·57 (0·45–0·75)	1·14 (0·90–1·32)	1·18 (0·89–1·40)	<0·001	<0·001	0·80 (0·50–1·44)	0·68 (0·49–1·00)
CSF glucose (mmol/L)	3·2 (2·8–3·7)	3·5 (3·2–3·9)	3·0 (2·5–3·5)	<0·001	1·1 (0·3–2·7)	0·5 (0·2–1·7)	1·1 (0·4–2·8)	0·02	3·0 (2·7–3·4)	3·1 (2·8–3·5)	3·0 (2·7–3·4)	2·9 (2·5–3·2)	0·009	<0·001	3·3 (2·7–3·9)	3·1 (2·8–3·4)
CSF:serum glucose ratio	0·58 (0·46–0·67)	0·63 (0·57–0·70)	0·52 (0·40–0·62)	<0·001	0·12 (0·03–0·41)	0·04 (0·01–0·26)	0·15 (0·05–0·42)	0·02	0·56 (0·49–0·63)	0·58 (0·53–0·64)	0·52 (0·48–0·61)	0·54 (0·45–0·63)	0·104	<0·001	0·57 (0·41–0·66)	0·57 (0·46–0·66)

Data are median (IQR) for continuous data and n/N (%) evaluable for categorical data. CSF=cerebrospinal fluid. HSV=herpes simplex virus. VZV=varicella zoster virus. NA=not applicable. *Significance values comparing not meningitis and all meningitis. †Significance values comparing enteroviral, HSV, and VZV meningitis cases. ‡Significance values comparing all bacterial meningitis and all viral meningitis cases.

Table 3: Clinical features of study population by cause of meningitis

McGill et al.. 2018. Lancet Infect Dis (18)30245-7.

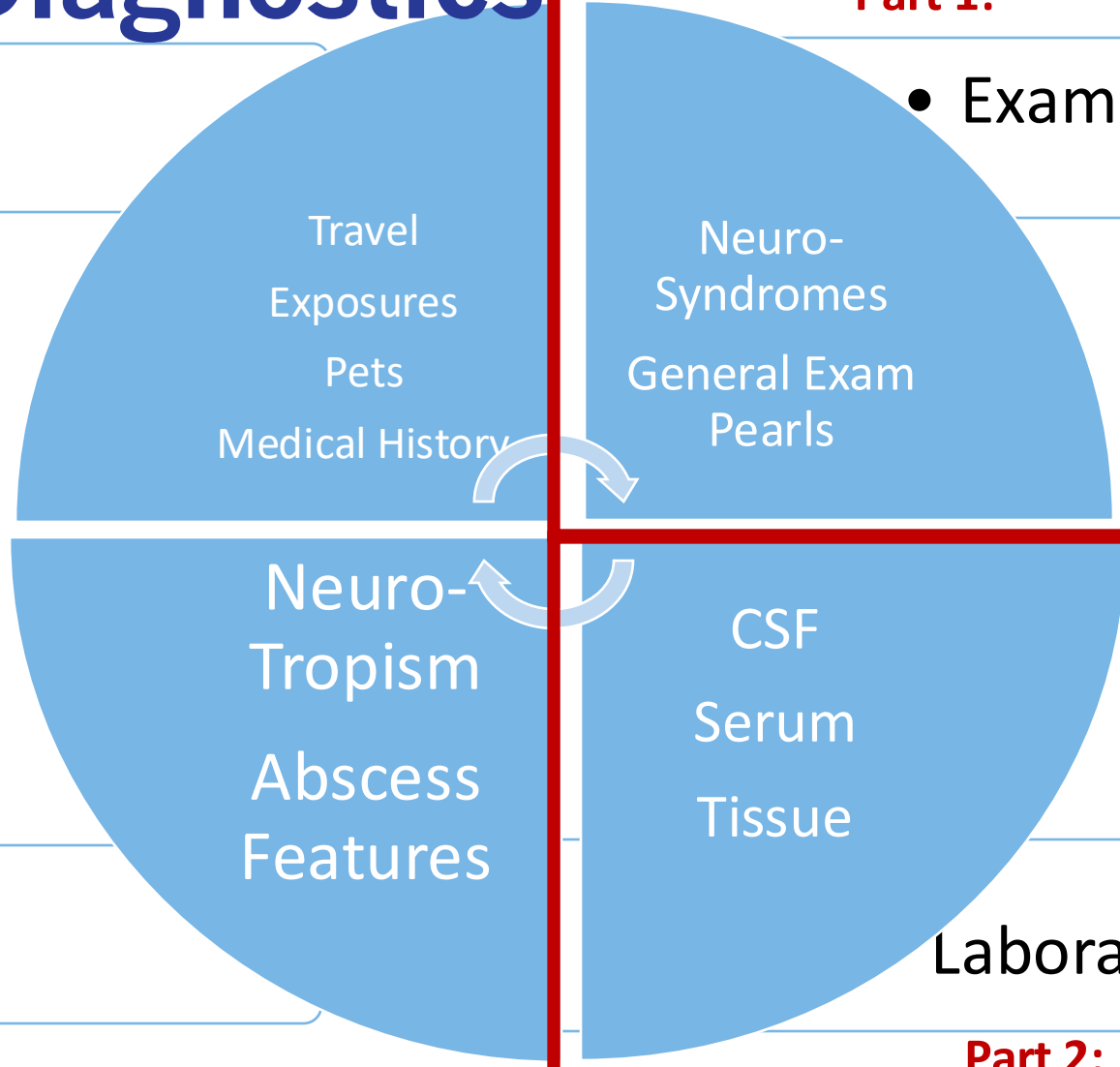
What about when we don't know?



