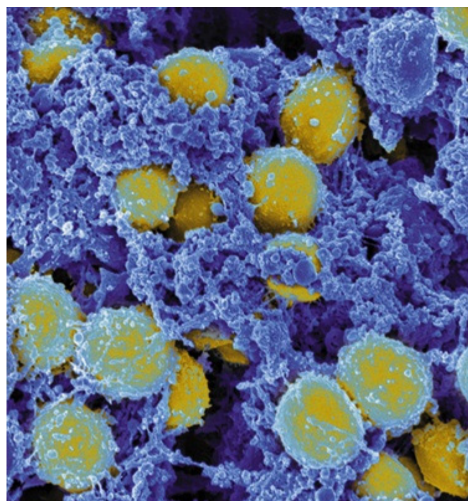
One Sample, Multiple Results

The Use of Multiplex PCR for Diagnosis of Infectious Syndromes

Author: Elizabeth Palavecino, MD // **Date:** APR.1.2015 // **Source:** Clinical Laboratory News

Topics: Cancer and Tumors,Infectious Diseases,Specializations,Molecular Pathology,Microbiology



Once confined to research and reference laboratories, molecular test methods for the diagnosis of infectious diseases have become part of routine testing algorithms in clinical laboratories of all sizes. Laboratory and clinical personnel have received this change with enthusiasm because molecular testing provides higher sensitivity and specificity, and in most cases a shorter turnaround time than traditional methodology. Based on the increased acceptance of these tests in clinical practice, manufacturers have developed a wide variety of options—both instruments and assays—from which laboratories can choose (1). The technology has evolved from manual to fully automated, closed systems that provide sample-to-result automation, enabling laboratories with limited prior experience to implement molecular testing.

Many studies have demonstrated the clinical effectiveness of molecular testing to detect one particular organism, such as Clostridium difficile or methicillin resistant Staphylococcus aureus (MRSA) (2). More recently, there has been a move toward developing molecular tests that detect multiple pathogens associated with an infectious syndrome rather than one particular organism. Molecular technologies with multiplexing capabilities may use traditional polymerase chain reaction (PCR) or real-time PCR and reverse-transcription PCR to amplify targets. They are usually offered as a panel that simultaneously detects the pathogens most commonly associated with a particular infectious syndrome, such as sepsis, meningitis/encephalitis, and urinary tract, respiratory, or gastrointestinal (GI) infections, using a single specimen.

Advantages and Limitations of Multiplex PCR Assays

This emergent technology is redefining the diagnosis of infectious diseases and can have a significant impact on patient management while streamlining the processing and testing of specimens in the clinical laboratory. The possibility of detecting multiple targets in a single sample is particularly important when clinical samples are difficult to collect or are limited in volume (e.g., spinal fluid), or when multiple different pathogens can cause the same clinical presentation—making it difficult for clinicians to narrow down the causative pathogen.

Until now, widespread implementation of multiplex molecular tests in clinical laboratories has been hindered by the high cost of the kits and by the need to acquire multiple instruments to cover the testing needs of different infectious syndromes. These assays also have some shortcomings. Table 1 summarizes the possible advantages and disadvantages of multiplex PCR assays.

T1 Potential advantages and disadvantages of multiplex PCR tests for diagnosis of infectious syndromes.

POTENTIAL ADVANTAGES	Increase diagnostic yield as multiple targets are tested in one sample
	Conserve and optimize analysis of samples difficult to obtain (spinal fluid, vitreous fluids, synovial fluids)
	Simplify ordering algorithm as only one test needs to be requested
	Streamline workflow in the laboratory and reduce hands-on time
	Potential saving in reagents by testing multiple organisms at once compared to testing each pathogen separately
	Standardize testing
POTENTIAL DISADVANTAGES	False positive results due to cross reactivity or unspecific amplification caused by multiple primers/targets present in the reaction
	False-negative results due to use of preferential amplification of one target over another
	Negative internal control due to exhaustion of reagents in samples with a high amount of one particular target
	Added cost of testing targets that may not be necessary in some patient populations
	High cost of commercial kits and instruments

However, even with their possible limitations, multiplex PCR assays are being adopted rapidly in clinical practice. I am hopeful that competition triggered by rapid market growth along with a reasonable reimbursement plan will lead to these tests being available at significantly lower cost and with an increased number of targets and improved automation. The paradigm shift from single to multiplex molecular assays will continue to occur as long as clinical benefits are observed.

