Direct nucleic acid diagnostic tests for bacterial infectious diseases:

Streptococcal pharyngitis, pulmonary tuberculosis, vaginitis, chlamydial and gonococcal infections

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OBJECTIVES:

1. Recognize three reasons for requiring rapid, accurate diagnosis for bacterial infectious diseases.
2. Recognize the advantages of a direct nucleic acid tests (NAT) over other methods.
3. Recognize the limitations of nucleic acid testing, and methods to identify and reduce them.
4. Discuss when culture is preferred to direct NAT testing.
5. Recognize the first NAT and nucleic acid amplification test (NAAT) to receive FDA clearance.
6. Determine the differences between protocols for NATs.
7. List the current direct NATs that are FDA approved.
8. Discuss methods of increasing sensitivity of NATs.
9. Recognize the difference between target amplification and signal amplification.
10. Given a scenario, determine the correct course of action from the standard algorithms.
11. List in order of preference the CDC recommendations for sexually transmitted disease testing and identify the rationale for the ranking.

Accurate and rapid diagnosis is key to implement adequate disease treatment and to prevent the spread of infectious disease. An infectious disease diagnostic assay should provide rapid, highly sensitive, and specific results. An insensitive assay can result in misdiagnosis of a truly infected patient, leading to lack of treatment and spread of disease. A nonspecific assay can result in false diagnosis of disease in a healthy patient, resulting in inappropriate treatment and, in some cases, psychological trauma. Absence of reliable diagnostic methods resulting in erroneous diagnosis may have serious consequences, both to the individual patient and to public health in general. Recent developments in molecular diagnostic methods have made possible more sensitive, specific, and rapid diagnostic tests for infectious disease compared to traditional microbiology techniques.

Evolution of diagnostic methods for detection of bacterial infections

For many decades, the standard methods for detection of bacteria in patient specimens were culture (liquid and/or solid media) and staining methods, such as acid-fast bacilli smear staining for *Mycobacterium tuberculosis* (Mtb) and Gram stain for *Neisseria gonorrhoeae* (GC). Less commonly, enzyme immunoassays (EIAs) and direct fluorescent antibody (DFA) tests are used to detect bacterial antigens using specific antibodies.

Clinical diagnostic methods were revolutionized by the development of nucleic acid tests (NATs), which use molecular biology techniques to detect microorganisms' nucleic acids. There are two main types of NATs used for microorganism identification: culture confirmation tests and direct tests. Culture confirmation NATs identify organisms grown in culture. Direct NATs detect organisms directly in the specimens without the need for culture. Direct NATs have a faster time to result compared with culture confirmation probe tests because they avoid culture altogether, and they are generally more accurate than direct immunological methods. The first direct nonradioactive NAT to receive Food and Drug Administration (FDA) clearance was the Gen-Probe PACE test for chlamydial[1] and gonococcal infections (1987).

A crucial technological improvement to direct NATs was made by adding a target amplification step, which amplifies the target sequence prior to probe detection. The first nucleic acid amplification tests (NAATs) received FDA clearance in

1993 for detection of *Chlamydia trachomatis*. Since then, improvements have been made in nucleic acid amplification technology with the development of second-generation NAATs that have minimized inhibition from sample components and improved workflow.

This review focuses on the commercially available, FDA-cleared or approved direct NATs for the detection of five bacterial infections: streptococcal pharyngitis (strep throat), pulmonary tuberculosis (TB), vaginitis, and chlamydial (CT) and gonococcal (GC) infections.

Direct NAT methodologies

Nonamplified direct NATs (see Table 1). Most commercially available direct NATS utilize nucleic acid probes that are specific for a unique nucleic acid sequence ("the target sequence") present in the organism to be detected ("the target organism"). The probes are usually labeled with fluorescent or chemiluminescent labels. The sample is treated to release

Table 1. Commercially available direct NAT technologies for bacterial detection

Type of NAT	Technology	Nucleic acid target	Label type	Commercial source
Nontarget-amplified	Hybridization protection assay (HPA)	rRNA	Chemiluminescence	Gen-Probe Inc.
	Solid phase capture	rRNA	Colorimetric	Becton-Dickinson and Company
	Hybrid Capture	DNA	Chemiluminescence	Digene Corp.
Target-amplified	PCR	DNA	Colorimetric	Roche Diagnostics
	SDA	DNA	Fluorescence	Becton-Dickinson and Company
	TMA	rRNA	Chemiluminescence	Gen-Probe Inc.

nucleic acids from the target organism, if it is present. Following this, the labeled DNA probe specifically combines with the target sequence to form a stable probe-target sequence hybrid. The hybrid is separated or discriminated from nonhybridized probes, and the signal emitted by label in the hybrid is measured.