McGill et al.. 2018. Lancet Infect Dis (18)30245-7
Glser et al. 2003 Clinical Infectious Disease. Mar 15;36(6):731

General Approach to Neuro-Infectious Disease Diagnostics

- History
- Exam
- Imaging
- Laboratory



Part 1:

Part 2:

Continuum (Minneap Minn).2021;27(4, Neuroinfectious Disease):836–854

CSF Basic Profile

- ✓Opening Pressure: 10 to 25 cm H_20 in the lateral decubitus position with legs EXTENDED
- ✓RBC: Adjust for Pleocytosis (700 -1000):1
- ✓WBC:
  - ✓Bacterial Neutrophilic Predominant Pleocytosis
  - ✓Viral Lymphocytic Predominant Pleocytosis
  - ✓Parasitic with elevated eosinophils
- ✓Glucose: 2/3 the Serum 1 Hour prior to LP

  hypoglycorrhachia is <40% of Serum (usually bacterial, fungal, mycobact
- ✓Protein: 15 to 60 milligrams per deciliter (mg/dL)

Continuum (Minneap Minn).2021;27(4, neuroinfectious disease),
Breiner A, J Neurol. 2019 Mar;266(3):616-624.
Zunt et al .Neurol Clin. 1999 Nov;17(4):675-89.

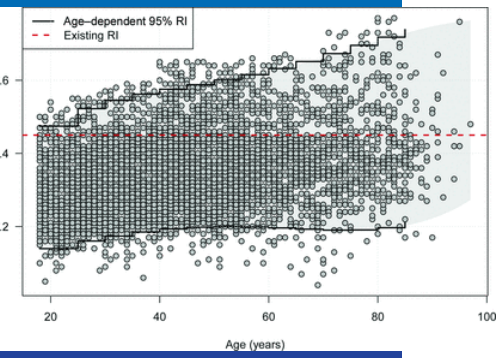
CSF Basic Profile*

- ✓ Opening Pressure: studies have shown ↑ with BMI, ↓ with age, +/- 1-15 cm H_2O with sitting
- ✓ RBC: Adjust for Pleocytosis (700 -1000):1
- ✓ WBC:
  - ✓ Bacterial Neutrophilic Predominant Pleocytosis → WNV Can Be Neurophilic
  - ✓ Viral Lymphocytic Predominant Pleocytosis → can start as Neutrophilic
  - ✓ Parasitic (or coccidioidomycosis) with elevated eosinophils → most automated analyzers have poor detection of Eosinophils, need manual microscopy
- ✓ Glucose: 2/3 the Serum 1 Hour prior to LP

  hypoglycorrhachia → can also be caused by SAH, parasitic disease of SAH, neurosarcoidosis, leptomeningeal carcinomatosis, mumps
- ✓ Protein: Age and sex -adjusted?

  obstruction/stagnation of CSF flow (FROINS Syndrome)

  --tumors, abscesses, stenosis, herniation



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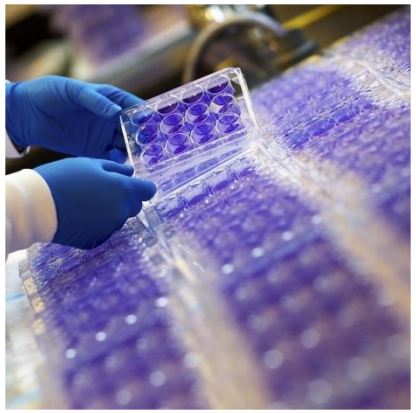
Breiner A, J Neurol. 2019 Mar;266(3):616-624.
Zunt et al .Neurol Clin. 1999 Nov;17(4):675-89.

Microbiology and Molecular Diagnostic Testing Modalities

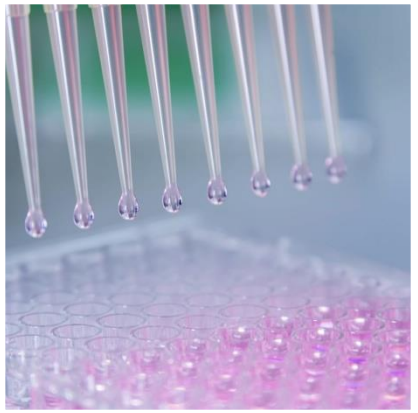
Culture/Direct Detection

Serology



Antigen Detection

PCR



Metagenomic Next Generation Sequencing

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CSF

Blood

Tissue

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Microbiologic and Molecular Diagnostics: Culture

- Little change in Culture Modalities in Decades
  - Sheep Blood and Chocolate Agar Plates in 3-5% Co2
  - Standard Culture Media ***Acceptable*** for:
    - Streptococcus pneumoniae, Neisseria meningitis, Streptococcus agalactiae (group B) and Listeria monocytogenes
- Turn Around Time:1-28 Days
- Increased yield with increased volume
- Exquisitely Sensitive to Antibiotics
  - Sensitivity of Bacterial etiologies (no ABX before LP): 75-90%
  - Sensitivity of Bacterial etiologies (ABX before LP): 40-60%

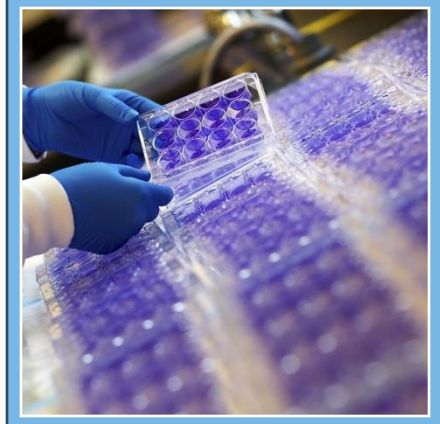
Gold Standard Diagnostic Test For: Bacterial Etiologies

Thomson et al Infect Dis Clin North Am. 2001 Dec;15(4):1047-71.