The remainder of this review will focus on the use of multiplex PCR tests in the following infectious syndromes: blood stream, respiratory, and GI infections. The need for rapid molecular testing with each type of infectious syndrome will be discussed, along with advantages of multiplex tests over conventional methods, and any possible disadvantages. In addition, the multiplex PCR assays that are commercially available and cleared by the Food and Drug Administration (FDA) for the diagnosis of these infections will be described and compared. Commercial Panels—Multiplex PCR Tests for Specific Infectious Syndromes

Blood Culture Panels

Blood stream infection—the presence of organisms in the blood—can trigger a systemic inflammatory response syndrome that can progress to severe sepsis and septic shock. Two factors drastically increase mortality in patients with bloodstream infections: progression of the infection to sepsis or septic shock, and delayed implementation of appropriate antimicrobial therapy (3,4). In addition, to prevent the emergence of antibiotic resistance, clinicians must start effective therapy early and avoid having patients unduly exposed to broad-spectrum antibiotics. For all these reasons, rapid laboratory identification of the pathogen and its resistance mechanisms is crucial in selecting appropriate therapy and is a decisive factor in patient survival.

While conventional culture and susceptibility testing may require 72 hours to produce results, multiplex PCR assays can do so in 1 to 3 hours after the blood culture is flagged as positive by the blood culture instrument. The benefit of such rapid testing will be seen if therapeutic decisions are made as soon as results are available; therefore, support from an antimicrobial stewardship team is paramount to achieve the goals of cost-effective testing and improved patient outcomes.

To date, there are two FDA-cleared molecular blood culture panels: the FilmArray Blood Culture Identification panel manufactured by Biofire Diagnostics and the Verigene blood culture test manufactured by Nanosphere. A sample from a positive blood culture bottle is tested on the corresponding system and all three reactions—sample preparation, amplification, and detection—are performed automatically by the instrument. The FilmArray assay is offered as a panel that detects gram negative, gram positive, and yeasts. The Verigene assay uses a separate detection panel for gram positive and gram negative, with the panel selected based on Gram stain results. Table 2 summarizes the details on the time to result for each of these blood culture panels and the group of organisms detected by each.

T2 Commercial molecular assays for detection of organisms in positive blood cultures of patients with bloodstream infection

ASSAY	ORGANISM GROUP DETECTED (SPECIES)
FilmArray BCID[1] The Biofire Diagnostics FilmArray Instrument Time to result: 1 hour	*Staphylococcus/S aureus*
	Streptococcus (3)
	Enterococcus
	Listeria monocytogenes
	Enterobacteriaceae (6)
	Acinetobacter baumanii
	Pseudomonas aeruginosa
	Haemophilus influenzae
	Neisseria meningitidis
	Candida (4) Resistance mechanisms (*mec*A, ESBL, KPC, *Van* A/B)
BC-GP[2] The Nanosphere, Inc. Verigine Reader and Processor SP Time to result: 2.5 hours	*Staphylococcus (3)*
	Streptococcus (4)
	Enterococcus (2)
	Listeria spp.
	Resistance mechanisms (*mec*A, *Van*A, and *Van*B)
BC-GN[3] The Nanosphere, Inc. Verigine Reader and Processor Time to result: 2 hours	*Enterobacteriaceae (6)*
	Acinetobacter spp
	Pseudomonas aeruginosa
	Resistance mechanisms ESBL (*CTX-M*), Carbapenamases (IMP, KPC, NDM, OXA, VIM)

Evaluation of both of these panels has shown that they accurately identify most leading causes of blood stream infections and provide results significantly faster than traditional methodologies, enabling clinicians to prescribe appropriate therapy much earlier (5).

