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REVIEW

Sample-to-result molecular infectious disease assays: clinical implications, limitations and potential

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ABSTRACT

Molecular infectious disease diagnostic tests have undergone major advances in the past decade and will continue to rapidly evolve. Assays have become extraordinarily simple to perform, eliminating the need for pre-analytic sample preparation and post-amplification analysis. This allows these tests to be performed in settings without sophisticated expertise in molecular biology, including locations with limited resources. Additionally, the sensitivity and specificity of these assays is superb and many offer extremely fast turn-around times. These tests have major impacts on patient care, but also have some limitations.

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Introduction

We have entered a new age of molecular diagnostic testing for infectious diseases. This follows nearly a century of traditional, culture-based methods that require the growth of organisms and analysis of their phenotypic properties. Molecular testing has proven to be faster and often times equally or more sensitive than its traditional counterparts. In many instances, there is no longer a need to wait days or weeks for an organism to replicate; instead, an identification of an organism is available in minutes to hours. This is possible due to technology that focuses on the genetic signatures of individual organisms.

Molecular diagnostics has undergone a complete renovation in the past two decades. With the invention of polymerase chain reaction (PCR) in the 1980s, it became possible to replicate nucleic acids so that a small amount of sample from a patient can provide enough genetic material to examine. PCR technology depends on numerous cycles of heating and cooling. Where laboratorians were once manually transferring the sample between hot and cool water baths over several hours, now automated instruments thermocycle with minimal hands-on time. Additionally, the discovery of *Taq* polymerase automated the process even more and allowed for the large-scale use of PCR technology.

The first step in a molecular assay is the extraction of nucleic acid from the patient's specimen. Many instruments are now available to carry this out in a relatively simple and cost-effective manner. However, this step does take some time and manual labor is needed to load and unload these instruments. Therefore, newer assays utilize 'on board' extraction, where no additional equipment or hand-on steps are required for nucleic acid extraction. The two stages in this process are lysis of the organisms and purification of the nucleic acid. Lysis can be done via pressure, heat, chemicals, sonication, or mechanical methods (bead beating'); purification can be done by immobilizing the nucleic acid on a binding material, washing several times, and then eluting the nucleic acid into water or buffer.

When using PCR, there must be a way to examine whether amplicons were made, and if so, how many. For several years, gel electrophoresis was the mainstay for this part of the process, which is laborious, time consuming, and uses potentially dangerous chemicals. Newer technology allows for the detection and quantitation of amplified DNA during amplification, for example, by intercalating fluorescent dyes into the newly created strands. Fluorescent intensity, which correlates to DNA concentrations, can be measured in real time. Additionally, methods for confirming the specificity of the amplification process are sometimes utilized. Melting curve analysis assesses the temperature at which the newly created double-stranded DNA dissociates (melts). This should be fairly specific for the target DNA targeted by that assay.

Molecular methods of the past required three distinct geographical areas within a laboratory: pre-PCR

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(extraction and purification), PCR (amplification), and post-PCR (analysis such as gel electrophoresis and melting curves). Laboratories that wished to partake in these methods had to have a lot of space to accommodate this. The reason for this is to minimize the risk of contamination of amplified nucleic acids, which plagued PCR technology until updated technology was developed. Even under extreme care, amplicons from one reaction could contaminate other reactions. Because so little nucleic acid is needed to begin with, a small amount of it from another patient's sample or another assay could be a serious disaster. Newer technologies offer a completely 'closed' reaction, where the sample is never opened and never touched by human hands after amplification. All detection and quantitation of the nucleic acid occur within the closed containers, which substantially minimizes the possibility of contamination.

While rapid identification of organisms is extraordinarily beneficial to patients, knowing the causative organisms alone is sometimes not enough. Treatment often depends on the antimicrobials that the particular strain of the organism will respond to. Thankfully, numerous molecular assays can detect genes that confer resistance to various antimicrobials.

Although PCR is the most commonly used technology in these systems, other methods of sequence-specific nucleic acid amplification are also used. Some of these alternative approaches are referred to as sequence-specific isothermal amplification protocols because they do not require changing the reaction temperature. Some common advantages are that isothermal techniques are extremely fast and do not require thermocyclers.[1] Transcription-mediated amplification (TMA) and nicking enzyme amplification reaction (NEAR) are two examples of isothermal amplification protocols.[2] These will be described in further detail below as they pertain to specific assays.

With all of these advances in PCR technology, diagnostic methods have become smaller and simpler. The phrase 'sample-to-result' describes technology that entails on-board nucleic acid extraction, amplification, and analysis. These assays require minimal hands-on time. Oftentimes, all reagents including controls are stored directly in individual cartridges. Entirely closed processes substantially reduce the risk of contamination. Some of the instruments are as small as a shoebox, while higher-volume instruments are floor-models, which allows labs of all sizes to partake in this new technology. This review is limited to truly sample-to-result assays and therefore does not include all molecular infectious disease tests. We will examine the use of this technology for infectious diseases in nearly all organ systems – gastrointestinal, genitourinary, central nervous system (CNS), respiratory, and more. A summary of the manufacturers is provided in Table 1. We will explore the clinical implications – we ask the most important question, 'How does this benefit our patients?'

Respiratory testing

Upper respiratory illness testing

Perhaps the most robust area of molecular infectious disease testing is in the field of upper respiratory infections. Numerous manufacturers and instruments are available for testing several different organisms. Influenza virus PCR has many advantages over conventional diagnostic techniques, which include culture and rapid antigen tests. Recovering influenza in culture takes several days, which is far too long to wait to begin therapy. Rapid antigen tests, although fast and easy to perform, are plagued with low sensitivity. Direct fluorescent antibody (DFA) testing, although relatively fast, is dependent upon collecting cells in a sample and it requires a fluorescent microscope. Nucleic acid tests can be performed in a time frame that is meaningful to patient management, is highly sensitive and specific, and many manufacturers offer assays that require minimal hands-on time and minimal expertise in the basics of molecular diagnostics. Molecular tests (unlike antigen tests) for influenza are considered diagnostic and no confirmatory tests are performed.

The first assay that we will discuss is the Cepheid Xpert Flu, which runs on the GeneXpert instrument.[3] The GeneXpert systems run with Xpert assay cartridges, with all necessary reagents and controls included in the cartridge. As we will see further in this review, numerous assays are offered, all of which use the same instrument, and all cartridges fit the instrument. Instruments are available in varying sizes, from a single-cartridge system all the way up to a system that holds 80 cartridges at one time. The GeneXpert Infinity systems hold either 48 or 80 cartridges and are equipped with a robotic loading system, further reducing the hands-on time in high-volume laboratories. Each module within a system operates individually, so the system is random-access, and can run as many different assays as the lab would like at the same time (e.g. an influenza assay can run at one time, and at any time later, a *Clostridium difficile* assay can run in the module next door, etc.). Therefore, this system is extremely flexible for laboratories of all sizes. A sample is pipetted into the sample area of a cartridge, and the cartridge is loaded into individual modules within the system by simply opening the drawer and inserting the cartridge, similar to

Table 1. Sample-to-answer molecular infectious disease systems.