Microbiology and Molecular Diagnostic Testing Modalities

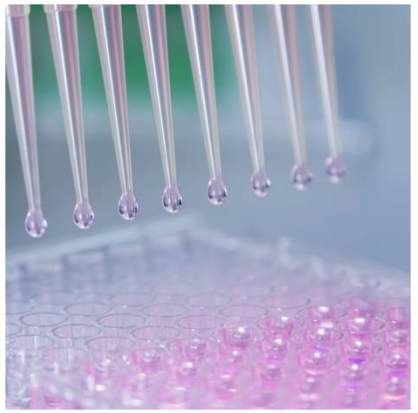
Culture/Direct Detection

Serology



Antigen Detection

PCR



Metagenomic Next Generation Sequencing

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CSF

Blood

Tissue

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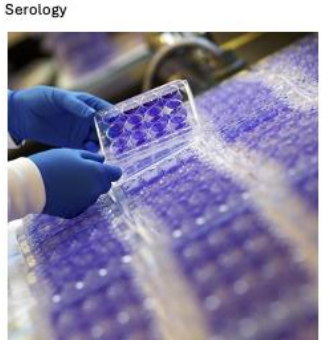
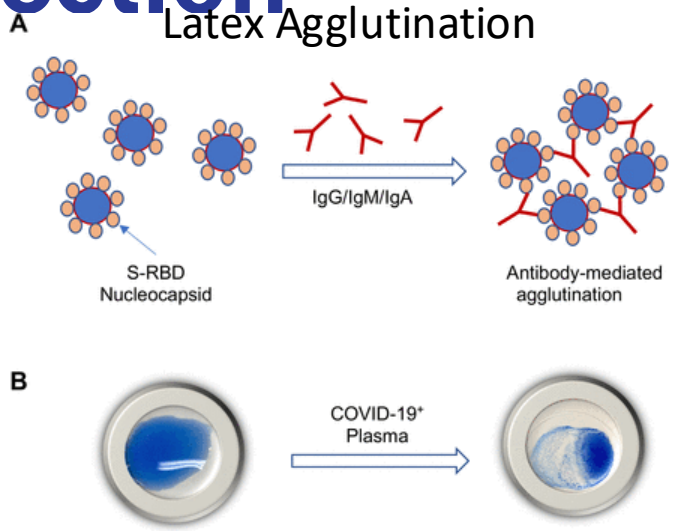
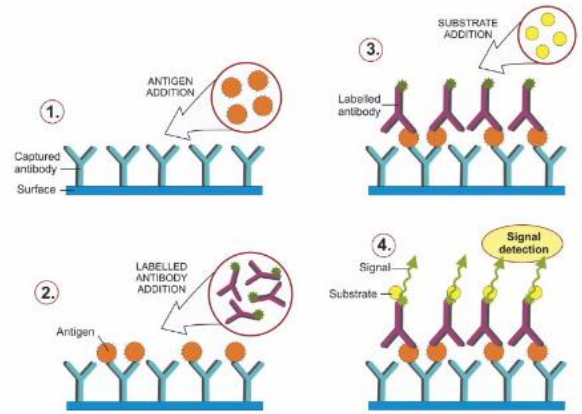
Microbiologic and Molecular Diagnostics: Serology and Antigen Detection





Enzyme-Linked Immunosorbent Assay



- Serology -- Refers to detection of antibodies
- Introduced 1960-1970s
- Turn Around Time: 15 minutes -1 hour

Esmail et al. Cell Rep Methods. 2021 Jun 21;1(2):100011.

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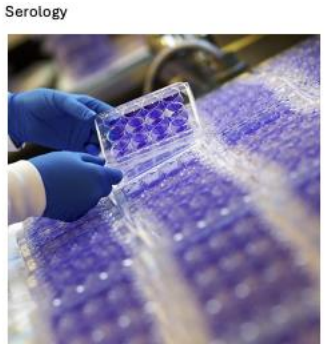
CSF

Blood

Microbiologic and Molecular Diagnostics: Serology



Advantages

- Short Turn Around Time
- Easy to Perform and Operate
- Low Cost
- Minimal Storage Constraints

Disadvantages

- Low Throughput
- Difficulty Distinguishing between chronic exposure vs active disease
- Cross-reactivity within Species
- Often requires CSF/Serum ratio to confirm intrathecal production

