Molecular Bacteriology in the Clinical Laboratory

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LEARNING OBJECTIVES:

1. Discuss the importance of rapid diagnostic assays in the clinical bacteriology laboratory.
2. Compare and contrast the GeneXpert system and the SmartCycler system.
3. Describe clinical applications for both the GeneXpert and SmartCycler systems.
4. Discuss the importance of institutions implementing a MRSA screening and surveillance program.
5. Explain the methodology of the COBAS AMPLICOR CT/NG assay.
6. State the principle of the AMPLIFIED *Mycobacterium tuberculosis* Direct Test
7. List FDA cleared molecular diagnostic bacterial assays.

ABBREVIATIONS: AFB, acid-fast bacilli; CDC, Centers for Disease Control and Prevention; CT, *Chlamydia trachomatis*; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; GBS, group B Streptococcus; GX16, GeneXpert 16; HPA, hybridization protection assay; IVD, *in vitro* diagnostics; MRSA, methicillin-resistant *Staphylococcus aureus;* MTB, *Mycobacterium tuberculosis,* NALC-NaOH, N-acetyl-L-cysteine-sodium hydroxide; NG, *Neisseria gonorrhoeae;* nm, nanometers; PCR, polymerase chain reaction; RLU, relative light units; RNA, ribonucleic acid; STI, sexually transmitted infection; TMA, transcription-mediated amplification; TMB, tetramethylbenzidine; WHO, World Health Organization

INDEX TERMS: Molecular Methods, Real-Time PCR, Cepheid GeneXpert, Cepheid SmartCycler, Methicillin-resistant *Staph aureus*, *Mycobacterium tuberculosis*, Vancomycin-resistant Enterococcus, COBAS AMPLICOR CT/NG test, AMPLIFIED *Mycobacterium tuberculosis* Direct Test, FDA cleared assays

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Molecular diagnostic assays are becoming the "norm" in the clinical microbiology laboratory, especially in bacteriology. Routine cultures using conventional media require 24 to 72 hours or longer to generate a final report. Acid-fast bacilli (AFB) cultures for *Mycobacterium tuberculosis* (MTB) using standard AFB media require up to six weeks to grow and be identified. Although health care providers are aware cultures may take several days or weeks before a definitive identification and susceptibility test can be made, this turn around time is not acceptable when dealing with virulent organisms such as methicillin-resistant *Staph aureus* (MRSA), MTB, and vancomycin-resistant *Enterococcus* (VRE). Physicians need to know within hours whether their patient is suffering from a dangerous infection that requires isolation and aggressive treatment. Fortunately, there are several FDA cleared assays and platforms that can generate results of pathogenic and fastidious organisms within a few hours. For example, some of the most widely used FDA cleared *in vitro* molecular diagnostic systems and tests on the market are the GeneXpert® and SmartCycler® Systems by Cepheid (Sunnyvale, California), the

COBAS AMPLICOR *Chlamydia trachomatis* (CT)/ *Neisseria gonorrhoeae* (NG) test by Roche Diagnostics, Inc. (Pleasanton, California), and the AMPLIFIED *Mycobacterium tuberculosis* Direct Test by Gen-Probe, Inc. (San Diego, California).[1] There are other assays on the market which are FDA cleared and will be mentioned briefly in this article.

GeneXpert® System by Cepheid

The GeneXpert® System uses real-time polymerase chain reaction (PCR) technology to identify various organisms within 30 minutes to a few hours. The GeneXpert® is a closed, contained system, with the ability to perform specimen preparation, target amplification, and detection all in one sample device (cartridge).[2,3]

The system can process a variety of unprocessed samples such as whole blood, human cells or tissue, or swabs that are placed directly in the sample cartridge.[4] DNA or RNA extraction takes place in the "microfluidic" cartridge that contains the reagents necessary to extract and purify the nucleic acids.[4] Once DNA or RNA is extracted, the system begins the amplification process using real-time PCR technology.[3,4]

During the detection process, various optics detect numerous target sequences concurrently, stopping the reaction when enough copies are generated.[4] The system performs reflex testing on positive samples for confirmation purposes all within the same cartridge. Contamination rates are virtually eliminated since the sample is added at the beginning to the self-contained cartridge with no additional human interaction.[4]

The GeneXpert® system can be configured with 1 to 16 modules, depending on workload demand.[3,4] According to the manufacturer's brochure, "each module includes a 6-channel optics system capable of exciting and detecting multiple fluorescent dyes in the same reaction tube."[4] Because of this feature, the reaction can be stopped once enough target is detected. Laboratories with high volume may utilize all 16 sites since each site runs independently and can be used to run various tests simultaneously. Smaller laboratories may only opt for 1-4 modules depending on need. Some laboratories may also consider the automated GeneXpert® Infinity System, This system applies robotic technology and can utilize up to 48 modules. Test throughput (theoretical maximum) is 1300 tests in a 24-hour period.[5]

Applications

The GeneXpert® system is used to monitor nosocomial and infection rates of select organisms. Organisms include *Clostridium difficile*, Group B Streptococcus (GBS), MRSA, and VRE. MRSA, VRE, and *Clostridium difficile* infections require patient isolation, continuous monitoring, and in most cases aggressive treatment.