Viral Respiratory Panels

One area that has experienced a dramatic change from conventional to molecular methodologies is virology testing. Respiratory tract infections are one of the most common causes of morbidity and mortality in all age groups, and the clinical presentation of different organisms can be similar, making it impossible to reliably predict the causative pathogen based solely on clinical symptoms. The Influenza A H1N1 epidemic in 2009 clearly demonstrated that rapid and specific diagnosis of viral respiratory infections is important for patient and public health management. Clinicians are becoming more aware that viral respiratory infections other than influenza can be very severe, driving interest in a rapid and comprehensive multiplex PCR test that can detect the most common pathogens—both for patient management as well as surveillance monitoring of respiratory viral infections. Nevertheless, the variable sensitivity observed with rapid antigen influenza tests limit their clinical utility.

Currently, there are many molecular testing options for influenza, including one assay recently FDA-cleared as a point-of-care test (6). In addition to the widely available molecular assays designed to detect influenza A (with and without subtyping), influenza B, and/or respiratory syncytial virus from nasopharyngeal swabs, multiplex PCR assays that detect multiple respiratory viruses are being used more frequently by clinical laboratories. At the time of this review, there were four FDA-cleared multiplex molecular respiratory viral panels (RVP). Table 3 lists the capabilities of these assays.

T3 Characteristics of multiplex PCR panels for detecting respiratory pathogens in nasopharyngeal swab samples.

ASSAY NAME (MANUFACTURER)	ANALYSIS PLATFORM	DETECTION METHODOLOGY	NO. OF TARGETS	TIME TO RESULT*	BATCHING
FilmArray Respiratory Panel (Biofire Diagnostics)	Film Array System	Endpoint melt curve analysis	17 viral targets 3 bacterial targets	1 hour	**No.** One sample in 1 hour per instrument
eSensor Respiratory Viral Panel (GenMark Dx)	eSensor	Electrochemical	14 viral targets	7 hours	**Yes.** 21 samples in 8 hours per instrument
xTAG Respiratory Panel v1 (Luminex Molecular Diagnostics)	Luminex 100/200	Fluorescence-labeled bead array	12 viral targets	8 hours	**Yes.** 21 samples in 8 hours per instrument
xTAG Respiratory Panel Fast (Luminex Molecular Diagnostics)	Luminex 100/200	Fluorescence-labeled bead array	8 viral targets	5 hours	**Yes.** 21 samples in 8 hours per instrument

*The time to result listed includes the time required for pre-extraction of nucleic acids using the NucleiSENS easyMAG instrument (BioMerieux) for the eSensor and xTAG assays.

Sensitivity varies by assay and by target, but all show high specificity for all targets included in the panels (7). Each laboratory should determine which system is appropriate for its specific needs, based on instrumentation available, experience and competency of personnel, and the patient population served.

GI Panels

Infectious diarrhea can be caused by bacterial, viral, or parasitic pathogens and remains a significant healthcare burden worldwide (8). Although most GI infections are self-limiting, they can be severe and even fatal in young children, the elderly, and other immunocompromised individuals. Because the pathogen cannot be ascertained by clinical presentation, clinicians often order a bacterial culture and also an ova and parasite (O&P) exam in stool samples from patients with diarrhea.

However, this conventional testing approach has at least three significant diagnostic limitations. First, culture and identification of bacterial pathogens in stool is labor intensive and can take 3–5 or more days for results. In addition, the CDC recommendation of simultaneous testing for Shiga toxin and culture has increased the cost of bacterial cultures in stool with little benefit in those areas with low prevalence of Shiga toxin-producing Escherichia coli (9). Second, the O&P exam lacks sensitivity, is time consuming, and requires highly trained personnel for meaningful interpretation and detection of parasites causing diarrhea (10). Third, although viral infections are an important cause of GI illness outbreaks, the majority of laboratories do not perform viral culture or antigen testing—except for rotavirus—so the infection goes unnoticed and potentially spreads.