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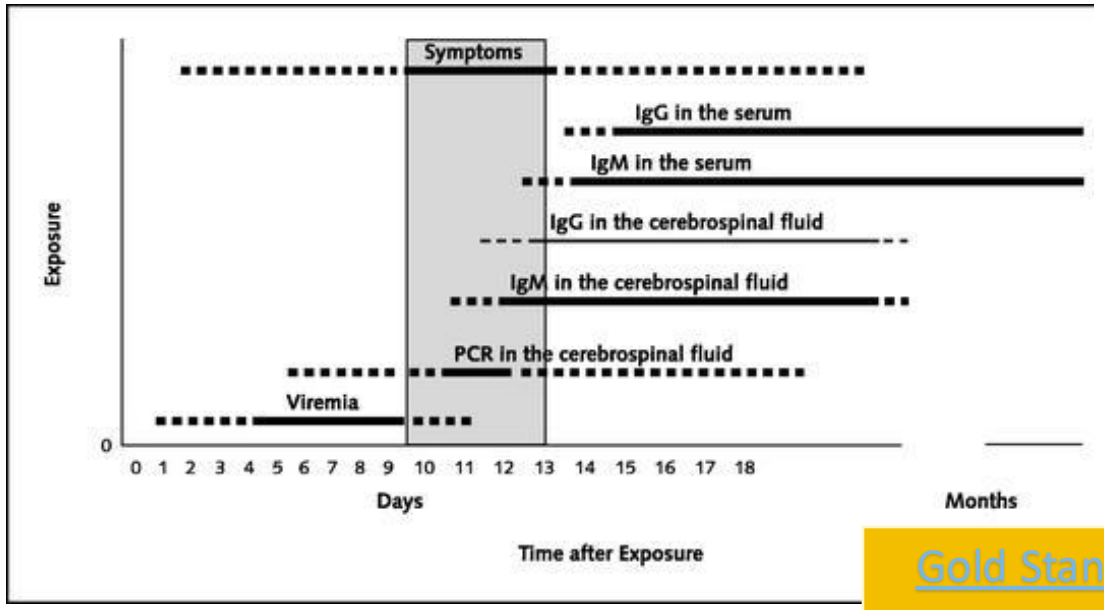
Ramachandran et al Neurol Clin. 2018 Nov;36(4):687-703.

Pearls of CSF Diagnostics with Serology

West Nile Serology Dynamics



Intrathecal Synthesis Ratio

VZV-specific IgG in serum / VZV-specific IgG in CSF

- Low ratio suggest intrathecal synthesis

Case no.	Age, y	Sex	Relevant underlying disorders	Rash	CSF pleocytosis	MRI/CT focal lesions	Angiographic or MRA abnormalities	CSF VSZ DNA PCR	CSF VZV IgG	Reduced serum/CSF ratio of VZV IgG	Ref.
1	18	F	None	−	+	+	ND	−	+	+	5*
2	54	M	AIDS	−	+	+	ND	+	+	+	5*
3	34	M	AIDS	+	−	+	ND	−	+	+	5*
4	28	M	HIV	−	+	+	−	+	+	+	6*
5	71	M	Leukemia	+	−	+	+	−	+	+	7*
6	76	F	None	+	−	−	−	+	+	+	7*
7	51	F	CREST syndrome	−	−	+	+	−	+	+	8*
8	56	F	None	−	−	+	+	−	+	+	†
9	77	F	None	+	−	+	+	−	+	+	†
					+	+	+	−	+	+	†
					+	+	−	+	+	+	†
					+	+	−	−	+	+	†
					−	+	+	−	+	+	†
					+	?‡	ND	−	+	+	†

Gold Standard Diagnostic Test For:

WNV (IgM)

La Crosse Virus

VZV (Myelitis/Vasculitis)

Neuroborreliosis

Neurosyphilis

Nagel et al. Neurology 2007.68 (13) 1069-1073

Gea-Banacloche et al. Ann Intern Med.2004;140:545-553.

Kronenberg et al. *Clinical Infectious Diseases*, 2002. 35 (3): 330–333

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CSF

Blood

Body Fluids

Microbiologic and Molecular Diagnostics: Antigen Detection

Advantages

- Short Turn Around Time
- Easy to Perform and Operate
- Low Cost
- Minimal Power Requirements
- Minimal Storage Constraints

Disadvantages

- Average Performance
- Not all Serogroups (Neisseria) Detected
- Cross Reactivity
- Specimen Preparation Requires Power
- Low Throughput

Gold Standard Diagnostic Test For:
Cryptococcal Meningitis (Lateral Flow)

Feagins et al. J Infect. 2020 Nov;81(5):712-718.

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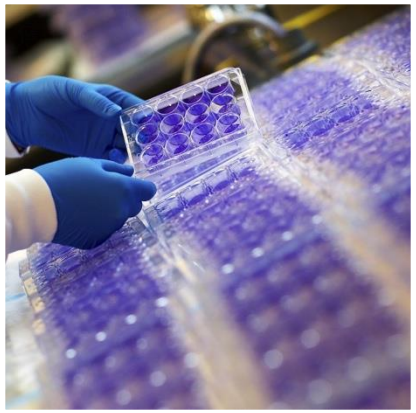
Microbiology and Molecular Diagnostic Testing Modalities