Manufacturer/ System	FilmArray (Biofire)	Verigene (Nanosphere)	Simplexa Direct (Focus)	GeneXpert (Cepheid)	Cobas Liat (Roche)	Alere i (Alere)	Panther and Tigris (Hologic)	ARIES (Luminex)
Selected Panels	Respiratory Panel Gastrointestinal Panel Meningitis/Encephalitis panel	Respiratory Panel Plus (RV+) Enteric Pathogens Test (EP) C. difficile (CDF)	Flu A/B & RSV HSV-1 and HSV-2	MRSA SA Nasal Complete MRSA/SA SSTI C. difficile/Epi C. difficile Norovirus MTB/RIF Flu Flu/RSV XC EV CT/NG Xpert GBS *Trichomonas*[a]	Strep A Influenza A/B	Strep A Influenza A/B	CT NG HPV *Trichomonas*	HSV 1 & 2
Run Time	1 hour	2–2.5 hours	1 hour	Variable; 30 minutes–2.5 hours	15–20 minutes	15 minutes	3.5 hours until first result	2 hours
Complexity	Moderate	Moderate	Flu A/B & RSV: Moderate HSV: High	Moderate	Influenza A/B: Waived Strep A: Waived	Waived	High	Moderate
Batch vs. Random Access	Random Access (single test per instrument)	Random Access (single test per instrument)	Batches of 8	Random Access (instruments of capacities ranging from 1 to 80 cartridges)	Random Access (single test per instrument)	Random Access (single test per instrument)	Random Access	Batches of 6

Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus*; SA, *Staphylococcus aureus*; EV, Enterovirus; CT/NG, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*; GBS, Group B Streptococcus; HPV, Human papillomavirus; HSV, Herpes simplex virus.

[a]The GeneXpert Trichomonas assay is CE marked but not FDA approved.

how an ink-jet printer is loaded with an ink cartridge. Within the instrument, a plunger descends into the cartridge, which forces a valve body to rotate. Multiple plunges and rotations occur, which isolates the organisms onto a solid membrane and adds reagents to the solution. Lysis occurs via sonication and additional rotations of the valves occur to add more reagents and force the sample into the reaction chamber, which juts out from the cartridge similar to a shark fin. Following one round of thermocycling, the solution is pulled back into the cartridge, additional reagents are added, and the solution is forced back into the reaction chamber to undergo another round of thermocycling, which is accompanied by color detection to complete the real-time PCR reaction.

The Xpert Flu assay detects and differentiates Influenza A, Influenza A/H1N1, and Influenza B from nasopharyngeal swabs and nasal aspirates/washes in less than 75 minutes. It has an overall reported sensitivity upward of 95% [4,5] when compared with other molecular methods; however, some studies report a lower sensitivity for Influenza B.[6] A separate Xpert assay, the Xpert Flu/RSV XC, detects and differentiates Influenza A, Influenza B, and Respiratory syncytial virus in approximately one hour. When compared with other molecular methods, the reported sensitivity for this assay is greater than 95% [7,8] for Influenza A, 94.8% for Influenza B,[9] and 89.3% for RSV.[7]

The Focus Simplexa Flu A/B & RSV Direct assay consists of a flat disk that resembles a compact disk and the 3 M Integrated Cycler instrument. There are eight wells on each disk, each covered by an adhesive. Prior to beginning the test, the adhesive cover is partially pulled back to expose a well. One single-use reaction mix vial per sample is allowed to thaw to room temperature from storage at −10 to −30°C; then fixed-volume pipettes are used to inoculate 50 µL each of the reaction mix and the patient sample into the appropriate sections of the reaction well. The well is re-covered by the adhesive; the disk is loaded onto the 3 M Integrated Cycler; and the run takes approximately 1 hour to complete. Compared with a laboratory-developed reverse transcription PCR, one study reported sensitivities for Influenza A, B, and RSV of 96.6%, 97.6%, and 99.2%, respectively.[10]

Another commonly used system for upper respiratory infections is the FilmArray Respiratory Panel (RP), which was first approved by the US FDA in May 2011 (subsequent versions have since been approved). The FilmArray instrument is a random-access single-module system with each reaction occurring within one disposable pouch. Multiple instruments may be connected to one laptop. The FilmArray pouch contains freeze-dried reagents in discrete blisters; steps within the multiplex, nested PCR process, and melting curve analysis are completed in each blister. The pouch is entirely closed, including waste, and the pouch is disposed of after a single use. The user adds a provided hydration solution to the pouch by drawing up the solution into a syringe and then placing the syringe into a port on the pouch; the pouch draws up the required volume with a vacuum so that measurement is not necessary by the user. The patient sample is mixed by a sample buffer and similarly inoculated into the pouch. The instrument is closed, the assay is begun, and no further hands-on time is required. The instrument drives the procedure in a set protocol using mechanical force, thermal changes, and optics. Cells in the patient sample are disrupted by mechanical bead beating in the first blister; then, the nucleic acid is extracted. After multiple wash steps, all of the waste is pushed into the first blister. In a subsequent blister, the first stage of PCR takes place, which is a large-volume, highly multiplexed reaction. The amplicons are diluted and a fluorescent DNA binding dye is added and this solution is distributed among the wells of the array, which contain specific primers for each target. The second (nested, singleplex) stage of PCR occurs in triplicate in the array, followed by a DNA melting curve analysis. Each pouch contains two controls. The associated software interprets the analysis and qualitative results are displayed on the laptop monitor.

The current version of the FilmArray RP contains 20 targets, including numerous strains of influenza, parainfluenza, and coronavirus; RSV; human metapneumovirus; rhinovirus/enterovirus; adenovirus; and three bacterial targets: *Bordetella pertussis, Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae* (see Table 2). Reported sensitivities range for the various organisms on the panel and the comparison method. Previous versions of this assay were reported to have a low sensitivity for adenovirus, but this appears to have substantially improved in the more current versions. [11,12] Couturier *et al*. reported sensitivities of 90–100% for all analytes except Influenza B (73%) and adenovirus (83%).[12] One study reported a sensitivity of 65% for *Bordetella pertussis* when compared with Focus's analyte-specific reagents.[13] High sensitivities have also been reported for off-label use with sputum, [14] nose and throat swabs,[14] and lower respiratory specimen.[15] The assay takes approximately 1 hour with minimal hands-on time.[11,16–18]

The Nanosphere Verigene Respiratory Viral Plus (RV+) is another relatively large panel (see Table 2). The Verigene system contains two instruments: a processor and an analyzer. Unlike the completely self-contained

Table 2. Comparison of sample-to-answer molecular assay for upper respiratory infection.

Platform Name	FilmArray	Verigene	3 M™ Integrated Cycler	GeneXpert	GeneXpert	Alere i	Cobas Liat
Assay Name	Respiratory Panel	Respiratory Virus *Plus*	Simplexa Flu A/B & RSV Direct Kit	Xpert Flu	Xpert Flu/RSV XC	Influenza A & B	Influenza A & B
Manufacturer	Biofire	Nanosphere	Focus	Cepheid	Cepheid	Alere	Roche
Targets	Influenza A Influenza A/H1 Influenza A/H3 Influenza A/H1-2009 Influenza B Respiratory syncytial virus Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Adenovirus Coronavirus HKU1 Coronavirus NL63 Coronavirus 229E Coronavirus OC43 Human metapneumovirus Human rhinovirus/Enterovirus *Bordetella pertussis* *Chlamydophila pneumoniae* *Mycoplasma pneumoniae*	Influenza A Influenza A/H1 Influenza A/H3 Influenza A 2009 H1N1 Influenza B H275Y (oseltamivir resistance) Respiratory syncytial virus A Respiratory syncytial virus B	Influenza A Influenza B Respiratory syncytial virus	Influenza A Influenza A H1N1 Influenza B	Influenza A Influenza B Respiratory syncytial virus	Influenza A Influenza B	Influenza A Influenza B
Run-time	1 hour	2.5 hours	1 hour	75 minutes	1 hour	15 minutes	20 minutes
CLIA Complexity	Moderate	Moderate	Moderate	Moderate	Moderate	Waived	Moderate
Throughput	Random access (1 pouch per instrument)	Random access (1 cartridge per analyzer)	Batch of 8 per run	Random access – platform capacity from 1 to 80 cartridges	Random access – platform capacity from 1 to 80 cartridges	Random access (1 cartridge per instrument)	Random access (1 cartridge per instrument)

