

Multiplex PCR for Detection and Identification of Microbial Pathogens



Mark A. Poritz and Beth Lingenfelter

Introduction

Over the last decade, a number of manufacturers have developed multiplexed in vitro diagnostic (IVD) platforms that can detect the nucleic acid signatures of many of the organisms responsible for infectious disease. Some of these testing platforms offer limited test menus (i.e., influenza A, influenza B, and RSV), while others are designed to detect a more comprehensive set of potential pathogens that can cause a particular infectious disease syndrome (e.g., respiratory, gastrointestinal, sepsis, meningitis) [1–3]. This chapter will describe FDA-cleared and/or CE-marked multiplex assays that are designed to detect a comprehensive set of pathogens associated with a particular infectious disease syndrome (≥ 10 assays/test). These include the BioFire (Salt Lake City, UT) FilmArray[®] System [4], the GenMark (Carlsbad, CA) eSensor XT-8[®] [5] and ePlex[®] [6], and the Luminex (Austin, TX) xTAG[®] [7], nxTag[®] [8], and Verigene[®] systems [9].

In addition to being comprehensive with respect to pathogens responsible for a particular syndrome, these multiplex panels offer the advantage of superior test sensitivity and specificity. Many of these panels have been designed to be easy-to-use, allowing molecular testing to be performed in moderate or low complexity settings and eliminating barriers that prevented many laboratories from being able to perform molecular assays on-site. Another important benefit of multiplex panel is the fast time to result. Molecular multiplex tests require hours to perform instead of the days required for culture-based methods.

M. A. Poritz (✉)
BioFire Defense, Salt Lake City, UT, USA
e-mail: Mark.Poritz@BioFireDefense.com

B. Lingenfelter
BioFire Diagnostics, LLC, Salt Lake City, UT, USA

These systems differ in particular details (the exact turnaround time, sample throughput, cost per sample, and number of target pathogens detected). These differences are due to the underlying technologies used for the detection of the pathogen nucleic acid. However, all these systems share the common attribute that the incremental cost of each additional assay in the test cartridge (in materials, labor, and quality control (QC) testing) is small compared to the total manufacturing costs for the disposable. This has enabled IVD manufacturers to develop broad test panels that include organisms which have not been a part of standard testing protocols because of the technical limitations of existing methods.

Nonetheless the availability of syndromic infectious disease panels poses hard questions for clinicians and the healthcare system overall: Does the wealth of information in a comprehensive test improve the treatment of an individual patient, and how can the economic value of this improved treatment be measured?

Comparison of Multiplex Nucleic Acid IVD Systems

Commercially available FDA-cleared multiplex nucleic acid-based tests for infectious agents include systems from Luminex, GenMark, and BioFire (now a subsidiary of bioMérieux) (Table 1). At present, all such systems combine the sequential steps of:

1. Nucleic acid purification from the appropriate human sample matrix (e.g., nasal swab, blood or blood culture, stool)
2. cDNA synthesis (reverse transcription) to convert viral RNA to DNA, if necessary
3. Multiplex PCR to amplify molecules of the pathogen nucleic acid
4. Specific detection of the expected amplicons to confirm that the correct target nucleic acids have been identified

The different systems vary mainly in whether the nucleic acid purification steps are integrated into the same cartridge that is used for amplification (Verigene, ePlex, and FilmArray) and in how the specific detection of amplicon is achieved. The Luminex xTag and NxTag systems use a fluorescent signal generated after hybridization of the amplicon to fluorescently encoded bead arrays to detect a specific amplicon [10, 11]. The Verigene system uses hybrid capture of the amplicons on a microarray with detection by gold nanoparticle probes [12]. The GenMark eSensor XT-8 and the ePlex systems use electrochemical detection of the target amplicon hybridized to a specific gold microelectrode [13]. The FilmArray system is described in more detail below.