Culture/Direct Detection

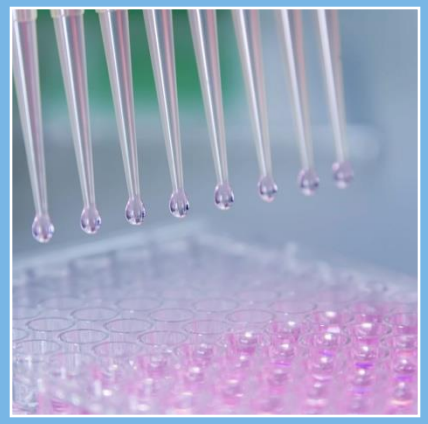
Serology



Antigen Detection

Nucleic Acid Testing

PCR



Metagenomic Next Generation Sequencing

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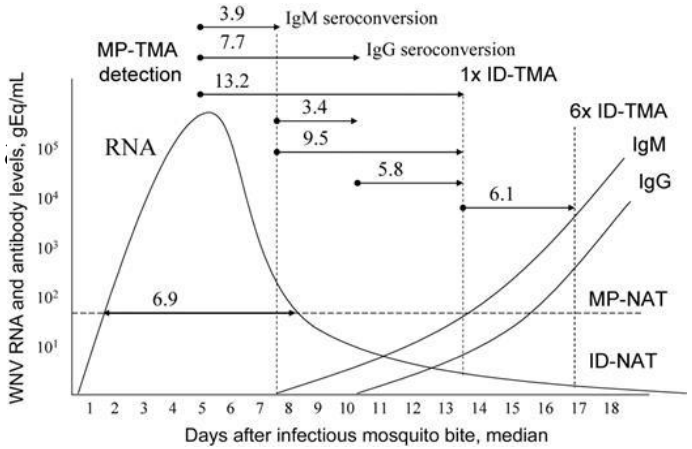
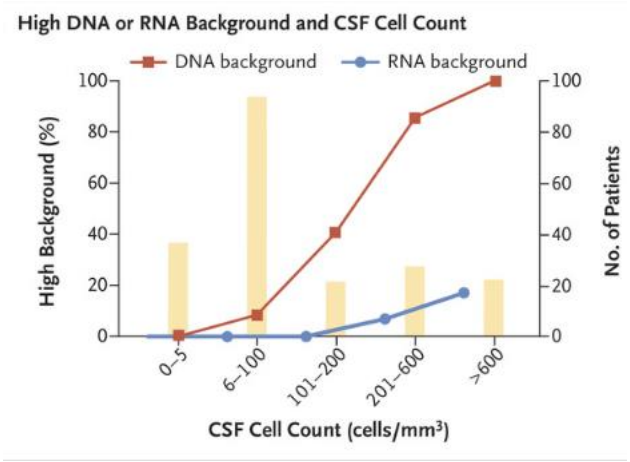
Pearls of CSF Diagnostics with Nucleic Acids

- Requires *Presence of Nucleic Acids* (RNA or DNA)
  - Timing of CNS Infection
    - Genetic Viral Load Highest In Acute Ph
    - Empiric Antimicrobials
    - Syphilis Stage
    - Paucibacterial Infections: TB
  - CSF Penetration
    - Localized Abscess
    - Overall Amount of DNA/RNA
  - Contamination Prone
    - RBCs "bystanders"
    - Environment
  - Storage
    - Degradation of nucleic acids (RNA >DNA) secondary to storage conditions
    - Avoid Unnecessary Freeze-Thaw





			Half-life (no. of days)			% loss of target after 2 weeks		
Pathogen	**Specimen type**	**No. of days stored**	**RT**	**4°C**	**−80°C**	**RT**	**4°C**	**−80°C**
N. meningitidis	EDTA blood	16				No loss	No loss	No loss
CMV	Serum	16	<1	2	3	79.7 ± 5	50 ± 10	36.9 ± 12
	EDTA blood	16				No loss	No loss	No loss
Human enterovirus	CSF	16	9			82.1 ± 9	No loss	No loss
Influenza A	Nasopharyngeal aspirates	14	6	6	10	74 ± 23	70 ± 25	66 ± 27

Busch et al. J Infect Dis. 2008, Oct;198(7): 984–993
Marra J Infect Dis. 1996 Jul;174(1):219-21
Ramachandran et al. Nat Rev Neurol. 2020 Oct;16(10):547-556
Hasan et al. Clin Microbiol. 2012 Dec;50(12):4147-50

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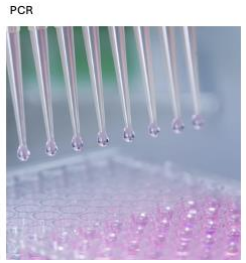
CSF