NATs use various ways to increase the analytical sensitivity of the tests for direct detection of microorganisms in clinical specimens. One strategy patented by Gen-Probe is to target ribosomal RNA (rRNA), which exists in thousands of copies in most microorganisms. For example, rRNA is present in up to 2,000 copies in *Chlamydia trachomatis*, in contrast to useful target sequences in genomic DNA, which are present in only one to a few copies per bacterium. This greatly increases the sensitivity of the assay because there is so much more target molecule available to form hybrids. In the case of the Gen-Probe assays, sensitivity was further increased by use of the hybridization protection assay (HPA) method for hybrid detection (described below). The combination of rRNA targeting and HPA technology led to the first DNA probe tests widely used in the clinical laboratory for both culture confirmation, as well as direct detection.

A second strategy used to increase the sensitivity of DNA probe assays is to amplify the signal molecules. An example of this is the Hybrid Capture assay (Digene Corp.), which uses antibodies that bind to RNA:DNA hybrids to detect hybrid formation. Each antibody carries an enzyme label that is used to generate a colored product. Each enzyme molecule makes multiple colored product molecules for each hybrid molecule to which it binds. Methods such as this, in which multiple signal molecules are generated from one hybrid molecule, are

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The role of esoteric reference laboratory as a partner for growth

By Ronald A. Blum, PhD, and Linda Dearing, MA

As the lab industry moves closer to "personalized medicine," the complexity of testing and information management will increase significantly. It is expected that within the next five to six years, molecular diagnostics will represent about 20% of total lab testing, up from around 7% currently.

With this projected increase in sophisticated testing and the shortage of certified staff, the esoteric reference lab is likely to become even more important to hospital and clinic laboratories. Laboratories are looking for ways to expand their menu, offer unique testing services, and grow their client base, while at the same time to reduce or hold the line on costs. The hospital laboratory can resolve many of these issues by partnering with an esoteric reference laboratory, which can serve as a partner to the referring lab in three important ways by:

- providing specialized testing needed in meeting the needs of the community;
- helping educate physicians and medical staff on the latest developments in diagnosis of disease and management of patient therapy; and
- supporting outreach, education, and consultation programs and — in some relationships — offering test-ordering-and-resulting system technologies.

A partner for comprehensive test services

The esoteric reference laboratory traditionally provides specialized testing that requires instrumentation and/or technical staff that are not financially feasible for most hospitals to sustain. While routine test volume is sufficient to warrant expenditures for improved methodologies, most hospitals prefer to send out rare or complex assays to a laboratory that specializes in esoteric testing. The major esoteric labs serve a national client base and generally offer:

- state-of-the-art, automated instrumentation for optimal test efficiency and quality control;
- a comprehensive or niche organized menu of assays to supplement the hospital's test offerings;
- highly trained professional staff with expertise in particular arenas of technology or medical specialty; and
- new testing [by a few esoteric labs] on a regular basis to meet the needs of the community either through research and development or technology transfer of existing technology

As advances are made in laboratory testing, esoteric labs are moving to provide more:

- multianalyte testing on limited specimen quantities;
- molecular diagnostics (genomics, proteomics, pharmacogenomics) for patient-specific risk assessment, treatment selection, and treatment monitoring; and
- automation of processing and analysis for more cost-efficient, accurate results and customized reporting.

One example of the variety and complexity of esoteric testing is in the realm of HIV patient management. The physician managing an HIV-positive patient must monitor him closely

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known as "signal-amplification" methods. Signal-amplification methods are usually significantly less sensitive and specific than those using a third strategy for improving assay performance called "target amplification."

Target-amplified direct nucleic acid amplification tests (see Table 1). Target amplification increases assay sensitivity by producing millions of copies of the target sequence in a test tube, similar to the way culture increases detection sensitivity by producing more copies of the target DNA or RNA inside the bacteria as they multiply. Target amplification, however, is much faster than culture; and it can be used to detect bacteria that are difficult or impossible to grow in the laboratory. The replicated sequences, or "amplicons," are

Table 2. Commercially available FDA-cleared direct NATs for bacterial infectious diseases