Table 1 Multiplex nucleic acid tests: FDA-cleared and/or CE-marked

Manufacturer	System	Technology	Nucleic acid extraction	Detection method	Panels	CLIA complexity	Hands-on time	Test run time	Samples/run
BioFire Diagnostics	FilmArray	Nested multiplex PCR	Integrated	DNA melt analysis	Respiratory Blood culture Gastrointestinal Meningitis/ encephalitis	Moderate	2–5 min	45–65 min	1
Luminex	xTag NxTag	PCR and liquid phase bead array	External	Fluorescently labeled bead array	Respiratory Gastrointestinal	High	15 min per 24 samples	4–8 h	96
Luminex	Verigene	PCR with low-density nucleotide array	Integrated	Hybridized gold nanoparticle probes	Respiratory Blood culture Gram positive Gram negative Gastrointestinal	Moderate	<5 min	2–3 h	1
GenMark	eSensor XT-8	PCR and hybridization	External	Microarray hybridization and solid-phase electrochemical	Respiratory	High	55 min	6 h	18
GenMark	ePlex	PCR and hybridization	Integrated	Microarray hybridization and solid-phase electrochemical	Respiratory	Moderate	<2 min	90 min	3 to 24

The FilmArray Pouch and Chemistry

The FilmArray system performs sample-to-answer multiplex nucleic acid testing for infectious disease. To accomplish this, the FilmArray pouch (Fig. 1) integrates all of the steps of nucleic acid purification, nested multiplex PCR amplification, and automated data analysis into a closed system [4]. The pouch is created by welding two sheets of plastic film together with heat in such a fashion that fluid can move between the working areas of the pouch via channels left in the plastic. A hard plastic fitment attached to the film (indicated in Fig. 1b) provides the enzymes and buffers needed to perform the biochemical reactions in the pouch. Other reagents (#1, #2, and #3 in Fig. 1a) are inserted between the film layers during manufacture of the pouch.

The FilmArray reagents are lyophilized in the pouch, and the pouch is stored under vacuum before use. This benefits the end user in two ways. First the freeze-drying process stabilizes the PCR reagents so that the pouch has a shelf life, at ambient temperature, in excess of 1 year – which simplifies the logistics of acquiring and storing the pouches. Second vacuum storage ensures that the wells of the fitment are also under vacuum. Thus the user does not need to control the volume of hydration fluid or sample that is injected into the pouch – which simplifies the steps needed to load a pouch.

Lysis and homogenization of bacteria, spores, and viruses occur in the FilmArray pouch by means of vigorous agitation in the presence of zirconium beads (#1 in Fig. 1a) and a denaturing buffer. DNA and RNA in the sample are purified by binding to silica magnetic beads (#2 in Fig. 1a), the beads are washed to remove proteins and other PCR inhibitors, and the nucleic acids are eluted into a buffer compatible with reverse transcription and PCR [14].

The eluted material hydrates a pill (#3 in Fig. 1a) that contains all of the primers needed for reverse transcription (for RNA targets) and first stage PCR. The primers in this pill are specific to each pathogen target (assay) contained in the pouch.

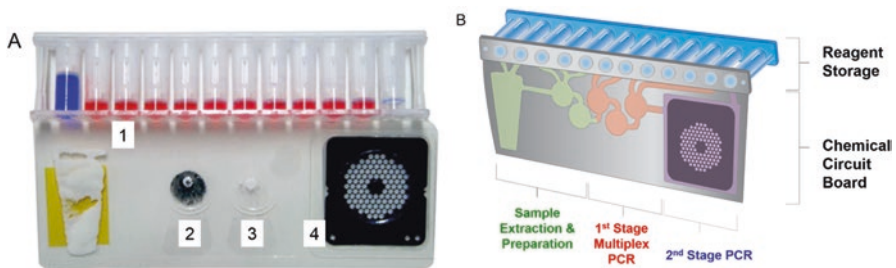


Fig. 1 The FilmArray Pouch: (a) Picture of a pouch hydrated with blue dye (through the left-side sample port) and red dye (through the right-side hydration port). In the film portion of the pouch, white zirconium beads (#1) are located in front of a yellow rectangle of tape which protects the pouch during bead beating. Black silica magnetic beads (#2) bind nucleic acid from the sample. A white pill of PCR primers (#3) is used in the first stage PCR. The black array (#4) contains 102 wells of 1 microliter each for 96 PCR reactions plus control empty wells. (b) Schematic of the pouch indicating the different functional sections of the pouch