cartridges and pouches used by other systems, the Verigene disposables consist of a cartridge, in which the patient sample is inoculated, and separate reagent packs, all of which are loaded onto the analyzer. PCR occurs, followed by hybridization of target DNA onto capture oligonucleotides on a microarray and addition of gold nanoparticle probes. The user transfers the cartridge from the analyzer to the reader, where analysis of hybridized DNA occurs and qualitative results are reported. Although the sensitivity and specificity are reportedly > 95%,[18–20] a study comparing the FilmArray RP and Verigene RV+ showed a high rate of invalid tests (15.8%) on the Verigene compared with only 1.2% on the FilmArray. [21] This study also showed a sensitivity of the FilmArray of 90.2% and Verigene of 84.7% for Influenza A when both assays were compared with another molecular method (Prodesse). Butt *et al*.[18] performed a workflow analysis and determined that the total hands-on time (including set up, all steps, and reporting results) was 21 minutes and the total turnaround time (TAT) was 156 minutes for the Verigene. For comparison, this study found that the FilmArray RP hands-on time was 5 minutes and the TAT was 70 minutes. They attributed some of the longer hands-on time for the Verigene RV+ panel to thawing frozen reagents and returning to the instrument to move the cartridge from the analyzer to the reader.

One potential limitation of these rapid molecular tests is the cost. However, the fast TAT of these new assays has implications on patient care and outcomes, which could lead to savings in overall health-care costs. A study comparing an influenza season using the Focus Simplexa assay with the previous influenza season using a traditional real-time PCR with a TAT of 25.2 hours showed that patients who tested negative for influenza in the conventional assay season were given a median duration of 1.1 days of oseltamivir, while patients who tested negative during the Focus assay season were given a median of 0 day of treatment. [22] Rogers *et al*.[23] compared the FilmArray RP to conventional methods in patients less than 21 years old who were admitted for uncomplicated respiratory illnesses, mostly via the emergency department. The average TATs were 6.4 hours for the FilmArray RP and 18.7 hours for the conventional methods. There were no differences in whether antibiotics were prescribed (to cover for possible bacterial infection), but the duration of antibiotics was shorter in the FilmArray group if they obtained results within 4 hours and if the test was positive. The length of hospital stay and time in isolation were also lower in patients who had a positive FilmArray RP compared with patients who had a positive test using the conventional methods. The authors found that using the FilmArray RP costs a total of $178 less per patient than conventional methods when all outcomes were factored in. The Focus study [22] found that despite a higher laboratory cost per test, the overall cost in the Focus assay season was $2.29 less per patient.

Additionally, studies that have compared the FilmArray RP with laboratory-developed, batched, multiplex RT-PCR assays and the Luminex xTAG RVP have shown that the RP is more likely to give a diagnosis as more pathogens can be detected. [17,24] This may especially be important in immunocompromised patients [24] and could have downstream effects such as decreased hospital length of stay, decreased unnecessary antimicrobial treatment, decreased diagnostic tests, and decreased readmission rates or unnecessary outpatient encounters. Butt *et al*.[18] reported the list price per test for the FilmArray RP at $129.00 and the cost per reportable analyte at $6.45; they found that the Verigene RV+ cost per test was $85.00 and cost per reportable analyte was $14.17.

Because of the minimal hands-on time, fast TAT, and lack of analysis or interpretation required to perform this test, the location in a specialized microbiology or molecular laboratory may not be necessary. Xu *et al*. reported their successful experience with the assay in the core lab, reporting a high rate (81%) of oseltamivir prescriptions or doses given in the emergency department in a timely manner to patients positive for Influenza. [16]

Another potential use for molecular respiratory tests is at the point-of-care (POC). Most POC assays have obtained a Clinical Laboratory Improvement Amendments (CLIA) waiver, which substantially decreases the regulatory requirements associated with performing the test in a non-lab setting, for instance, in an emergency department or a physician's office. The Alere i was FDA approved in June 2014 and was granted CLIA waiver in January 2015. Although this assay is CLIA waived, it is substantially more complex than any other waived test currently on the market (e.g. rapid antigen tests, beta-HCG tests). The system comprises an instrument that performs one test at a time and a kit, which contains a sample receiver, a test base, and a transfer cartridge. The instrument has a screen that directs the user to follow steps of the procedure. The sample receiver and test base are inserted into the Alere i instrument and the user waits 3 minutes. Then the sample (nasopharyngeal swab) is inserted into the sample receiver, which contains an elution buffer. The transfer cartridge is used to pipette the sample into the test base, which contains two reaction tubes, each containing lyophilized reagents. The lid of the

instrument is closed and a result is given in approximately 15 minutes.

This instrument uses an isothermal nucleic acid amplification technology called NEAR, which employs a strand-displacing DNA polymerase initiating at a nick created by a nicking enzyme, rapidly producing many short nucleic acids from the target sequence. It does not require lengthy and complex thermocycling or DNA purification. NEAR technology can deliver PCR-caliber results significantly faster and can generate an abundance of amplified products (up to trillion times) in a very short amount of time, enabling molecular detection in minutes; this can also compensate for suboptimal sample collection.[25,26]

The manufacturer's package insert claims a sensitivity and specificity of 97.9% and 86.2% for Influenza A and 92.5% and 96.5% for Influenza B, respectively, when compared with viral culture.[25] Bell *et al*. found a 97.8% sensitivity when the Alere i Influenza A & B assay was compared to viral cell culture.[26] Alere also claims a 94.5% and 98.4% positive percent agreement and 97.7% and 99.4% negative percent agreement with PCR for Influenza A and Influenza B, respectively.[27] Recent studies have shown a sensitivity of 77.8–88.8% when compared with PCR.[28–31] However, one study reported a specificity of 62.5% for Influenza A and 53.6% for Influenza B when compared with the GeneXpert (Cepheid) PCR and discrepancies resolved with the xTAG RVP (Luminex).[32] Finally, two recalls on two different lots for different reasons were issued within the first year after the CLIA waiver was issued.[33] Unlike many of the other molecular respiratory tests, the Alere i uses nasal swabs instead of nasopharyngeal swabs, potentially allowing collection to be substantially more comfortable for patients.

The Roche Cobas LIAT (Lab in a tube) Influenza A and B assay was recently (September 2015) granted CLIA waiver. This system is composed of a single-module instrument and a wand-like cartridge. The instrument has a screen that prompts the user through the process. The sample (nasopharyngeal swab in universal transport media) is added to the sample tube using an included non-precise pipette. The sample tube is inserted into the instrument and the reaction occurs within 20 minutes. The sample tube is divided into several sealed segments containing all necessary reagents. The sample moves from one segment to the next by compression from the instrument and undergoes all steps required for real-time reverse transcriptase PCR within the closed Liat tube. Binnicker *et al*. reported a 99.2% sensitivity for Influenza A and 100% for Influenza B and a specificity of 100% for both, when compared to Focus Simplexa Flu A/B & RSV assay (described above).[34] Further studies showing the clinical impact of POC tests are needed.