Infectious disease	Organism detected	Manufacturer, assay	Assay technology	Specimen type(s) (FDA-cleared/approved)
Group A streptococcal pharyngitis (strep throat)	*Streptococcus pyogenes*	Gen-Probe, GAS Direct Test[3]	HPA	· Throat swabs
Pulmonary tuberculosis	*Mycobacterium tuberculosis* complex	Roche, AMPLICOR MTB[4]	PCR	· Respiratory specimens · Smear-positive samples only
		Gen-Probe, AMPLIFIED *Mycobacterium tuberculosis* Direct Test (MTD)[5]	TMA	· Respiratory specimens · Smear-positive and -negative samples
Chlamydial infection	*Chlamydia trachomatis*	Gen-Probe, PACE 2 CT[1]	HPA	· Female endocervical swabs · Male urethral swabs · Conjunctival swabs
		Digene, Hybrid Capture II CT	Hybrid Capture	· Female endocervical swabs
		Roche, AMPLICOR CT[1]	PCR	· Female endocervical swabs · Male urethral swabs · Male and female urine
		Becton Dickinson, BDProbeTec ET CT/GC[1]	SDA	· Female endocervical swabs · Male urethral swabs · Male and female urine
		Gen-Probe, APTIMA Combo 2[1]	TMA	· Female endocervical swabs · Male urethral swabs · Male and female urine · Vaginal swabs (pending FDA approval)
Gonococcal infection	*Neisseria gonorrhoeae*	Gen-Probe, PACE 2 GC	HPA	· Female endocervical swabs · Male urethral swabs
		Digene, Hybrid Capture II GC	Hybrid Capture	· Female endocervical swabs
		Roche, AMPLICOR GC	PCR	· Female endocervical swabs · Male urethral swabs, symptomatic patients only · Male urine
		Becton Dickinson, BDProbeTec ET CT/GC	SDA	· Female endocervical swabs · Male urethral swabs · Male and female urine
		Gen-Probe, APTIMA Combo 2[1]	TMA	· Female endocervical swabs · Male urethral swabs · Male and female urine · Vaginal swabs (pending FDA approval)
Vaginitis	*Gardnerella vaginalis*, *Trichomonas vaginalis*, *Candida spp.*	Becton Dickinson Affirm VPIII Test[1]	Solid Phase Capture	· Vaginal swabs

usually identified using labeled DNA probes. Several target-amplification methods are currently in widespread use for the identification of bacteria, including the polymerase chain reaction (PCR), strand displacement amplification (SDA), and transcription-mediated amplification (TMA) (Table 1).

PCR.[2] The DNA target sequence is heat denatured and then amplified by a DNA polymerase. Primers that bind specifically to the ends of the target sequence direct the DNA polymerase to copy only that sequence. Each round of DNA synthesis is followed by a heat-denaturation step to separate the newly synthesized DNA strand from the strand from which it was copied. A special instrument called a "thermocycler" is needed to carry out the PCR process. The number of target molecules doubles each round. PCR copies only DNA; therefore, if the target sequence is RNA, the RNA must first be converted to DNA using a separate enzymatic reaction. In most of the FDA-approved PCR tests, detection of amplicons is performed using a colorimetric reaction, which is detected in a spectrophotometer.

SDA[3] is an amplification process that, in contrast to PCR, is carried out at one temperature. This "isothermal" method also uses primers to define the DNA sequence to be amplified; however, instead of using heat denaturation to separate the double-stranded DNAs that are made, a special enzyme called a "restriction endonuclease" is used to cleave the primer of the newly synthesized DNA. DNA polymerase recognizes the break and re-initiates DNA synthesis at that point, displacing the previously made strand as it proceeds to copy the DNA once more. In this way, multiple DNA copies are generated that can, themselves, re-enter the SDA process. Like PCR, SDA only amplifies DNA. If the target is RNA, it must first be converted to DNA in order to be amplified. Homogeneous detection* is performed using a special fluorescent probe called a "molecular beacon" that is present in the SDA reaction. This probe produces signal when it hybridizes to amplicon generated as the amplification reaction proceeds and changes its configuration.

TMA[4,5] is an isothermal process that amplifies RNA or DNA molecules, using primers to define the target sequences that is amplified and two enzymes: reverse transcriptase (RT) and RNA polymerase. The reverse transcriptase makes double-stranded DNA copies that are used by the RNA polymerase to make multiple single-stranded RNA copies. The RNA copies are then used by the reverse transcriptase to make more DNA copies to be copied by the RNA polymerase. The result is an automatic amplification process in which TMA generates up to 10-billionfold amplification of the target sequence within 15 to 30 minutes. Homogeneous detection* of the amplicon is performed using the HPA method. HPA uses acridinium ester-labeled probes, which hybridize specifically with the amplicons. After amplification, a "selection reagent" destroys the acridinium ester on the unhybridized probes, while the acridinium ester on hybridized probes survives because the label is protected within the hybrid structure. A special instrument called a "luminometer" is used to add reagents that cause the acridinium ester to emit light and to measure the amount of light produced.