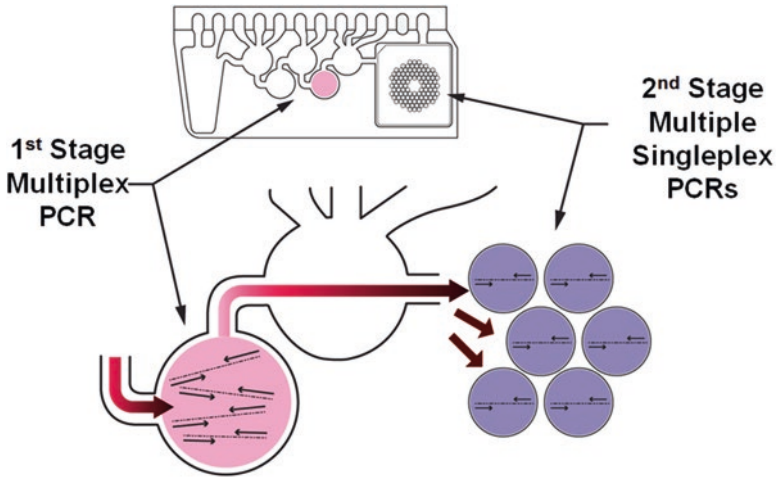


Fig. 2 Schematic of nested multiplex PCR as performed in the FilmArray pouch. Reverse transcription and the first-stage multiplex PCR occur in a blister of the pouch (shown in pink). Primers spotted into wells of the array (shown in blue) allow singleplex nested PCRs to occur in each well

The FilmArray system performs nested, multiplex PCR in two stages (Fig. 2). Reverse transcription and first stage PCR (PCR1) are performed in the same reaction volume and in a multiplex format, combining primer sets for all assays for a FilmArray panel in one mix. Following PCR1 amplification, the reaction is diluted approximately 100-fold to reduce the concentrations of the outer primers, nonspecific products, and first-stage PCR chemistry. The diluted reaction is then combined with a fresh PCR master mix and flooded over a 102-well array, where a second stage PCR occurs (PCR2). Each well of the array contains assay-specific primers that anneal within the first-stage outer amplicon. At the end of PCR2, a DNA melt curve analysis of the amplicons is performed using a nonspecific DNA-binding dye, LCGreen® Plus [15], that fluoresces in the presence of double-stranded DNA. The presence of a specific melt curve with T_m (melting temperature) in the range predicted for that specific amplicon confirms the presence and identity of the products made in each well.

The mechanics and chemistry of the FilmArray pouch have been described in greater detail elsewhere [4]. Here we highlight some of the features of the system that have contributed to the robustness of the test. The combination of a denaturing lysis buffer and “bead beating” with zirconium beads has been shown to lyse organisms and release intact total nucleic acid (DNA and RNA) from spores [16] as well as from the cerebral spinal fluid (CSF), stool, blood culture media, and whole blood. The nested PCR of the multiplex chemistry makes the FilmArray test remarkably resistant to PCR inhibitors that may remain after nucleic acid purification. Even modest levels of amplification in first-stage PCR can still be detected as an amplicon in the inner, nested product of PCR2. The dilution step following the first-stage amplification reduces the complexity of the input material to the second-stage

reaction enough that, in most cases, the DNA-binding dye detects the presence of a single amplicon in the reaction (the melt curve analysis serves as an additional filter for the correct product).