Pulmonary tuberculosis testing

Tuberculosis, caused by *Mycobacterium tuberculosis* (TB), is the second most common infectious cause of death worldwide after human immunodeficiency virus syndrome (HIV).[35] The organism first infects the lung and then can disseminate to any organ system. There are an estimated 9.4 million new cases globally, with more than 80% of all cases occurring in 22 low-income countries.[36] In 2013, 1.5 million people died from the disease.[37]

A rapid, highly accurate, and sensitive diagnostic test is the global priority to control tuberculosis.[38] TB control strategies and efforts have been impeded by slow, complex diagnostic methods.[39] Acid-fast bacilli (AFB) smear testing has been widely used for the detection of TB for 127 years;[40] however, it can detect mycobacteria other than TB, and hence it has a low specificity. In addition, many patients with negative AFB smears have a subsequent positive culture; therefore a negative smear does not exclude TB disease.[41] Conventional culture-based methods can take 2–6 weeks for the mycobacteria to grow and additional 3 weeks for antimicrobial susceptibility testing.[41,42] Because of these characteristics, a sample-to-result molecular test for TB has numerous advantages over conventional smear and culture, even in areas of low prevalence.

The Xpert MTB/RIF assay runs on the GeneXpert system (described above). All necessary reagents and controls are included in a disposable cartridge.[43] The assay detects TB and resistance to rifampin in sputum specimens in about 2 hours with minimal hands-on time.[42] It uses real-time PCR assay to amplify a TB-specific sequence. Additionally, mutations within the *rpo*B gene are detected, which confers resistance to rifampin.[44] Rifampin resistance often correlates with isoniazid resistance; therefore, presence of the *rpo*B gene-resistance mutation is sometimes an early marker of multidrug-resistant (MDR) tuberculosis, which requires a more aggressive approach to treatment and infection control.

The WHO reviewed published data from papers, large multicentric studies as well as unpublished and single-center studies in late 2010. Review of the results from 12 single-center evaluation studies showed that the assay has a sensitivity range of 70–100% in smear-positive patients and about 60% in smear-negative patients compared with culture. The specificity was reported to range from 91% to 100%. Rifampin-

resistance detection was 95.1% sensitive and 98.4% specific.[45] Results from a controlled clinical validation trial of 1730 suspected TB individuals showed the sensitivity of a single direct Xpert test to be 72.5% in smear-negative/culture-positive and 90.2% when three smear-negative samples were tested. The Xpert specificity was 99%. Sensitivity and specificity of rifampin resistance detection were 99.1% and 100%, respectively.[43,45] In February 2013, the WHO published additional evidence including a systematic review of a total of 27 studies involving 9558 participants. This review showed a sensitivity of 88% and a pooled specificity of 99% compared with culture when Xpert MTB/RIF was used as an initial diagnostic test replacing smear microscopy. Xpert MTB/RIF yielded a pooled sensitivity of 68% and a pooled specificity of 99% when used as an add-on test following a negative smear microscopy. Using Xpert MTB/RIF in smear-positive, culture-positive TB showed a pooled sensitivity of 98%. Additionally, Xpert MTB/RIF achieved a pooled sensitivity of 95% and a pooled specificity of 98% in detecting rifampin resistance.[46]

To perform the Xpert MTB/RIF assay, the user adds bactericidal buffer to sputum before transferring a defined volume to the cartridge. This reduces the viability of *M. tuberculosis* in sputum by at least 6–8 $\log_{10}$ units after 15 minutes of incubation and therefore eliminates the requirement to have a biosafety cabinet.[43,47,48] The Xpert MTB/RIF can be used under varying temperature and humidity conditions and requires minimal personnel training. [45] For these reasons, this assay could be used in regional, non-specialized laboratories or potentially outside of laboratory settings in developing countries.

This novel molecular test has a few limitations. The need for conventional culture and drug resistance test is not eliminated by Xpert MTB/RIF technology. These tests are still required for monitoring response to treatment and for detecting resistance to antibiotics other than rifampin.[45,49] A negative Xpert MTB/RIF does not exclude the diagnosis of TB. The CDC reports that 15–20% of TB cases in the United States that are reported to have negative cultures may also have negative nucleic acid amplification test.[49] This may be due to the low bacterial load or the presence of inhibiting substances in the sample. From the operational aspect, one limitation is that the assay needs uninterrupted electrical power supply as well as annual validation of the system, which may be problematic in underdeveloped areas.[45]

Although the test is more expensive than smear and culture-based methods, the rapidity and high sensitivity and specificity make it appealing to be used as an alternative to conventional serial sputum microscopy.[50] Implementation of this assay may lead to budgetary increases for laboratories in the short term compared with smear microscopy; however, rapid results from the Xpert MTB/RIF assay may contribute to substantial cost saving by avoiding unnecessary treatment and infection control practices, such as airborne respiratory isolation.[40] Millman *et al*. suggested that routine use of Xpert MTB/RIF could contribute to a decreased cost of $2278 per inpatient admission, which would in turn save approximately $533,520 per year in a medium-sized urban public hospital.[50]

In December 2010, WHO endorsed the Xpert/MTB RIF for use in endemic countries by strongly recommending this test to be used as the initial diagnostic test in individuals suspected of having MDR TB or HIV-associated TB.[51] In 2012, several agencies including the Bill and Melinda Gates Foundation formed an agreement with Cepheid to provide discounted costs to eligible developing countries. The initial cost of a four-module instrument with a laptop is approximately $17500 and the cost per cartridge is $9.98.[52,53]

This rapid molecular testing has shown promising impact on TB control strategies worldwide. One study estimated that implementation of nucleic acid amplification tests globally will reduce the TB incidence rate by 28% by 2050.[39,54] With earlier diagnosis, earlier initiation of treatment, and earlier infection control strategies, appropriate implementation of the test can substantially benefit patients, health-care providers, hospitals, and public health systems. [49] More studies need to be performed in the future for the application of Xpert MTB/RIF, particularly in the pediatric population, extrapulmonary tuberculosis, and the usefulness of the assay for monitoring response to treatment.[45]

In conclusion, studies have shown that sample-to-result molecular testing for respiratory pathogens is rapid and simple and can positively impact patient care.

Central nervous system

Patients who present with signs and symptoms that could be consistent with an infection of the CNS often undergo a lumbar puncture, with numerous tests performed on their cerebrospinal fluid (CSF). CNS infections can be caused by viruses, bacteria, fungi, and rarely parasites. Enterovirus is the most common cause of aseptic meningitis when an etiology is found.[55] The disease is self-limited and specific antiviral therapy is not available. However, because viral meningitis can be difficult to differentiate from bacterial meningitis and other causes of CNS disease, patients are often admitted and treated empirically for other

conditions until a firm diagnosis is made. Therefore, it would be reasonable to assume that a rapid diagnosis of enterovirus would substantially decrease the length of stay, antimicrobial administration, and hospital costs. This was seen in a 2015 study using the Cepheid Xpert EV assay.[56] Giulieri and colleagues compared patients with aseptic meningitis diagnosed via the Xpert EV assay (TAT from collection to result, 5 hours), a conventional home-brew PCR (TAT, 60 hours), and patients with no diagnosis. They found that patients with a diagnosis confirmed on the GeneXpert had a significantly shorter duration of empiric antibiotics and fewer patients received empiric acyclovir. These patients had an average length of stay of 0.5 days, compared with 2 days and 4 days for patients in the group with conventional PCR or no diagnosis, respectively. This assay has an observed sensitivity of 94.7–100% and a specificity of 100%.[57–59] The test takes 2.5 hours to run with minimal hands-on time. It uses 140 µL of CSF and is categorized by CLIA as moderate complexity.