There are two FDA-cleared multiplex PCR assays that can detect bacterial pathogens in stool—Hologic's ProGastro SSCS and the BD MAX Enteric Bacterial Panel. However, a more comprehensive, rapid, sensitive, and specific assay for the diagnosis of infectious diarrhea caused not only by bacterial but also viral or parasitic pathogens may be desirable for several reasons, including minimizing additional testing, quickly implementing infection control practices to decrease spread, and potentially avoiding unnecessary antibiotics. Table 4 lists three such FDA-cleared GI assays and the target organisms included in each of these panels.

T4 Pathogens detected by gastrointestinal panels FDA-cleared for testing stool samples in patients with infectious diarrhea.

ASSAY	ORGANISMS DETECTED	
FilmArray GI (BioFire Diagnostics)	Bacteria	*Campylobacter (yeyuni, coli, and upsaliensis), Clostridium difficile Plesiomonas shigelloides, Salmonella, Yersinia enterocolitica. Vibrio (parahemolyticus, vulnificus, and cholera), Diarrheagenic E. coli/Shigella (EAEC, EPEC, ETEC, STEC, EIEC), Shiga-producing E. coli, E. coli 0157.*
	Viruses	Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus.
	Parasites	*Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia.*
xTAG GPP* (Luminex Molecular Diagnostics)	Bacteria	*Campylobacter, Clostridium difficile, Salmonella, Yersinia enterocolitica. Vibrio cholera, Enterotoxigenic E. coli (ETEC), Shigella, Shiga-producing E. coli, E. coli 0157.*
	Viruses	Adenovirus F40/41, Norovirus GI/GII, Rotavirus A.
	Parasites	*Cryptosporidium, Entamoeba histolytica, Giardia lamblia.*
Verigene EP (Nanosphere Inc.)	Bacteria	*Campylobacter group, Salmonella, Shigella, Vibrio group, Yersinia enterocolitica, Shiga-toxin 1 and 2.*
	Viruses	Norovirus GI/GII, Rotavirus A.
	Parasites	None

EAEC: Enteroaggregative *E. coli; EPEC:* Enteropathogenic *E. coli; ETEC:* Enterotoxigenic *E. coli; STEC:* Shiga-toxin–producing *E. coli; EIEC: Shigella*/Enteroinvasive *E. coli.*
*This assay requires pre-extraction of nucleic acids using the NucleiSENS easyMAG instrument (BioMerieux).

These assays have high sensitivity and specificity, and they allow the detection of viral pathogens which are usually not detected by routine work up of stool samples. The clinical and financial impact of GI panels has not yet been fully evaluated, and there are some concerns that molecular testing may be detecting colonization rather than infection in some cases, making it difficult for clinicians to interpret the results. With more laboratories using GI panels, I am hopeful that the clinical efficacy and cost effectiveness of these panels will be revealed in the near future.

Conclusion

Molecular testing is replacing conventional methodologies for the diagnosis of infectious diseases. The improved instrumentation and the availability of fully automated systems with sample-to-answer design have helped clinical laboratories implement these assays. More recently, the availability of multiplex PCR assays that can detect a large number of targets in a single sample has shifted the paradigm from ordering a series of individual tests to detect many different pathogens to using one sample and one test to make a diagnosis of infectious syndromes.

Multiplex PCR assays are even more useful clinically when there is support from an antimicrobial stewardship team that can act on results in real time. The high cost of these

molecular panels compared to conventional methodology is still hindering them from being implemented widely, but the rapid turnaround time and higher sensitivity and specificity are factors that could make these tests a powerful tool. The cost savings of implementing multiplex PCR assays may not be seen directly in the clinical laboratory, but rapid diagnosis can be translated into savings for the institution due to decreased length of stay and better clinical outcomes for patients.

Acknowledgments: I thank Carlos A. Fasola for helpful editorial suggestions to the manuscript.

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