Many medical centers have implemented MRSA screening and surveillance programs to monitor health-care associated and community associated MRSA infections. New Jersey state law mandates that patients be screened for MRSA upon admission to determine whether they are colonized or infected with the organism.[6] With this law in effect since March 12, 2007,[6] the need for an accurate and reliable screening method is crucial. The Xpert MRSA assay with its ability to turn around results in just over an hour is a simple and accurate solution for the screening and surveillance of MRSA carriers.[3,7,8] With such control measures in place, reduced nosocomial infections and spread of the illness is expected.

The Association for Molecular Pathology lists the various tests that are FDA cleared and run on the GeneXpert® system. Turn-around times of each test are included.

SmartCycler® System by Cepheid

Cepheid also markets the SmartCycler® System which uses real-time PCR technology and detection. With the SmartCycler, DNA or RNA extraction and master mix preparation are manual. Manual preparation adds as much as 20 minutes.[9,10]

The SmartCycler® system is used in research-based facilities and reference laboratories. One feature the SmartCycler® has that the GeneXpert® does not is the ability to run home brew assays and other manufacturers' assays on the instrument.[9] The SmartCycler® also has a maximum capacity of 96 tests with a 20-40 minute turn-around.[10] Table 2 lists *in*

vitro diagnostic (IVD) assays from outside manufacturers that are FDA cleared to run on the SmartCycler®.

Table 1. FDA Cleared Assays that run on the GeneXpert® System by Cepheid.[9]

Assay	Turn Around Time
Xpert MRSA	75 minutes
Xpert MRSA-SSTI	Less than one hour
Xpert MRSA-BC	Less than one hour
Xpert EV	2.5 hours
Xpert GBS	30+ minutes
Xpert *C.difficile*	45 minutes
Xpert MRSA-SA	Less than one hour
Xpert vanA	45 minutes

Abbreviations
MRSA: methicillin-resistant *Staphylococcus aureus*
SSTI: skin and soft tissue infections
BC: blood culture
EV: enteroviral meningitis
GBS: group B Streptococcus
SA: *Staphylococcus aureus*
vanA: vancomycin A

Table 2. FDA-Cleared IVD Assays that run on the SmartCycler® by Cepheid.[9]

Company	Assay
Prodesse	ProFlu
BD	GeneOhm MRSA
BD	GeneOhm StrepB
BD	GeneOhm C.diff
BD	GeneOhm VRE
BD	GeneOhm StaphSR
Cepheid	Smart GBS

Abbreviations
IVD: *In vitro* diagnostics
BD: Becton Dickinson
MRSA: methicillin-resistant *Staphyloccus aureus*
StrepB: *Streptococcus* group B
C. diff: *Clostridium difficile*
VRE: vancomycin-resistant *Enterococcus*
StaphSR: Staphyloccus methicillin sensitive and resistant
GBS: Group B streptococcus

COBAS AMPLICOR *Chlamydia trachomatis* (CT)/ *Neisseria gonorrhoeae* (NG) Test, Roche Diagnostics, Inc.

According to the Centers for Disease Control and Prevention (CDC), *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection (STI) in the U.S. with more than one million new cases reported in 2008.[11] The CDC also reports that approximately 700,000 new infections of *Neisseria gonorrhoeae* occur each year.[12] Several studies report high incidence rate of co-infections in both males and females.[13,14,15] Since not all infected individuals are symptomatic, it is important to test for both organisms. If untreated, infertility and ectopic pregnancies can occur.[11]

The first FDA-cleared PCR test in the United States was the AMPLICOR CT test in 1993.[16] The assay has been modified to include the detection of both *Chlamydia trachomatis* and *Neisseria gonorrhoea*. The advent of a molecular diagnostic test, which can test for both organisms simultaneously using multiplex technology, is a great improvement over routine culture and serological assays.