Compare direct NATs with culture/immunological methods

The advantages of direct NATs over other common microbiology methods are:

- Rapid turnaround time (one to six hours). Although this is similar to EIA, it represents a significant improvement over culture, which takes between 24 and 72 hours (CT detection) to over eight weeks (Mtb detection). Rapid assays lead to faster patient treatment and, in some cases, can increase public health safety (e.g., patient isolation to avoid spreading of disease).
- Higher throughput compared to culture. Hundreds of samples may be processed at the same time with some direct NATs, saving time and lowering cost. An increasing trend towards assay automation is also reducing the workload associated with these assays.
- High sensitivity of NAATs. NAATs can theoretically detect as little as one organism per sample, while the detection threshold of other methods, such as EIA, is often greater than 1,000 organisms per sample.[1,6] With clinical sensitivities generally greater than 95%, NAATs are usu-

*In homogeneous detection, hybridized and nonhybridized probes are not physically separated using a wash step, but can be discriminated based upon the different properties of the hybridized and unhybridized states.

ally equivalent or more sensitive than standard culture methods, especially with certain types of specimens (e.g., detection of CT in urine specimens).
- Specimen stability is usually much improved for direct NATs since viable organisms are not required for detection. Specimens for direct NATs can be stored for longer periods of time and at greater temperature ranges than culture specimens.

The potential limitations of direct NATs are:
- In the case of some first-generation NAATs, amplification inhibition may lower the sensitivity of the assays, such as with CT assays using urine specimens.[6,7] Inhibition is usually sample-specific and may cause false-negative results. Inhibition may be controlled by monitoring with internal or amplification controls[2,3], or it can be eliminated by sample processing using techniques such as target capture.[4,5,7] Despite occasional inhibition, most NAATs are still clinically much more sensitive than nonamplified direct NATs or EIAs.
- Carry-over contamination of amplicon between samples may be an issue with NAATs, but not with nonamplified direct NATs.[6] Carry-over contamination can be minimized by carefully performing the test procedure and by implementing good laboratory practices.
- Current FDA-approved NAATs do not test for drug resistance. In cases where drug-resistance data are needed, such as for Mtb diagnosis, both NAAT and culture are performed. The NAAT rapidly identifies Mtb leading to timely treatment decisions, while culture (which takes up to eight weeks) allows confirmation of diagnosis and determination of drug resistance.
- The cost of NAATs is often higher than traditional microbiology and EIA methods. One exception is CT culture, which is typically difficult to perform and more costly than NAATs.

Direct NATs for bacterial infectious diseases (see Table 2)

Group A streptococcal pharyngitis. The gold standard for group A streptococcus (GAS) detection in patients with pharyngitis is culture from a throat swab specimen on multiple media due to its high sensitivity. Throat culture, however, requires 24 to 48 hours for results. Rapid antigen detection assays (RADTs) have been developed for faster turnaround time and convenience, but the assays are generally less sensitive than culture. Most authorities recommend that all negative RADTs be confirmed by throat culture when the patient is a child or adolescent, and also whenever the physician is uncertain that the RADT being used is as sensitive as throat culture in his practice setting.

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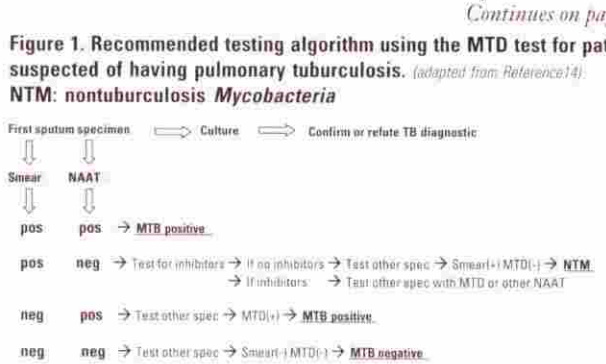
Figure 1. Recommended testing algorithm using the MTD test for patients suspected of having pulmonary tuburculosis. *(adapted from Reference14)*
NTM: nontuburculosis *Mycobacteria*



and respond quickly to changes in drug efficacy. Rapid advances in viral load testing, genotyping, and drug-level monitoring can provide hospitals with the very latest testing to meet the urgent needs of physicians. Highly complex, rapidly changing modalities require a high level of expertise to perform and interpret within the context of a changing clinical picture. In addition, patient management also necessitates careful assessment for the opportunistic infections that can develop in this patient population. The hospital laboratory can partner with a full-service esoteric laboratory to provide single-source send-out testing for greater economy and faster turnaround times.

A partner in education and outreach

The reference lab may also be a key source of information and education on emerging diseases, advances in testing, and changes in patient-management standard-of-care algorithms. The reference lab can work with the hospital to provide educational materials, speakers, and online resources to help educate physicians on the use and interpretation of esoteric tests. These activities can help drive additional test volume through the hospital to the benefit of both the hospital and the send-out esoteric laboratory. The physician benefits not only from the knowledge transfer but also from the ease of using his local hospital to order both routine and esoteric testing.