The FilmArray pouch and instrument contribute to the sensitivity of the overall system in several important ways. First the nucleic acid sample purification starts with a relatively large volume of the sample (100 μ l for the RP and GI panels, 60 μ l for the BCID), and, nominally, all of the nucleic acid purified from this volume is delivered to the combined reverse transcription – first-stage PCR reaction. Unlike some benchtop protocols, there is no dilution from the purified nucleic acid into an RT step and a second dilution into a PCR. Secondly the pouch is controlled by pneumatic actuators (described below) that can move liquid between blisters of the pouch in seconds. This minimizes the time during which nucleic acid could be degraded or PCR primers could bind to an incorrect DNA or RNA target and thus generate specific “nonspecific” amplicons. To the same end, the FilmArray pouch achieves a true mechanical hot start. In both the first stage and second stage PCRs, the primers do not come in contact with the DNA polymerase/Mg, dNTP mixture until both components are at or above the temperature at which polymerization will take place. This minimizes the formation of primer dimers, and higher-order multiplex primer structures in the first-stage PCR, which compete with the correct amplicons for PCR reagents. This enhances the specificity of the amplification reactions and thus increases the sensitivity of the individual PCR assays.

All FilmArray pouches have at least two internal controls that demonstrate the proper functioning of the pouch. One is a small amount of synthetic DNA spotted onto the second stage PCR array along with primers to amplify this target. This “PCR2” control only monitors the function of the second stage PCR. A more important control for pouch function is generated by freeze-drying a small number of cells of the yeast *Schizosaccharomyces pombe* into the well of the pouch fitment that receives the sample. Nucleic acid purified from these yeast cells must pass through all the steps of the pouch. Outer primers in the first-stage PCR and inner primers spotted onto the second-stage PCR array are designed to amplify a spliced messenger RNA from the *S. pombe* cells (in the FilmArray BCID pouch which does not contain reverse transcriptase, the *S. pombe* target is a genomic DNA sequence). BioFire has shown during the development of several different pouches that artificially induced failure modes that prevent detection of a pathogen organism in a sample also prevent detection of the yeast control.

The FilmArray instrument controls the movement of liquid through the pouch using pneumatically-actuated bladders and seals that force liquid from a pouch blister or prevent liquid from leaving a blister, respectively. The hydrated reagents in the pouch fitment are introduced into the pouch blisters via additional pneumatically actuated pistons.

The amplification reactions are thermocycled using 1 inch square Peltier devices situated adjacent to the first- and second-stage PCRs (#3 and #4 of Fig. 1a, respectively). To detect the melting of the second-stage PCR amplicons, a blue LED illuminates the array, and a camera with a filter to detect green light observes the signal generated by the DNA-binding dye LCGreen® Plus.

Clinical Utility of Multiplex Panels

Multiplex panels are particularly attractive to clinicians because they provide a comprehensive and accurate test result in a short period of time, and the test panels have been designed to match the clinical syndrome (i.e., respiratory infection, infectious gastroenteritis). However, given the increased cost of these tests, it is important to demonstrate their impact to patient management and their cost-effectiveness. It is intuitive to believe that a rapid, accurate, and comprehensive diagnostic test should improve patient management by shortening the time to the most effective therapy, by preventing inappropriate therapy (especially empiric antimicrobials), by reducing additional diagnostic testing (e.g., imaging studies), by improving the use of infection control measures, and by reducing patient length of stay. Of particular importance, these tests can reduce the unnecessary use of empiric antimicrobials by reducing the time to pathogen-directed therapy. Use of broad-spectrum antibiotics for patients with serious illnesses and the inappropriate use of antibiotics in the outpatient setting “just in case” are important drivers of antibiotic resistance which is one of the major healthcare threats of our time. In this section, we will review what is known about the clinical utility and cost-effectiveness of these multiplex panels. Because the clinical implication for each syndromic panel is different, the discussion is presented by syndrome.