HSV type 1 and type 2 can cause encephalitis, with HSV-1 predominating in the postneonatal period. Patients who present with signs and symptoms that could be consistent with these infections should be placed on acyclovir as soon as the diagnosis is considered. Untreated, mortality is approximately 70%.[60] PCR has vastly improved patient management and outcomes compared with traditional culture-based or serologic methods. Empiric antivirals will not interfere with PCR as they may with culture. Serology takes 2–4 weeks to confirm an acute infection, which is of limited value for care of the patient. Finally, PCR is substantially less invasive than brain biopsy, which at one time was considered the 'gold standard' for the diagnosis of HSV infections of the CNS.[61] HSV PCR can be performed using the moderately complex Focus Simplexa HSV 1 & 2 Direct assay on the 3 M Integrated Cycler (described above).

FilmArray's Meningitis/Encephalitis Panel was FDA approved in October 2015. This assay is performed similar to the RP as described above. It uses 200 µL of unprocessed CSF, takes about 2 minutes of hands-on time, and results are available in 1 hour.[62] The panel tests for the following organisms: *Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae*, cytomegalovirus, enterovirus, HSV-1, HSV-2, HHV-6, human parechovirus, varicella zoster virus, and *Cryptococcus neoformans*. Currently, there are no peer-reviewed published studies stating the performance or clinical implications of this assay. Since the identification of some of these organisms in CSF (e.g. human parechovirus) has in the past been limited, it will be important to study how this new diagnostic technique impacts patient care.

Gastrointestinal infections

Multiplex GI panels

Infectious diarrhea affects millions of people each year around the globe with high morbidity and mortality and a substantial impact on the health-care system. The WHO reports an estimate of 2195 children deaths per day due to infectious gastroenteritis.[63] In 2010, the U.S. national bill for the 237,000+ patients suffering from gastrointestinal infections was over $6 billion.[64] Rapid and accurate detection of GI pathogens is important for appropriate therapy and proper infection control strategies.[65]

Clinical presentation of infectious gastroenteritis is not of much help in terms of specifying the etiology, as diarrhea is the primary symptom regardless of the causative agent. This makes it challenging for the clinician to decide what test to order. Also, for many of these agents, there is no diagnostic test available.[66] Currently, in about 80% of cases of infectious diarrhea, the etiologic agent remains unidentified, resulting in improper therapy.[67]

Conventionally, bacterial culture, antigen detection, microscopy for ova and parasites, viral culture, and singleplex real-time PCR assays have been used for identifying various organisms. Some of these methods are time consuming, labor intensive, and can only test for a limited number of organisms. Despite the fact that the causative agents are sometimes indistinguishable clinically, the conventional methods require the clinicians to select an appropriate test or utilize a 'shotgun' approach, where they order numerous tests at one time. Moreover, the results for some of these tests may not be available for several days. The prolonged TAT makes these tests less attractive, particularly with respect to patient management.[68]

Etiologic diagnosis of infectious diarrhea will be extremely beneficial for patient management, infection control, and public health strategies.[66] As a result, the interest in utilizing multiplex molecular assays for the identification of the causative agents in infectious diarrhea has been increasing.[66,69]

Multiplex molecular assays use PCR to simultaneously detect and identify multiple gastrointestinal pathogens' nucleic acids extracted from stool specimens.[69] There are two FDA-approved commercially available multiplex sample-to-result assays: FilmArray GI panel and the Verigene enteric pathogens (EP) panel. The FilmArray GI

panel includes 22 targets (13 bacterial, five viral, and four parasitic) within a single pouch (See Table 3). The Verigene EP panel has the ability to identify nine targets (five bacterial, two viral, two toxins) (see Table 3). FilmArray's GI panel works almost identical to its RP as described above.[65] Khare *et al*. demonstrated that the majority of targets represented on the FilmArray GI panel show high sensitivity and specificity (>90%).[65] Buss *et al*., in their study of 1556 cases, reported the FilmArray GI panel to have 100% sensitivity/positive predictive value in 12 out of 22 targets (*Plesiomonas shigelloides, Salmonella* spp., *Yersinia enterocolitica*, Enterotoxigenic *Escherichia coli* (ETEC), Shiga Toxin-producing *E. coli* (STEC), *E. coli* O157, *Cryptosporidium* spp., *Cyclospora cayetanensis, Giardia lamblia*, Astrovirus, Rotavirus A, and Sapovirus) .[66] For the other 10 targets the FilmArray sensitivity/positive predictive value was > 94.5%. The FilmArray specificity/negative predictive value for all the targets was >97.1%. Conventional methods identified at least two pathogens in 8.3% samples, while the FilmArray showed an overall rate of mixed infection of 27%. Buss *et al*.[66] detected multiple pathogens in 31.5% of the specimens.

Verigene's EP panel is also FDA approved and includes targets for five bacteria, two viruses, and two toxins (see Table 3). At the time of this writing, there were no peer-reviewed published studies demonstrating the sensitivity and specificity or workflow analysis for the assay. The manufacturer reports a hands-on time of <5 minutes and a run-time of about 2 hours.

There are a few limitations regarding gastrointestinal panels. First, as they can detect a large number of possible pathogen combinations and because asymptomatic infections with some of the targeted pathogens are not uncommon, especially in the pediatric population, interpreting positive results and identifying the main culprit for the symptoms can be challenging. [66,70] The second limitation is that conventional bacterial cultures may still be required to determine susceptibility to antibiotics and for epidemiological tracking of possible outbreaks [71] (e.g. Salmonella serotyping). However, given the rapidity of obtaining a positive PCR result, it may still be possible to culture the organism from the same specimen.

One major limitation to these panels may be the cost. The cost of the FilmArray GI panel per sample is estimated to be $115.00–$150.00 with less than 10 minutes of total hands-on technologist time for setup and reporting results. The cost of running FilmArray GI panel that can detect and identify 22 pathogens is comparable to the average of three tests ordered by conventional methods per specimen (Barney T, Hopper A, Nelson-Miller C, *et al*, Unpublished data).

GI panels have significant infection control implications. By placing patients with unsuspected GI pathogens in appropriate isolation while removing patients with a negative GI panel from unnecessary isolation, FilmArray GI panels can potentially lead to a more logical approach to patient isolation. This would not only minimize the risk of nosocomial infections, but also significantly reduce the associated costs and increase patient satisfaction. [72,73] Rand *et al*.[72] saved 158 inpatient residual samples that had been originally submitted and tested negative for *Clostridium difficile* and/or rotavirus and tested them on the FilmArray GI panel. In this study, 22.2% of samples had at least one other infectious agent detected, and 60% of those patients were never placed in isolation. Additionally, they identified 20.3% of patients who could have been removed from isolation based on a negative FilmArray GI panel. This study indicated that numerous pathogens are currently underdiagnosed and there may be inappropriate use or underuse of isolation.