Outreach programs are a way for existing hospital-based labs to grow their business by garnering a greater share of a currently aligned physicians' test ordering and by attracting the work of physicians not previously using the hospital laboratory for routine or esoteric testing. By partnering with an esoteric reference lab, the hospital lab can significantly expand its effective test menu and offer additional services to draw work from general and specialist physicians throughout the community.

A partner for information technology

One way of supporting outreach is to offer improved methods for electronic test ordering and resulting to provide the physician with ease and adaptability. Ours was one of the first reference laboratories to develop a PC-based program that a physician can access via the Internet, thereby increasing the hospital's connectivity with its client base. The next generation of outreach laboratory information system or LIS links are Web-based order-entry-and-resulting systems, specifically designed for hospitals to connect to physicians' offices, nursing facilities, and clinics. Web-based outreach can increase the hospital's laboratory revenue, capture patient billing information from point of care, supply regulatory coding guidelines, and help create a competitive edge for the hospital in the medical community.

Summary

Partnering with a reference lab can actually help grow a referring lab's own business. The reference lab provides a hospital's physicians with access to unique tests and clinical information and broadens the scope and depth of the referring lab's menu. As medicine moves toward prescribed treatments based on a patient's individual genotype or phenotype, the reference lab will play an even bigger role in helping the hospital to educate physicians and their patients to the most efficient testing strategies consistent with good patient management. □

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The Gen-Probe GASDirect Test[8] (Table 2) is the only FDA-cleared direct NAT for the detection of GAS in throat samples. GASDirect uses the HPA method to detect GAS rRNA sequences. With a sensitivity of about 90% and a specificity ≥98%, GASDirect is superior to RADTs and similar to culture with regard to sensitivity.[8,12] The GASDirect test provides results in 60 minutes and has a cost per sample lower than throat culture but similar to that of RADTs. Thus, GASDirect may be a useful alternative to RADTs for the rapid initial detection of GAS.[12]

Pulmonary tuberculosis (TB). The current guidelines from the American Thoracic Society and the Centers for Disease Control and Prevention (CDC) for laboratory testing require fluorescent acid-fast staining (AFB smear), as well as culture on both liquid and solid media. Smears are rapid (several hours) but have poor sensitivity. Culture is sensitive but requires two to eight weeks for results. The Gen-Probe AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) Test[10] (the first FDA-cleared NAAT for Mtb detection) and Roche AMPLICOR MTB[9] tests (Table 2) have been integrated into the arsenal of methods for TB diagnosis. The two assays are approved for respiratory specimens only and can be used for the initial diagnosis of TB but not for monitoring treatment.[10]

When compared with culture or with the patient final diagnosis, these assays have a sensitivity and specificity similar to culture[9,10,13], but unlike culture, commercially available NAATs can be performed in one day, thus providing rapid diagnostic results to the physician. NAATs were initially approved by the FDA only for patients with a positive AFB smear. The MTD assay has also been approved for testing of smear-negative patients suspected of having TB.[14] This application of MTD allows the rapid detection of infected individuals from samples that contain low numbers of mycobacteria that may be very difficult to detect when examining stained smears. The CDC-recommended algorithm for MTB detection using the MTD assay is summarized in Figure 1.

Vaginitis. Organisms associated with vaginitis include *Gardnerella vaginalis*, *Trichomonas vaginalis*, and/or *Candida* (yeast). Traditional methods for the diagnosis of vaginitis include culture and slide microscopy (wet mount) from vaginal specimens. Culture is sensitive, but slow, costly, and labor-intensive. Slide microscopy is rapid, but has a low sensitivity for some of the organisms involved in vaginitis (e.g., ≤55% for *Candida* and *Trichomonas*), and the accuracy of the method often depends on the expertise of the microscopist. Becton-Dickinson's BD Affirm VPIII assay[11] (Table 2) is a NAT that uses two probes for each organism: a capture probe and a detection probe. The target sequence first hybridizes with the capture probe, which is immobilized onto a bead, and then with the detection probe. Unbound sample material and probes are then washed away, and the detection probe is bound to a detection enzyme. After several additional washes to remove excess enzyme, enzyme substrate is added and is converted into a blue-colored product. The test results are determined visually. The Affirm VPIII test is capable of consistently detecting all three organisms with a high sensitivity (sensitivity vs. culture is 81 % for *Candida*, 89% for *Gardnerella*, and 92% for *Trichomonas*).[11]

Table 3. Comparison of four FDA-cleared direct NATs for the detection of CT

Assay	Technology	Target sequence	Swab specimens CT sensitivity #	Urine specimens CT sensitivity
Gen-Probe PACE 2 CT	HPA	rRNA	70% to 90%	Not applicable
Roche AMPLICOR CT	PCR	DNA	>90%	80% to 90%
Becton-Dickinson BDProbeTec CT/GC	SDA	DNA	>90%	80% to 90%
Gen-Probe APTIMA Combo 2 CT/GC	TMA	rRNA	>95%	>90%

 # Sensitivity ranges were estimated using published results, reviews, comparative studies, meta analyses, and the manufacturers' package inserts.