The COBAS AMPLICOR CT/NG assay, which utilizes end-point PCR and colorimetric detection, allows for simultaneous capture of both infections using complementary biotinylated specific primers and oligonucleotide probes specific for each target sequence.[17,18] DNA extraction procedures are performed manually, requiring separate rooms for both pre-amplification and post-amplification processing. Workflow must be in a unidirectional manner in order to ensure minimal contamination. Once the extraction process is complete, the sample is placed in the COBAS AMPLICOR Analyzer where the fully automated amplification and detection process occurs.

After PCR amplification, the detection process begins. Biotinylated amplicons are denatured and allowed to hybridize to target-specific oligonucleotide probes bound to magnetic particles.[17,18] Avidin-horseradish peroxidase is then added which will bind to the biotinylated amplicons. Once unbound material is washed away, tetramethylbenzidine (TMB) is added to the reaction, which forms a color complex that is measured by the COBAS AMPLICOR Analyzer at 660 nm. [17,18] Turn-around time once the sample is added to the COBAS is approximately 3.5 hours—1 hour for the amplification process and 2.5 hours for detection.[19]

Studies performed on urogenital swabs using the COBAS AMPLICOR CT/NG test for both organisms reported high sensitivity and specificity rates confirming the assay is well suited for screening and diagnosing *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections.[20,21]

Please note; the assay is FDA cleared for urine from males and endocervical specimens in females. The test is not available in the U.S. for testing female urine specimens for *Neisseria gonorrhoeae* or for testing urethral swab specimens from asymptomatic males for *Neisseria gonorrhoeae*.[22]

AMPLIFIED *Mycobacterium tuberculosis* Direct Test, Gen-Probe, Inc.

According to the World Health Organization (WHO), approximately one-third of the world's population is infected with MTB.[23] It is estimated that every second someone in the world is newly infected with the organism.[23] If the proper diagnosis and antimicrobial treatment is not administered in a timely manner, the disease can be fatal, especially multi-drug resistant strains. Conventional AFB cultures require six or more weeks to grow. Since MTB is contagious via airborne droplets, there is a need for a rapid, accurate molecular diagnostic test, which can detect the organism on prepared specimens and not on isolated colonies, was essential in the monitoring and management of infected individuals.

Fortunately, there is an FDA-cleared assay which can detect AFB in sputum, bronchial specimens, or tracheal aspirates.[24] The assay is the AMPLIFIED *Mycobacterium tuberculosis* Direct Test by Gen-Probe, Inc.

Specimens are prepared using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) digestion. Nucleic acids are released using sonication. Amplification and detection are done using transcription-medicated amplification (TMA) and the hybridization protection assay (HPA), respectively detecting *Mycobacterium tuberculosis* complex ribosomal RNA.[24] The principle of both TMA and HPA are discussed in the accompanying article "Molecular Virology in the Clinical Laboratory."

This assay can detect members of the MTB complex, which include *Mycobacterium tuberculosis, Mycobacterium bovis* (including bacillus Calmette-Guerin strain), *Mycobacterium africanum*, *Mycobacterium canettii*, and *Mycobacterium micoti*.[25]

This assay however cannot differentiate among the various species in the complex. The assay should be performed only on individuals showing signs and symptoms consistent with active MTB infection, those who have not received antituberculous therapy, or have not received therapy in the previous 12 months.[24]

Same day results are possible for this assay however several studies have been performed that confirm a specificity of greater than 95% but variable sensitivity (61% to 100%).[26,27,28] Inhibitory substances in the specimen can affect the sensitivity values. One study found that by diluting the sample 1:10, inhibiting substances were reduced while maintaining detectable MTB complex numbers (≥30,000 RLU).[24] By utilizing the researchers dilution protocol, sensitivity, specificity, positive predictive value, and negative predictive values were 100% versus culture.[29]

FDA Cleared Diagnostic Tests

A list of all FDA cleared bacterial molecular assays to date including the manufacturer, test name, and methodology utilized can be found in Addendum B in the online version of *Clin Lab Sci* 2010;23(4) at www.ascls.org. There are many other assays that are available outside the U.S. or for research purposes only that are not FDA cleared and are therefore not included in this list.

Summary

Identifying organisms in a timely manner has always been one of the greatest challenges for clinical microbiologists. Healthcare providers want rapid results in order to prescribe the appropriate antimicrobial therapy and improve patient care. Some of the more virulent pathogens such as MRSA, VRE, and MTB must be identified as quickly and accurately as possible in order to prevent the spread of the disease to other patients and employees. The need for rapid, molecular methodologies in the clinical bacteriology laboratory was essential to improve patient outcomes. Organisms

that use to take days or weeks to identify are now detected in hours or within the same day. The advent of molecular assays has enabled clinical laboratory scientists to report out accurate, sensitive and timely results confirming the critical role they play on the healthcare team.

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