Respiratory Multiplex Panels

Currently there are three vendors with multiplex respiratory panels that are both FDA-cleared and CE marked (BioFire Diagnostics, Salt Lake City, Utah; Luminex, Austin, Texas; and GenMark, San Diego, California) and several more that are CE-marked (Seegene, Seoul South Korea; Curetis, Holzgerlingen, Germany, Fast-Track Diagnostics, Sliema, Malta). These panels include assays for several viral pathogens (e.g., influenza, respiratory syncytial virus (RSV), human rhinovirus, parainfluenza viruses, adenovirus, coronaviruses, etc.) and some also include selected bacterial targets (e.g., *Mycoplasma pneumoniae*, *Bordetella* spp, *Legionella pneumophila*). All of the FDA-cleared test are limited to testing nasopharyngeal swab (NPS) samples, while several of the CE-marked tests include a larger range of respiratory sample types (e.g., bronchoalveolar lavage, sputum, etc.).

Prior to the availability of multiplex respiratory panels, testing for viral respiratory pathogens relied on viral culture, direct fluorescent antigen (DFA) testing, enzyme immunoassays (EIAs), and traditional PCR assays. While viral culture was the gold standard, it has several limitations, including that it is a complex test requiring highly skilled laboratory workers to both set up the test and interpret the results, it is slow (taking days to complete), and only a limited number of human pathogens can be grown in viral cultures. DFA tests can be performed directly on the patient sample and can have a fast turnaround time; however, these tests are also technically

complex, and the range of pathogens is limited. EIA assays can be performed in a variety of ways and are used for rapid antigen tests. Rapid antigen tests are fast and simple to use but are known to have poor test sensitivity and a limited test menu. Traditional PCR assays are highly sensitive and specific; however, they are complex and require highly trained laboratory staff and specialized laboratory facilities. In addition, each test is ordered independently placing a large burden on the ordering clinician to select the correct test or to order multiple individual tests. Due to their complexity, these tests are commonly sent to specialized reference laboratories which can increase cost and slows the time to result. The introduction of multiplex respiratory panels has allowed for comprehensive testing (ability to test viral pathogens that do not grow in cell culture and the ability to simultaneously test for bacterial and viral pathogens) in a shorter time frame. The easy-to-use systems allow the testing to be performed by laboratory workers without specialized molecular skills and in laboratories without specialized equipment and facilities. These systems allow testing to be performed closer to the patient and therefore further reduce the time to test result by reducing the need to transport samples and eliminating the delays associated with batch testing.

Multiplex respiratory panels have the potential to improve patient management and lower overall healthcare costs by improving use of influenza antivirals, reducing inappropriate use of antibiotics and antivirals, reducing use of healthcare resource (e.g., additional laboratory or imaging procedures), informing appropriate infection control practices, and reducing length of hospital, emergency department, and intensive care unit (ICU) stay.

Several studies have evaluated the effect on patient and healthcare outcomes linked to the use of a multiplex respiratory panel. Xu et al. [17] showed that replacing DFA testing with on-demand use of the FilmArray RP for children presenting to the emergency department (ED) resulted in dramatic reductions in test turnaround time (7 vs 1.4 h), timely (defined as within 3 h of discharge from the emergency department) administration of oseltamivir for 81% of patients testing positive for influenza, effective use of cohorting for admitted patients, and a potential saving of 900 h of ED boarding time. Similarly, in a pre-/post-intervention study of pediatric patients admitted through the ED, Rodgers et al. [18] compared several outcome measures when the FilmArray RP replaced the use of three clinician-ordered traditional PCR tests (Prodesse assays for, FluA/B/RSV, PIV 1,2,3, and hMPV). The study demonstrated that use of the FilmArray RP resulted in a significant increase in the number of patients with positive test results (77.9% vs 59.8%, $p < 0.001$) and a 65% reduction in time to result (6.38 vs 18.65 h, $p < 0.001$) when compared to use of traditional PCR tests. These improvements lead to a mean reduction in length of hospital stay of 0.3 days for patients with positive test results, reduced duration of antibiotics for patients with positive test results (2.7 vs 3.2 days, $p < 0.001$) or when results were reported in <4 h (2.8 vs 3.2 days, $p < 0.001$), and an overall reduction in healthcare costs of \$231/patient. Another study of 4779 pediatric patients reported significant reductions in the duration of antibiotic use (4 vs 5 days, $p < 0.01$), use of chest radiographs (59% vs 78%, $p < 0.01$), and an increase in appropriate use of isolation measures [19].