Chlamydial and gonococcal infections. Laboratory tests for CT and GC detection include Gram stain for GC, culture, non-amplified NATs, NAATs, EIAs, and DFA tests.

Nonamplified NATs. Gen-Probe's DNA probe assays for the detection of CT, GC, or both (PACE 2 CT[1], PACE 2 GC, or PACE 2C) are approved by the FDA for diagnosis from endocervical, male urethral specimens, and conjunctival specimens (CT only). Using a single swab specimen, the PACE 2 test detects either organism or both together (PACE 2C). PACE 2 continues to be the most commonly used test for CT and nonculture GC detection in clinical laboratories in the United States. The PACE 2 assays use the HPA method to detect rRNA from CT and GC. The sensitivity and specificity of the PACE 2 assays compare favorably to culture. The sensitivity of the PACE 2 GC assay is similar to NAATs, but the PACE 2 CT assay is somewhat less sensitive than NAATs (Table 3).

NAATs. The Roche AMPLICOR test is a first-generation NAAT based on PCR.[2] The BDProbeTec assay is a first-generation NAAT based on SDA.[3] These assays target sequences present in genomic DNA or in plasmids that are usually, but not always, present in the organisms. Two BDProbeTec assays are available: BDProbeTec CT[3] which detects CT only, and BDProbeTec CT/GC which detects both CT and GC simultaneously in one specimen. The most recently introduced CT/GC NAAT is the Gen-Probe APTIMA Combo 2 assay. This is a second-generation NAAT based on TMA, which allows the detection and identification of both CT and GC in a single specimen by targeting CT and GC rRNA molecules simultaneously.[4,5] The assay includes a unique target capture method of sample preparation, which purifies and concentrates the target molecules, thereby removing inhibitors that may be present in the specimen. The target-capture method uses a specific capture probe to capture target rRNA molecules onto magnetic particles, which allows unwanted sample components to be washed away. The purified and concentrated target is then amplified by TMA.

Table 4. CDC recommendations for screening men and women for CT and GC genitourinary infections

(adapted from Reference 15)

Organism	Tests recommended (in order of preference)
C. trachomatis	- NAAT on swab or urine - Nonamplified NAT, EIA, or DFA on swab - Culture on swab
N. gonorrhoeae	- Culture on swab - NAAT or nonamplified NAT on swab - NAAT on urine

Note: Swab specimens recommended are endocervical swab and male intraurethral swab.

Sensitivity and specificity of direct NAATs for CT and GC detection. NAATs for CT are usually more sensitive than nonamplified NATs and CT culture, while NAATs for GC are similar in sensitivity to both culture and nonamplified NATs. All CT NAATs generally have a high sensitivity with swab specimens (>90%), with APTIMA Combo 2 often showing the highest sensitivity (>95%).[16,17]

For urogenital specimens, PACE 2 assay kits are approved for the detection of CT and GC in male urethral and female endocervical swab specimens only. Testing with NAATs, however, may be performed not only on swabs but also on urine specimens. Urine collection is noninvasive and results in better patient acceptance, reduced staff workload, and decreased cost of testing. The sensitivity of first-generation NAATs is typically, however, 5% to 10% lower with urine specimens than with swab specimens (especially female urine specimens), due to a high level of inhibitory substances. The PCR method (AMPLICOR) is not cleared for GC detection in female urine specimens. The SDA method is approved for testing male and female urine specimens, but the assay sensitivity with female urine is typically lower than with endocervical swabs (by approximately 10%). The target capture method of APTIMA Combo 2 decreases inhibition[7], therefore ensuring a consistent high performance of the assay with urine specimens (sensitivity 91% to 100%).[16,17]

The CDC recently published guidelines for CT and GC testing (Table 4).[15] For CT infections, NAATs are recommended due to their high sensitivity, but probe tests and EIAs are also acceptable. For GC infections, culture on swab specimens is highly sensitive and can also assess drug resistance. NAATs and probe tests have similar sensitivity to GC culture and are particularly recommended, if transport and storage conditions are not conducive to maintaining the viability of GC organisms for culture. For both CT and GC infections, male urethral and female endocervical swabs are the recommended specimens for all testing methods. NAATs may be conveniently performed on urine specimens if the invasive swab-collection method is not acceptable or not possible. The CDC has recommended verifying some NAAT-positive results to rule out false-positive results due to carry-over contamination or to nonspecific reaction of some first-generation NAATs with nongonococcal *Neisseria* species. Additional NAAT testing should be performed to confirm positive CT/GC NAAT results in a low-prevalence population to rule out the possibility of false-positive results that can have adverse medical, social, or psychological impacts. Verification of positives should be performed with a different NAAT, or a NAAT using the same technology but targeting different CT/GC sequences, to help rule out false-positive results.