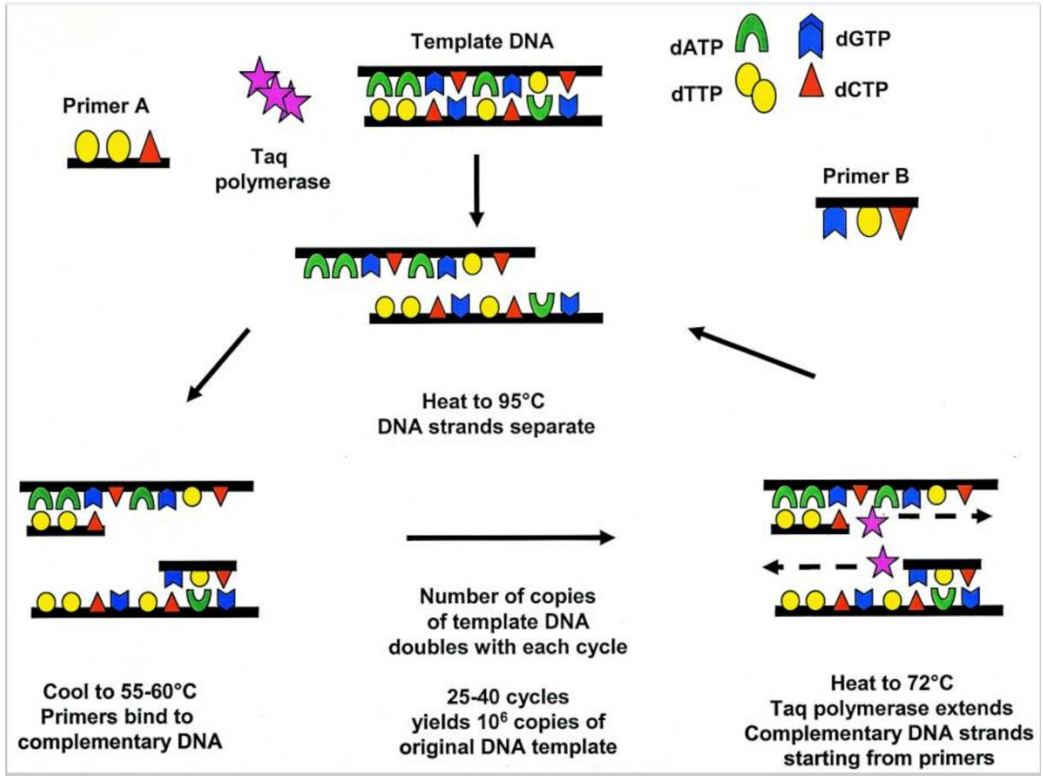
Blood

Tissue

Microbiologic and Molecular Diagnostics: PCR



- Introduced 1985
- Turn Around Time: <24 hours
- Revolutionized CSF Viral Diagnostics
  - Previously Required Brain Biopsy
- Widely Available
- Quantification Available (monitoring)



DeBiasi et al Clin Microbiol Rev. 2004 Oct;): 903–925. 17(4

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CSF

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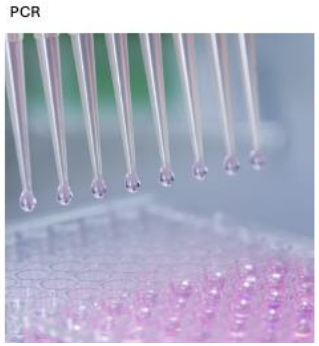
Tissue

Microbiologic and Molecular Diagnostics: PCR



Advantages

- Retains Positivity after Antimicrobial Treatment Acutely
- Copy Number useful for re-infection
- Focused/Targeted Approach
- High Throughput

Disadvantages

- False-positives
  - Mis-priming (temp based)
  - Non-pathologic results (i.e. HHV-6)
- Cost (Relatively)

Gold Standard Diagnostic Test For:
CMV
EBV
Enterovirus
HSV
VZV (Meningitis/Encephalitis)

Wang et al. Clin Microbiol. 2012 Mar;50(3):702-8.
Walls et al *J. Med. Virol.2017. 89:559–561*

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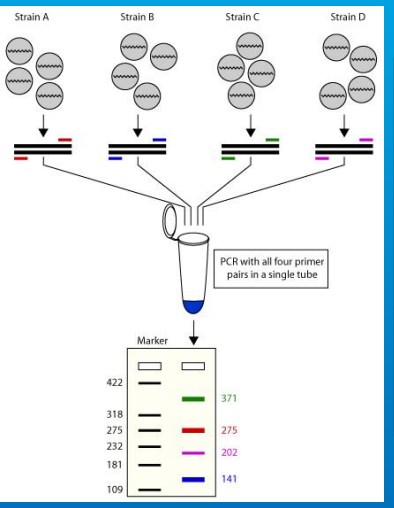
CSF

Blood

Tissue

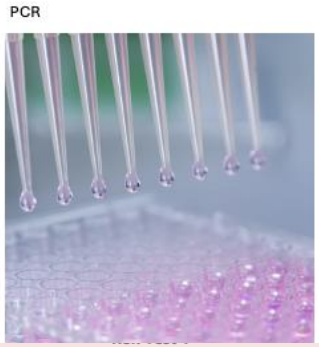
Microbiologic and Molecular Diagnostics: PCR



- **Multi-Plex vs Single-Plex**

- Meningitis Encephalitis Panel
  - Biofire (multi-plex PCR Assay)
    - 6 Bacteria: Ecoli, H.flu, Listeria, N. Meningitidis, S. agalactiae, S. Pneumoniae
    - 6 Viruses: Enterovirus, Parechovirus, CMV, HSV 1&2, VZV, HHV-6,
    - Yeast: C. neoformas/C.gatii

  - If pre-test probability remains high despite negative multi-plex result send single plex PCR assay
    - Cryptococcus
    - HSV 1 and HSV 2



	No. Studies / No. Patients (Ref. studies)	Sensitivity (95%CI) X^2; p value§	Specificity (95%CI). X^2; p value§
All bacteria	16/6183[10,17,22,24–27,29–33]	89.5 (81.1–94.4) 6.00; 0.98	97.4 (94–98.9) 251.9; <0.0001
S. pneumoniae	16/7090[10,17–22,24–26,30,34]	87.5 (77–94) 3.71; 0.999	98.5 (97–99.3) 144.7; <0.0001
H. influenzae	10/4959[10,17,18,20–22,24,25,30,32]	64.9 (39.5–84) 4.91; 0.842	99.4 (98.9–99.6) 22.4; 0.07
S. agalactiae	10/5266[10,17,18,20,22,25–27,31,33]	71.5 (49.6–86.5) 7.67; 0.56	99.5 (98.5–99.9) 7.67; 0.56
E. coli	11/4743[10,17–21,25,27,30,32,33]	70.9 (50.2–85.5) 4.93; 0.896	99.6 (99.1–99.8) 25.5; 0.0043
N. meningitidis	10/3501[17,18,20–22,24,25,29–31]	74.5 (52.9–88.4) 2.26; 0.986	99.1 (98.6–99.5) 20.9; 0.013
L. *monocytogenes*	7/1332[18,21,24,25,29,31,32]	70.4 (40–89.5) 0.504; 0.008	98.9 (96.9–99.6) 5.62; 0.22
Enterovirus	3/6883[10,22,23]	93.8 (87–97.2) 2.91; 0.23	99.3 (98.7–99.7) 28.53; <0.001
HSV-1	3/6883[10,22,23]	75.5 (51.2–90.1) 1.18;0.554	99.9 (94.7–100) 2.55;0.28
HSV-2	3/6883[10,22,23]	94.4 (83.9–98.2) 0.435;0.804	99.9 (99.7–100) 1.36;0.507
VZV	4/6897[10,21,23,29]	91.4 (78.9–96.9) 0.82;0.84	99.8 (98.7–100) 23.55;<0.001

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Waldrop et al..2022.Arch Pathol Lab Med.
Trujillo-Gómez .2022. E clinical Medicine. 2022;44:101275.