A very interesting study was conducted by Nebraska medical center during the cyclosporiasis outbreak that occurred in Iowa and Nebraska in 2013. The institution was carrying out an unrelated research study on the FilmArray at this time. The FilmArray GI panel detected *Cyclospora* prior to and during the early phases of the outbreak in specimens that went unidentified by the clinical laboratory because cyclosporiasis was not clinically suspected and therefore modified acid-fast staining was not initially ordered. The first positive *Cyclospora* specimen using the FilmArray GI panel was collected 1 week prior to the first reported positive case in Nebraska. This indicates that if FilmArray was utilized as a screening tool, the outbreak would likely have been detected earlier. [74] In a similar study, during a shigellosis outbreak in Rhode Island in 2013, the FilmArray GI panel not only was quicker than conventional culture but also detected 40% more cases of *Shigella*/EIEC (Enteroinvasive *E. coli*) compared to culture.[75]

Another advantage of the FilmArray GI panel is that it uses a closed system, so there is minimal risk of contamination.[66]

Routine use of these panels has the potential to allow the cost-effective, timely detection of multiple pathogens. This could lead to improved public health and cost savings due to optimal use of laboratory resources and reduced transmission in outbreaks. An additional area of cost savings may include the prevention of repeat health-care encounters because the etiologic agent would be known soon after the first visit. Further studies regarding cost analysis are warranted.

Table 3. Comparison of gastrointestinal panels and organism-specific tests.

	Gastrointestinal Panels		Organism-Specific Tests			
Platform Name	FilmArray	Verigene	GeneXpert	GeneXpert	Verigene	GeneXpert
Assay Name	Gastrointestinal Panel	Enteric Pathogens Panel	C. difficile/Epi	C. difficile	C. difficile	Norovirus
Manufacturer	Biofire	Nanosphere	Cepheid	Cepheid	Nanosphere	Cepheid
Targets	**Bacteria** *Campylobacter* (includes *C. jejuni, C. coli* and C. *upsaliensis*) *Clostridium difficile* (toxin A/B) *Plesiomonas shigelloides* *Salmonella* *Yersinia enterocolitica* *Vibrio* (includes *V. parahaemolyticus, V. vulnificus* and V. *cholerae*) *Vibrio cholerae* **Diarrheagenic E. coli/Shigella** Enteroaggregative *E. coli* (EAEC) Enteropathogenic *E. coli* (EPEC) Enterotoxigenic *E. coli* (ETEC) lt/st Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2 *E. coli* O157 *Shigella*/Enteroinvasive *E. coli* (EIEC) **Parasites** *Cryptosporidium* *Cyclospora cayetanensis* *Entamoeba histolytica* *Giardia lamblia* **Viruses** Adenovirus F 40/41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus (I,II, IV, and V)	**Bacteria** *Campylobacter* group *Salmonella spp.* *Shigella spp.* *Vibrio* group *Yersinia enterocolitica* **Toxins** Shiga Toxin 1 (stx1) Shiga Toxin 2 (stx2) **Viruses** Norovirus Rotavirus	Toxin B 027 strain	Toxin B	Toxin A Toxin B 027 strain	Norovirus genogroup I Norovirus genogroup II
Run-time	1 hour	2 hours	45 minutes	45 minutes	2 hours	1 hour
CLIA Complexity	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Throughput	Random access (1 pouch per instrument)	Random access (1 cartridge per analyzer)	Random access – platform capacity from 1 to 80 cartridges	Random access – platform capacity from 1 to 80 cartridges	Random access (1 cartridge per instrument)	Random access – platform capacity from 1 – 80 cartridges

Norovirus

Norovirus is an extremely contagious organism and the most common cause of acute gastroenteritis worldwide. Transmission is fecal-oral. Although the disease is most often self-limited, the virus can have major impacts on health care by causing large outbreaks.[76] These are often associated with close living conditions such as cruise ships, nursing homes, and day cares. Therefore, prompt diagnosis of the etiology can have a positive impact on patient care by preventing further spread of disease.

The Cepheid Xpert Norovirus assay is performed similarly as the other GeneXpert assays described above. It is performed directly from a stool sample in approximately 1.5 hours and detects and discriminates Norovirus genogroups I and II. One large study that tested 1,403 samples found the Xpert assay to have a positive percent agreement of 98.3% and 99.4% for genogroups I and II, respectively, and a negative percent agreement of 98.1% and 98.2% for genogroups I and II, respectively,[77] compared with a composite reference method at the CDC. Further studies are needed to determine the clinical utility of a standalone Norovirus assay versus a large multiplex panel.

Clostridium difficile

Toxigenic *Clostridium difficile* is the main cause of health-care- and antibiotic-associated diarrhea. It can cause a range of mild to severe disease, pseudo-membranous colitis, toxic megacolon, perforation of the colon, sepsis, and rarely death.[78,79] *C. difficile* has a large impact on health care with at least US $1 billion spent per year.[66] Detection of *C. difficile* quickly and accurately is important for appropriate antibiotic treatment and proper infection control strategies, especially during outbreaks.[78]

There are a variety of tests currently available for the detection of *C. difficile* from stool samples. These tests include culture, antigen detection, toxigenic culture, enzyme immunoassay, and molecular testing.[80] Stool culture is the most sensitive test available; however, it is laborious, has slow TAT (48–96 hours), and does not determine the presence of a toxin. Antigen detection tests are rapid tests (<1 hour) that use latex agglutination or immunochromatography to detect the presence of *C. difficile*. Antigen testing is nonspecific for pathogenic strains, as some organisms do not produce the toxin, and therefore they have been used in combination with more specific tests.[79] Some of these less-sensitive tests required three separately collected stool samples, which lead to the old ordering adage, '*C. diff* times 3'.

Tissue culture cytotoxicity assay for *C. difficile* for detection of toxin B was considered the 'diagnostic gold' standard for many years. However, this assay is expensive, has a slow TAT (24–48 hours), and requires substantial technical expertise. It does provide specific and sensitive results for *C. difficile* infection; however, it is considered less sensitive than PCR or toxigenic culture for detecting the organism in patients with diarrhea.[79,80] Enzyme immunoassay detects toxin A, toxin B, or both A and B. These assays are inexpensive and simple, and therefore are commonly used. However, they are relatively insensitive, and some institutions employ them in a two- or three-step algorithm with other diagnostic methods, such as assays for the enzyme glutamate dehydrogenase.[79,80]

Most molecular tests utilize PCR to detect the gene encoding toxin B with high sensitivity (94.4%), specificity (96.3%), and fast TAT.[81] Cepheid offers two FDA-approved tests: Xpert *C. difficile* and Xpert *C. difficile*/Epi, which differentiates the epidemic strain, North American profile 1 (NAP1). These assays are performed directly from a stool sample on the GeneXpert system, nearly identically as the other Xpert assays described above. The NAP1 strain is implicated in hospital outbreaks.[82] The Verigene *C. difficile* test has the ability to detect both toxin A and toxin B genes. It can also identify NAP1 strain.[83]

C. difficile is shed in feces; therefore spores can be transferred through any surface, device, or material that becomes contaminated with feces. The CDC recommends placing these patients in a private room to minimize the transmission.[79] One major associated cost with *C. difficile* infection is the length of hospital stay. Slow TATs of some of the available tests can attribute to the longer stay and perhaps unnecessary isolation. Rapid molecular testing has a higher sensitivity and specificity compared to other rapid tests and only one sample is required. Faster TAT leads to earlier detection, reduced repeat testing, and ultimately shorter hospital stay and unnecessary isolation.[84] Many institutions place patients in contact isolation when *C. difficile* is suspected. Often this is done by flagging patients who have a pending order for a *C. difficile* test. Therefore, they may stay in isolation until the *C. difficile* test is negative. In this approach, the longer the *C. difficile* test takes, the longer a patient may stay in isolation who may or may not need it. Isolation not only implies extra costs for the hospitals and patients but also leads to patient dissatisfaction.[84,85] A study that was published in Society for Health care epidemiology of America (SHEA) found that patients placed in contact isolation were likely to report

perceived care problems twice as much as patients not in contact isolation.[85]