Future development of direct NATs

Future developments include increased automation to make direct NATs easier to run. Most of the current direct NAT instrumentation platforms only automate one part of the assay (e.g., amplification or detection steps). New, fully automated instruments such as the TIGRIS DTS system (Gen-Probe Inc.) automate all assay steps with a throughput of up to 1,000 tests per 12-hour day.

Some NAATs that used cumbersome and lengthy detection methods are beginning to use probes that detect amplicons as they are made in the amplification reaction ("real-time detection"). Real-time amplification technologies are especially useful for quantitative assays, which are important in some diagnostic applications.

Progress is also being made in developing multiplex testing to allow detection of greater numbers of organisms in a single test. The use of amplification technologies, together with biochip and microfluidic technologies, may ultimately lead to the ability to rapidly and cost effectively detect dozens of different organisms in a single sample using a single test.

Conclusion

Direct NATs represent a breakthrough in diagnostic methods for bacterial infections. The use of direct NATs in clinical laboratories is constantly increasing because these assays offer a rapid turnaround time, high accuracy, and specimen flexibility. Direct NATs are increasingly replacing culture and other conventional methods for the diagnosis of many bacterial infections, such as CT infection. In the case of TB diagnosis, where species identification and drug-resistance profiling is required, NAATs have not completely replaced culture; instead, NAATs and culture, which complement each other, are both integrated in the diagnostic algorithm. Future development of NAATs for Mtb includes multiplex assays that can detect multiple mycobacterial species, together with certain types of drug resistance (e.g., rifampin resistance). Newer generations of direct NATs are constantly being developed to improve the clinical laboratory's ability to detect infectious diseases, and will ultimately result in better patient care. □

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CE test on DIRECT NUCLEIC ACID DIAGNOSTIC TESTS FOR BACTERIAL INFECTIOUS DISEASES

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This test was prepared by by Gail S. Williams, PhD MT(ASCP)SBB, CLS(NCA), Northern Illinois University, College of Health and Human Sciences, Clinical Laboratory Sciences Program, DeKalb, IL.

1. **Detection of bacterial pathogens should be accomplished quickly for all of the following reasons EXCEPT:**
   a. proper therapy can begin sooner
   b. prevent a possible epidemic
   c. probable decrease of time in the hospital for inpatients
   d. insensitive assays will have false negative results
2. **The *specificity* of an assay is important because:**
   a. false positives lead to inappropriate treatment for uninfected patient
   b. false negatives result in missed treatment and spread of disease
   c. false positives lead to reduced antibiotic resistance of normal flora
   d. false negatives may give a patient false hope
3. **When are probe tests on swabs for *Neisseria gonorrhoeae* superior to culture?**
   a. Always
   b. When delayed or poor transport conditions occur
   c. When immediate bedside testing is available
   d. Never
4. **Advantages of direct NATs over other microbiological methods include all of the following EXCEPT:**
   a. one to six hours turnaround time
   b. more tests per hour completed
   c. specimen can be dead
   d. antimicrobial drug resistance is determined quickly
5. **One limitation of NATs or NAATs is that:**
   a. they are too sensitive
   b. amplicon carryover can lead to false positives
   c. cost of testing for all pathogens is higher
   d. they all require expensive instruments and automation
6. **How is elimination of inhibition without losing sensitivity of NAATs accomplished?**
   a. Monitoring with internal controls
   b. Only using urine for *Chlamydia* testing
   c. Extract with a target capture method before amplification
   d. Dilution of the sample by 1000
7. **Culture is preferred to direct NATs for the diagnosis of:**
   a. *Chlamydia trachomatis*
   b. drug-resistant *Mycobacterium tuberculosis*
   c. *Neisseria gonorrhoeae*
   d. *Gardnerella vaginalis*
8. **In 1987, the first NAT probe to receive FDA clearance was used to diagnose:**
   a. *Streptococcus pyogenes* and *S. agalactea*
   b. *Mycobacterium tuberculosis* and *MAC*
   c. *Trichomonas vaginalis* and *Gardnerella vaginalis*
   d. *Chlamydia trachomatis* and *Neisseria gonorrhoeae*
9. **In 1993, the first target amplification test to receive FDA clearance was used to diagnose:**
   a. *Neisseria gonorrhoeae*
   b. *Chlamydia trachomatis*
   c. *Streptococcus pyogenes*
   d. *Mycobacterium tuberculosis*
10. **The majority of probe tests for bacteria look for which nucleic acid. Why?**
    a. rRNA, because it is more specific
    b. rRNA, because there are thousands of copies in each bacteria and that increases sensitivity
    c. DNA, because it is more specific
    d. DNA, because there are more polymorphic options to make specific probes
11. **How does the hybrid capture method increase sensitivity?**
    a. By using rRNA as a target
    b. By amplifying the signal after the capture probe has bound to its DNA target
    c. By amplifying the capture probe before it bends to target DNA
    d. By amplifying rRNA before using the probe
12. **Strand displacement amplification or SDA is different from the polymerase chain reaction or PCR by which of the following:**
    a. SDA uses DNA as the target molecule
    b. SDA uses DNA polymerase to extend primers
    c. in SDA, DNA must be denatured before primer binding
    d. SDA is isothermal