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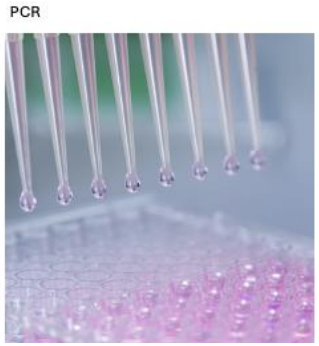
CSF

Blood

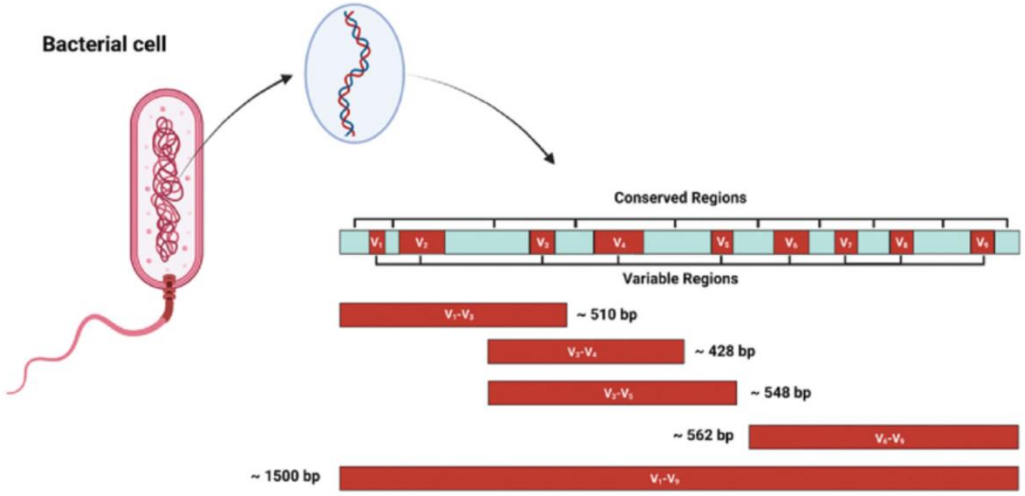
Tissue

Microbiologic and Molecular Diagnostics: PCR



- Universal Bacterial PCR
  - 16s recombinant ribosomal RNA (rRNA) highly conserved gene on all bacteria
    - Conserved Region
      - Present in All Bacteria
    - Hypervariable region
      - Specific to species
- Universal Fungal PCR
  - 18s and 28s rRNA
    - ITS1, ITS4
      - Present in all Fungi
    - ITS2
      - Specific to species

- Meta-Analysis of CSF 16s
- Sensitivity 47-100% | Specificity 77-100%
- Pooled Sensitivity 92% | Specificity 94%



Clarridge et al Clin Microbiol Rev. 2004 Oct;17(4):840-62
Srinivasan et al.Ann Emerg Med. 2012 Nov;60(5):609-620.e2
Lourenco et al Italian Journal of Animal Science 21(1):899-913

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CSF

Blood

Tissue

Microbiologic and Molecular Diagnostics: mNGS

- Introduced, *clinically for CSF,* ~5 years ago
- Turn Around Time: 1 -10 days
- There is no “Panel” nor Organism-Specific “Primers”
- All DNA and RNA (Human, Viral, Bacterial, Fungal, Parasitic, Amoebic Species *{with nucleic acid present in sample}* are sequenced and amplified.
- Computationally, human DNA are filtered out and then all amplified sequences matched to publicly available databases to confirm infectious etiology.

Wilson MR et al.,. N Engl J Med. 2019 Jun 13;380(24):2327-2340.;
Ramachandran PS et al Nat Rev Neurol. 2020 Oct;16(10):547-556.;.

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CSF

Blood

Body Fluids

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Microbiologic and Molecular Diagnostics: mNGS

Advantages

- Hypothesis Free

ORIGINAL ARTICLE BRIEF REPORT

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

- Promise for identifying novel neurotropic disease

JOURNAL ARTICLE

Diagnosis of Neuroinvasive Astrovirus Infection in an Immunocompromised Adult With Encephalitis by Unbiased Next-Generation Sequencing