There are a few limitations for *C. difficile* molecular PCR assay. A meta-analysis of online data from 1999–2010 reported that despite the high sensitivity and specificity of PCR for *C. difficile*, the accuracy of the assay depends on the *C. difficile* prevalence. When *C. difficile* prevalence in the tested population was <10%, the positive predictive value was only 71%. This can possibly lead to overtreatment and unnecessary isolation. On the other hand, at a *C. difficile* prevalence of >20%, the PPV was 93%. Therefore, PCR might not be the best diagnostic test in patient populations with low prevalence.[86] The other limitation is that the molecular assay very specifically detects the gene encoding the toxin and not the toxin itself. This may result in positive results in asymptomatic *C. difficile* carrier patients.[86] Hence, some institutions have instituted algorithms using both PCR and the toxin EIA in order to increase the positive predictive value. In addition, asymptomatic colonization with toxigenic *C. difficile* is common in children < 1 year old (50–70% of healthy neonates), which makes interpretation of a positive result complicated.[66,86]

Similar to the FilmArray GI panel, rapid molecular testing for *C. difficile* allows cost-effective, timely detection of the organism, optimized use of laboratory resources, and reduced transmission in outbreaks, which has a substantial impact on patient management, satisfaction, and public health.[84] Despite all the benefits of the molecular testing, the diagnosis of *C. difficile* continues to be challenging and the optimal diagnostic method remains difficult to achieve.[87,88] The limitations of these tests, such as detecting asymptomatic carriers, should be taken into account when interpreting a positive result.[87,88]

Sexually transmitted infections

Diagnosis and treatment of sexually transmitted infections (STIs) are important because complications can be serious, including pelvic inflammatory disease, adverse neonatal outcomes if associated with pregnancy, disseminated infection, and increased risk of acquiring HIV. Diagnosis can be difficult because many infections are asymptomatic; however, asymptomatic infections can still be transmitted to sexual partners. Additionally, signs and symptoms can be nonspecific and easily confused with other genitourinary diseases.[89] Diagnostic techniques that are rapid and highly sensitive and specific should increase the rate of treatment and time to treatment and partner notification, which should reduce the overall transmission and prevalence in a community. Conventional methods in this field include culture, laboratory-developed molecular assays, serology, and immunochromatographic POC tests, the latter of which are not routinely used due to poor performance. The Centers for Disease Control and Prevention (CDC) recommends nucleic acid amplification-based tests for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) over culture with some exceptions, which are explored below.[90] The CDC also recommends molecular testing for *Trichomonas vaginalis*.[91] *Trichomonas* is a flagellated protozoan parasite that causes vaginitis in women and urethritis in men, although it is often asymptomatic.

Cepheid offers an Xpert CT/NG assay on their GeneXpert platform (described above). Results are available in 90 minutes and the assay is considered moderately complex. Approved specimens include patient-collected vaginal swabs, endocervical swabs, and male and female urine. The sensitivity and specificity for CT and NG are reported to be very high (>95.6% sensitive and >99.4% specific), depending on the analyte, specimen type, and comparison method.[92,93] The test has performed well in POC settings. One study [94] reported that asymptomatic, mostly men who have sex with men (MSM) patients were tested on-site at a sexual health clinic in London and received treatment in 2 days after their test, compared with an average of 10 days for historical matched controls who were tested via conventional methods in the off-site, hospital-based laboratory. In this population, 14% of all patients were positive. Another study [92] involved staff performing this assay in a remote community of Australia, where delays in testing via conventional, lab-based methods and notification of patients lead to an average time to treatment of 21 days. The staff were given a 1-day training session regarding use of the assay and instrument. At the end of the study period, they reported that the GeneXpert was easy to use and feasible in their remote setting. This was a proof of concept study, so results of the GeneXpert were not used in patient care. Perhaps in the future, this assay will become CLIA-waived and use in the POC setting will be routine.

The Hologic Gen-Probe Panther operates differently than previously described sample-to-result molecular systems. Up to 120 samples can be initially loaded, followed by continuous-feed, random-access availability, for up to 275 samples processed in 8 hours. The first result will be available in 3.5 hours. Reagents and controls are loaded separately, usually once per day, at any time, as the controls are valid for 24 hours. Aptima assays are utilized for HPV, CT/NG, and *Trichomonas*, which can all be detected simultaneously on the same sample. These assays employ transcription-mediated

amplification. Reflex HPV genotyping for types 16, 18, and 45 can occur when HPV is detected. Approved samples for each assay include: CT/NG: clinician or patient self-collected vaginal swab, female endocervical swab, male urethral swab, male urine, ThinPrep test vial; *Trichomonas*: clinician-collected endocervical and vaginal swabs and PreservCyt liquid Pap specimen; HPV: ThinPrep vial only. Although female urine is not FDA approved on the Panther, studies have shown good performance for detection of CT/NG from this common specimen type.[95,96] Although this system is considered high-complexity by the FDA, Ratnam *et al*. found that this system had the lowest overall hands-on time, including operational and maintenance steps, compared with four other molecular CT assays.[97]

The Panther uses the TMA technique. In contrast to PCR, this method involves RNA transcription (via RNA polymerase) and DNA synthesis (via reverse transcriptase) to produce RNA amplicons from a target nucleic acid (either DNA or RNA). TMA has some advantages compared to PCR; for example, it is isothermal so it does not require lengthy and complex thermal cycling. TMA produces RNA rather than DNA amplicons. RNA is more labile in a laboratory environment, which reduces the possibility of carryover contamination. Lastly TMA produces 100–1000 copies per cycle in contrast to PCR, which produces only two copies per cycle. This results in a 10 billion-fold increase of DNA (or RNA) copies within about 15–30 minutes.[98,99]

One potential drawback to relatively recent advances in nucleic acid-based tests for the diagnosis of *NG* is the lack of an isolate to perform susceptibility testing on. Penicillin, tetracycline, and fluoroquinolones are no longer recommended for treatment of NG because of widespread resistance to these antibiotics. Therefore, susceptibility testing is not necessary for initial antibiotic selection; however, the CDC recommends culture and susceptibility testing in patients who demonstrate treatment failure. Also, susceptibility testing is necessary for surveying circulating isolates to monitor for emerging resistance.[90]

Another limitation to STI molecular testing and a potential area for future growth is the lack of FDA-approved tests for ocular, rectal, and pharyngeal specimen. Trachoma, caused by *Chlamydia* infection of the eye, is the leading cause of preventable blindness worldwide. It occurs mainly in resource-poor settings and infections can be seen even after prophylactic treatment. Presence of infection does not always correlate well with physical findings. One study collected ocular samples in Tanzania and tested them in a U.S. laboratory on the GeneXpert CT/NG assay, achieving 100% sensitivity and specificity compared with another molecular assay (Roche Amplicor CT/NG). In a subsequent study,[100] the researchers collected two swabs from the same eye on 144 children under 9 years old. One swab was tested on the Xpert CT/NG assay in the field in Tanzania, and the other swab was sent to the U. S. for testing on the Roche Amplicor CT/NG test. A total of 127 samples were available for analysis and the Xpert assay was found to have a sensitivity of 100% and specificity of 95%. The other 17 samples did not obtain results on the GeneXpert due to insufficient sample (n= 9), other material in sample (n = 4), or loss of electricity (n = 4).