Similar findings have been observed in studies of adult patients. Brendish et al. [20] performed a prospective randomized study of adult patients presenting to the emergency department (ED) with respiratory symptoms over two respiratory seasons. In the control arm, patients were tested for respiratory viruses at the clinician's discretion using nine traditional PCR assays performed at a reference laboratory. In the intervention arm, all patients were tested with the FilmArray RP, and testing was performed in the ED. The study demonstrated the expected increase in pathogen detection (45% vs 15%, $p < 0.0001$) and reduction in time to result (2.3 vs 37.1 h, $p < 0.0001$) but failed to show the expected reduction in the proportion of patients that received antibiotics (84% vs 83%, $p = 0.96$). However, there was an increase in the proportion of patients that received a short course of antibiotics (< 48 h, 17% vs 9%, $p = 0.0047$) especially for patients with positive FilmArray RP test results. Patients in the intervention group also had a mean reduction in hospital length of stay of 1.1 days (5.7 vs 6.8 d, $p = 0.0443$) with the shortest length of stay observed in patients with positive FilmArray RP results. The use of influenza antivirals was the same in both groups (18% vs 14%, $p = 0.16$); however, the intervention group had a significant increase in the number of influenza-positive patients that received influenza antivirals (82% vs 47%, $p = 0.0001$) and a reduction in the use of antivirals for patients that were influenza-negative (18% vs 53%). In another study evaluating adult patients with a positive influenza result on a multiplex respiratory panel, Rappo [21] reported a significantly lower odds ratio for hospital admission ($p = 0.046$), a reduced length of stay ($p = 0.040$), reductions in antimicrobial duration ($p = 0.032$), and a reduction in the number of chest radiographs ($p = 0.005$).

There is currently only one study evaluating the use of multiplex panels in an outpatient setting. Greene et al. evaluated the difference in use of antibiotics and antivirals for adult outpatients tested with the FilmArray RP that were [1] positive for influenza, [2] positive for non-influenza pathogen, or [3] negative for all pathogens [22]. They observed significant increases in the use of influenza antivirals (81.0% vs 5.5–2.5%, $p < 0.001$) and reduced use of the antibiotics (29.5% vs 48.6–49.3%, $p = 0.005$) for individuals with a positive result for influenza. However, detection of non-influenza viral pathogens did not lead to a reduction in the use of antibiotics when compared to those with no pathogen detected (48.6% vs 49.3%). The authors suggest that either influenza testing alone is more cost-effective for this patient population or that additional education and/or antibiotic stewardship is needed to drive appropriate use of antibiotics in the outpatient setting.

Blood Culture Panels

Blood culture panels are designed to test positive blood cultures with the aim to provide a faster time to organism identification. In addition, these panels include assays for selected antibiotic resistance genes providing important information to guide antibiotic therapy. There are currently three vendors with multiplex blood

culture panels that are both FDA-cleared and CE-marked (BioFire Diagnostics; Luminex and Accelerate Diagnostics, Tucson, Arizona), while GenMark (San Diego, California, USA) and Curetis (Holzgerlingen, Germany) have panels that are CE-marked. These panels include assays for gram-positive bacteria, gram-negative bacteria, yeast, and antibiotic resistance markers or, in one case (Accelerate Pheno™ System), antibiotic susceptibility test results. The Curetis Unyvero BCU Blood Culture Application Cartridge® panel is the most comprehensive (with identification of ~100 bacteria and 16 resistance markers) followed by the BioFire FilmArray Blood Culture Identification Panel (identification of ~27 bacteria and 3 resistance markers). The GenMark ePlex and Luminex Verigene systems provide separate panels that are specific to gram-positive bacteria, gram-negative bacteria, or yeast with selection of the appropriate panel determined by blood culture gram stain. All of these tests can be used with a variety of different blood culture media and blood culture systems.