13. One of the key advantages of TMA (used with HPA) over PCR and SDA is that:

a. it is the only isothermal assay
b. it is a homogeneous assay and uses either RNA or DNA
c. fluorescence is measured
d. no specimen processing is required

14. Which of the following is NOT an FDA-cleared test?

a. GASDirect by Gen-Probe
b. Affirm VPIII by Becton-Dickenson
c. Hybrid Capture II GC by Digene
d. TIGRIS DTS by Gen-Probe

15. Select the correct statement regarding the PCR and hybrid capture amplification methods.

a. PCR starts with DNA targets; hybrid capture starts with rRNA
b. Hybrid capture amplifies targets; PCR amplifies signals
c. Hybrid capture requires a thermocycler; PCR does not
d. PCR amplifies target DNA, and hybrid capture amplifies signals

16. Current guidelines for diagnosis of group A streptococcal throat infections are:

a. screen with RADTs or NAT. Follow up all tests with culture
b. culture all specimens. Confirm suspicious colonies with RADTs or NAT
c. screen with RADTs or NAT. Follow up negatives with culture
d. NAT only

17. First sputum specimen is positive with a NAAT and negative for acid-fast bacilli. What else needs to be done?

a. Nothing. It is reported as Mtb
b. Culture for confirmation and drug sensitivity: retest new specimen with AMPLIFIED MTD
c. Only retest new specimen with AMPLIFIED MTD
d. Culture for confirmation

18. What types of specimens for STD testing are most likely to result in false negatives?

a. Male urethral
b. Female endocervical
c. Male urine
d. Female urine

19. According to the CDC, the number one choice for testing *C. trachomatis* is_____ because:

a. NAAT on swab or urine; because it is more rapid, sensitive and specific
b. probe tests for rRNA; they are more specific
c. DFA on swab; you can actually see the elementary bodies
d. culture on swab; culture is always the most definitive method of diagnosis of live organisms

20. According to the CDC, the best test for *N. gonorrhoeae* is_____ because:

a. NAAT on swab; the organism can be dead and still get results
b. probe test on swab; false positives from normal *Neisseria spp.* are less of a problem than with NAAT
c. NAAT on urine; it is easier to obtain
d. culture from swabs; it is highly sensitive and drug sensitivities may be determined

MEDICAL LABORATORY OBSERVER MLO

TEST ANSWER FORM

DIRECT NUCLEIC ACID DIAGNOSTIC TESTS FOR BACTERIAL INFECTIOUS DISEASES — January 2004

(This form may be photocopied; it is no longer valid for CEUs after Jan. 31, 2005)

Shade circles like this: ● Not like this: ⊗

1. To what extent did the article focus on or clarify the objectives?
☐ Excellent ☐ Fair
☐ Good ☐ Poor

2. To what extent was the article well-organized and readable?
☐ Excellent ☐ Fair
☐ Good ☐ Poor

3. Type of lab:
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☐ other

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5. How will you use the CE units?
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6. How long did it take you to complete both the reading and the test? _____ minutes

	A	B	C	D
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5.	○	○	○	○
6.	○	○	○	○
7.	○	○	○	○
8.	○	○	○	○
9.	○	○	○	○
10.	○	○	○	○
11.	○	○	○	○
12.	○	○	○	○
13.	○	○	○	○
14.	○	○	○	○
15.	○	○	○	○
16.	○	○	○	○
17.	○	○	○	○
18.	○	○	○	○
19.	○	○	○	○
20.	○	○	○	○

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