Many gonococcal or chlamydial rectal and oropharyngeal infections are asymptomatic, and the CDC recommends that certain populations, such as MSM, undergo routine genital and extragenital screening. [90] However, no FDA-approved nucleic acid amplification tests are available for rectal and oropharyngeal sources, so laboratories must perform a validation if they wish to test samples from those sites. Goldenberg *et al*.[101] tested 409 rectal swabs on the Xpert CT/NG on the GeneXpert and the Aptima Combo2 assay on the Hologic Gen-Probe Tigris platform and found that the GeneXpert sensitivity and specificity were 86% and 99.2% for CT and 91.1% and 100% for NG, respectively; however, no discrepant analyses were performed on the samples that did not agree between the two assays, so it is possible that the sensitivity and specificity were higher if the Aptima assay delivered some false-positive or -negative results. Interestingly, rectal swab samples required dilution to avoid invalid results.

HSV 1 and HSV-2 most often cause genital and oral STIs. They establish latency after primary infection and can reactivate, causing recurrent local disease. Extragenital complications can occur, including disseminated disease, meningitis, and proctitis. Vertical transmission from mother to infant can occur during delivery, making the diagnosis of active disease important during pregnancy.

The Focus Simplexa™ HSV 1 & 2 Direct Kit was recently (August 2015) approved for genital swab samples in addition to CSF (see above). According to the package insert, the sensitivity and specificity for HSV 1 and HSV 2 are both greater than 97% from genital swabs. [102] The Luminex ARIES HSV 1 & 2 assay was FDA approved in October 2015 as moderate complexity. This PCR assay detects and discriminates HSV 1 & 2. The ARIES system holds up to 12 samples at a time in two magazines. [103] A cutaneous or mucocutaneous sample is inoculated into a cassette, which is loaded into a magazine. A magazine can hold up to six cassettes but does not need to be

full when loaded into the instrument. The user then starts the assay and additional cassettes cannot be added into that magazine during the 2-hour run. The other magazine can be loaded and started at any time. The package insert lists sensitivities for HSV-1 of 91.1% and 97% for cutaneous and mucocutaneous lesions, respectively, and sensitivities for HSV-2 of 95% and 98.5% for cutaneous and mucocutaneous lesions, respectively. The specificities for HSV-1 were 94.2% and 95.4% for cutaneous and mucocutaneous lesions, respectively, and specificities for HSV-2 were 88.8% and 93.2% for cutaneous and mucocutaneous lesions, respectively. The reference method was ELVIS HSV-ID and D3 Typing Test System. Peer-reviewed publications are needed to verify these characteristics.

Currently, there are no sample-to-result molecular assays for syphilis. New techniques for diagnosis and quantitation of HIV are undergoing evaluation. Perhaps we will see these become mainstream in the future.

Expert commentary

Sample-to-result molecular infectious disease assays perform with exceptionally high sensitivity and specificity, are fast and simple to perform, and require few resources such as space and personnel training. There are some limitations that accompany this type of testing. First, the scope of molecular assays ranges from targeting a single organism or a few organisms to extraordinarily large panels. Tests that target numerous organisms, such as the FilmArray gastrointestinal panel, will lead to a shift in how providers think about diagnostic tests, e.g. will large, multiplex panels make obtaining the patient's history and physical exam less important? Is there a need to elucidate that information when there is one test for most possible etiologies? Balancing these diagnostic techniques will be a challenge; remembering their positive and negative predictive values in different patient populations will be of upmost importance. We must continue to emphasize the appropriate interpretation of results. If tests are easy to perform and highly accessible, will we obtain results that are inappropriate for the patient's clinical context? How often will we find results that we are currently 'missing' with our conventional diagnostic tests? There may be substantial difficulty in assessing the clinical implication of results, for example, the possibility of *C. difficile* as a colonizing organism as discussed above. The utility of targets for which there is no treatment (e.g. rhinovirus) is also uncertain.

Some organisms, such as influenza, mutate over time and the theory that these mutations could lead to decreased sensitivity of an assay over time is a significant concern. However, the potential need for periodic revalidation is forbidding. It would be a large amount of work to have to revalidate an assay yearly. Also, each assay has specific specimen source for which it is approved, which may limit the utility of the test. For example, the FilmArray RP is only approved for use on nasopharyngeal swabs, but some patients would benefit from testing on lower respiratory tract specimen such as bronchioalveolar lavages, or less-'painful' specimen collection such as nasal swabs. Another limitation of molecular testing is the inability to perform resistance testing on isolates and surveillance for public health purposes.

Additionally, some platforms, such as the Alere i, FilmArray, Verigene, and Cobas Liat, run only one sample at a time. While this allows for customization based on needs of the laboratory, even small- to medium-sized labs may need numerous devices to keep up with testing volumes and to prevent a backlog of specimen.

Currently, the biggest area of concern is with the cost of these tests. As we have seen in this review, there are many studies that demonstrate positive downstream effects of the tests, such as shorter length of hospital stay, which lead to substantial decreases in overall health-care costs. However, large upfront costs or increases in per test costs can be challenging to overcome.

Five-year view

Molecular infectious disease testing has become so simple that the term 'sample-to-result' is now common terminology. This was the result of numerous, somewhat small changes that all added up to a substantial shift in the way molecular diagnostics is performed. Additional improvements will lead to broader use of these assays. Costs will decline and test menus will expand to include more organisms. Tests may include quantitative results in addition to qualitative results. Instruments will become smaller and more portable. This will all lead to a shift in the physical location of these tests from specialized molecular laboratories, to microbiology labs, to general core labs, and eventually to more direct patient testing sites, such as the patient's bedside.

Within five years, we are certain to see enormous growth in POC molecular tests, which can be

Key issues

- Sample-to-result molecular tests are in the process of replacing the earlier generations of nucleic acid tests, which require pre-analytic sample processing and post-analytic analysis, are in open systems in which contamination is of high risk, and take several hours to complete.
- Many sample-to-result molecular infectious disease assays have high sensitivity and specificity, are fast and easy to perform, and require minimal resources.
- Large, multiplex nucleic acid panels have led to and will continue to cause a major shift in the way we diagnose patients.
- When evaluating new molecular tests, an emphasis is placed on how the result of the test will impact the quality of patient care.
- Currently, the cost of these tests may be a barrier to implementation; however, demonstration of decreases in overall health-care costs may help overcome this barrier.

performed by any licensed personnel and not necessarily laboratory staff. This means that the tests can fairly easily be performed in a clinic, urgent care, pharmacy, or emergency department setting. In order for this to come to fruition, the tests will need to be faster (less than 30 minutes). They also will be modified to appeal to non-lab staff. While lab staff members are accustomed to common tasks such as precise pipetting and documenting quality control, these steps will need to be eliminated or automated when used by a wide range of professional and even nonprofessional providers. In addition, sample-to-result tests will become more routine in resource-limited settings, where the burden of disease tends to be exceptionally high. POC tests for tuberculosis are already in use (outside of the U.S.), and tests for HIV and Hepatitis C virus are in development.

Studies that demonstrate the sensitivity and specificity of new tests will continue to be important, but may be overshadowed by studies that demonstrate the impact on patient care. It is no longer good enough to have an assay that performs well; it is necessary to show that it leads to shorter hospital length of stays, faster time to appropriate treatment, elimination of unnecessary treatment, overall decreases in health-care costs, and other measures of best practices. This is all in line with the idea of 'personalized' or 'precision' medicine, a growing area of emphasis in today's practice of health care.

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• **This reference is of importance because it led to a change in practice. Many labs were concerned about the sensitivity of the test for adenovirus infections and were compelled to confirm negative adenovirus results with another assay. This study refuted that need.**

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