Prior to the availability of multiplex blood culture panels, pathogen identification was performed using classic standard culture-based systems with phenotypic (or MALDI-TOF) identification followed by traditional growth-based antimicrobial susceptibility testing. These tests are the gold standard methods and are very reliable; however, they suffer from a slow time to result (1–3 days after the positive blood culture) and technical complexity. Molecular assays for specific pathogen identification, such as *Staphylococcus aureus* and *Enterococci*, have been in use for some time and have shown improvements in patient outcomes [23–26]. Another method that reduces time to organism identification is using MALDI-TOF to identify bacteria directly from minimally processed blood cultures without the need to subculture to agar plates. The MALDI-TOF identification is very comprehensive, and the reduced time to bacterial identification has also been shown to improve patient outcomes [27, 28].

While individual molecular assays and MALDI-TOF identification have both been shown to improve patient outcomes, they are both technically complex and require specialized skills. As a result, these test methods are typically performed during standard laboratory working hours and are typically not used on night shifts when staffing is limited.

Molecular multiplex panels offer the advantage of a fast time to organism identification along with ease of use. All of the FDA-cleared and CE-marked panels use unprocessed blood culture media and provide identification results within 1–7 h of test initiation. Due to the ease of use, these panels can be used by laboratory staff without specialized molecular biology skills; however, because these panels do not identify all possible pathogens, the results must be interpreted in conjunction with the blood culture gram stain, and traditional culture and sensitivities must still be performed. Furthermore, appropriate adjustments to antimicrobials rely on proper test interpretation and a good understanding for the local patterns of antimicrobial resistance (e.g., antibiogram). Treatment adjustments should be based on local guidelines developed by an antimicrobial stewardship program (ASP) team with an in-depth understanding of the capabilities of the test being used, the local antibiogram, and the local patient populations.

As with the individual molecular assays and the MALDI-TOF identification, numerous studies have shown that use of multiplex molecular blood culture panels dramatically reduces the time to organism identification [29–32] which drives more appropriate pathogen-directed therapy. Pathogen-directed therapy includes antibiotic escalation (the addition or change of dose when the current therapy is ineffective against the identified organism) and antibiotic de-escalation (discontinuation of unnecessary empiric antibiotics). A prospective randomized study conducted at the Mayo Clinic showed that antibiotic escalation occurred more quickly with or without real-time ASP oversight (5 h with BCID and ASP, 6 h with BCID only, and 24 h without BCID or ASP, $p = 0.04$); however, optimal antibiotic de-escalation requires oversight by an ASP (21 h with BCID and ASP, 34 h with BCID only, and 38 h without BCID or ASP, $p < 0.001$) [29]. The finding that multiplex molecular blood culture panels paired with an ASP results in a faster time to optimal antibiotic therapy (most narrow effective therapy) has been confirmed in several additional studies [31, 33]. The use of molecular multiplex blood culture panels has also been shown to reduce unnecessary treatment due to contaminated blood cultures [29, 34]. Reducing the use or duration of unnecessary antibiotics is important to reducing the incidence of antibiotic resistance. The cost of molecular multiplex blood culture panel and the fact that they do not replace existing testing have been raised as reasons to not use them; however, studies of the overall healthcare cost prove the panels to be cost neutral [29–31] or to reduce overall healthcare cost [30, 31, 34–37].