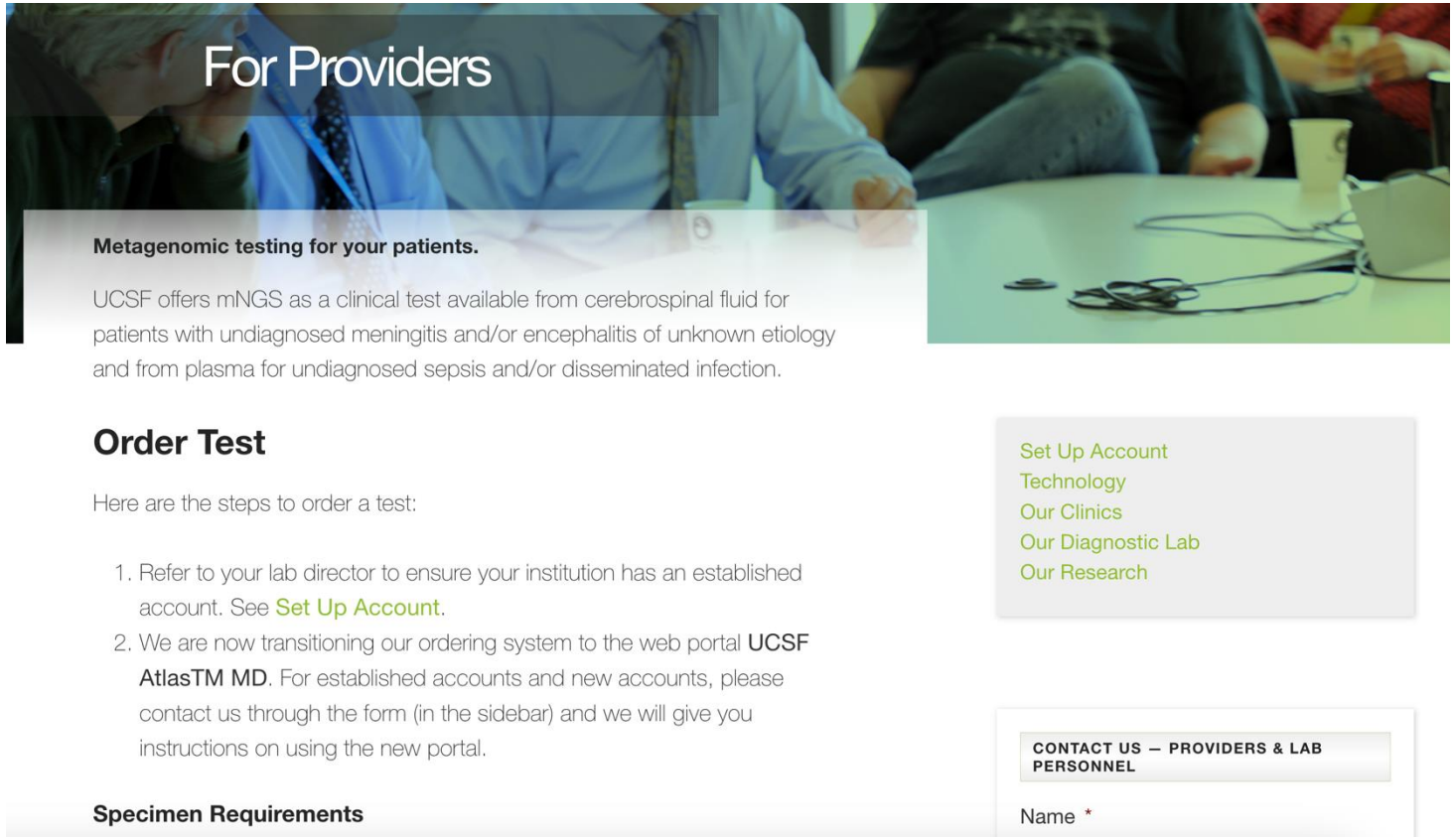
- Contamination Controls
  - Local water samples
- Low sample volume required
  - 600uL

Disadvantages

- Insensitive When
  - Low Pathogen Loads
    - Thresholds for reporting
  - High human DNA (CSF WBC >500)
- Long Turn Around Time
  - Bioinformatics Pipelines developing
- Often Requires Interpretation and Orthogonal Confirmatory Testing
  - No Gold Standard Comparison
- Cost ($1000 – $2,500)
- Not Widely Available

Ramachandran et al. Neurol Clin. 2018. Nov; 36(4):687 -703
Wilson MR et al.,. N Engl J Med. 2019 Jun 13;380(24):2327-2340
Ramachandran PS et al Nat Rev Neurol. 2020 Oct;16(10):547-556.

Referral Logistics for *Clinical* mNGS at UCSF



Bacterial Meningitis: Beyond the Basic Profile

Organism	CSF Gold Standard	Sensitivity/Specificity (%)				Serum Testing	Notes
		GS/Cx	Serology	Antigen	PCR		
S. pneumoniae	Culture	90	--	69-100 / 96	88 / 100	+ Blood Cx in 80%	Antigen/PCR particularly useful after ABX
N. meingitidis	Culture	75	--	33-70 / 100	75 / 99	+ Blood Cx in 90%	
Group B Streptococcus	Culture		--	79/100	72 / 100		
H. influenzae	Culture	86	--	78-86 / 100	65 / 99	+Blood Cx in 94%	
L. monocytogenes	Culture	<50	--	--	70 / 99	+ Blood Cx in 46%–63%	
T. pallidum	Serology * (VDRL)	--	49-88 / 74-100	--	43 / 97	Required for Diagnosis	Diagnosis guidelines suggest combination testing (treponemal, non-treponemal) for highest sensitivity/specificity. Varies by Stage of Disease

Viral Meningitis: Beyond the Basic Profile

Organism	CSF Gold Standard	Sensitivity/Specificity			
		Cx	Serology	Antigen	PCR
EBV	PCR	--	--	--	97 / 100
HSV 1/2	PCR	--	--	--	>95 / 100
VZV	PCR – Encephalitis/Meningitis Serology – Myelitis/Vasculitis	--	77-100% / --	--	80-90 / 100
WNV	Serology* (IgM)	--	86%–96% / 100%	--	50-60 / --
JCV	PCR	--	--	--	74-92 / 92 -96

*consider Urine Serology in Acute Period

Questions?