While the evidence is strong that multiplex panels dramatically reduce time to organism identification and time to optimal antibiotic therapy, the evidence is inconsistent with regard to reductions in patient mortality and length of hospital stay, mostly likely because the management of patients with sepsis is complex and multifactorial. However, a recent meta-analysis [38] of studies using rapid molecular methods to test positive blood cultures found a small but statistically significant reduction in patient mortality when the results were used as part of an ASP (OR 0.64, 95% CI 0.51–0.79) but not when used outside of an ASP (OR 0.72, 95% CI 0.46–1.12). The improvements were seen for patients with gram-positive (OR 0.73, 95% CI 0.55–0.97) or gram-negative bacteremia (OR 0.51, 95% CI 0.33–0.78), but not for patients with candidemia (OR 0.90 95% CI 0.49–1.67). The study also found that time to effective therapy decreased by a weighted mean difference of -5.03 h (95% CI -8.60 to -1.45) and that length of hospital stay decreased by -2.48 days (95% CI -3.90 to -1.06).

GI Panels

Currently there are three vendors with multiplex gastrointestinal panels that are both FDA-cleared and CE-marked (BioFire Diagnostics; Luminex, and BD, Sparks, Maryland) and several more that are CE-marked (Seegene, Soul, South Korea; Mobidiag, Finland; Serosep, Limerick, Ireland). Some of these panels include assays for bacteria, viruses, and parasites in one test (FilmArray GI Panel, BioFire

Diagnostics; Luminex GGP, Luminex; Verigene Enteric Pathogens Test, Luminex), while others provide separate panels for bacterial, viral, or parasitic pathogens (Allplex™, Seegene; BD Max®, BD; AmpliDiag®, Mobidiag; EntericBio® real-time Dx, Serosep). Most tests are performed with raw stool samples or stool in transport media. The use of fecal swabs with transport media is also common, and direct testing of rectal swabs is desirable.

Current testing for infectious gastroenteritis includes many tests (stool culture, ova and parasite examination (O&P), enzyme immunoassays) that are technically complex and suffer from low diagnostic yield. The gold standard for stool pathogen testing is stool culture; however, stool culture has low diagnostic yield, is technical complexity, and has a long time to result. The yield for stool cultures is reported to be between 1.5% and 2.9% with a cost per positive result of \$952 to \$1200 [39]. Another commonly ordered test is O&P, which requires the collection and testing of three different stool samples. This method is also known to be technically difficult, to have low sensitivity, to be improperly used, and to have a low diagnostic yield of 1.4% [40]. As a result of the low diagnostic yield, clinicians often order multiple tests for the same stool sample, or perform testing sequentially until a causative pathogen is identified. As an example, a study conducted at a children's hospital found that a median of three tests (range 1–10) were ordered per stool sample [41]. To make matters worse, several studies have shown that clinician test ordering practices for gastroenteritis are problematic, in part due to the complexity of which pathogens are covered by what test [40, 42–44].

The use of culture-independent molecular multiplex panels has increased the diagnostic yield for stool testing due both to increased test sensitivity and an expanded test menu. Studies using the FilmArray GI Panel identified a pathogen in 40–50% of stool samples [41, 45, 46]. Some of the assay requires specialized molecular laboratories and personnel (BD Max, Luminex GGP, Allplex); however, some are designed to be simple to use (FilmArray GI Panel, Verigene Enteric Panel) and to provide a fast time to result (as little as 1 h from test initiation).

While these tests have the benefits of offering a comprehensive and accurate result in a relatively fast time period, the impact to patient care is largely unknown; however, one recent pre-/post-implementation study highlighted several important improvements when the FilmArray GI Panel was used to test pediatric and adult inpatients [47]. These included an increase in diagnostic yield from 6.7% to 32.8% and an improved time to result from a mean of 54.75 h to 8.94 h when compared to traditional clinician ordered tests. When compared to a matched historical control group, implementation of the FilmArray GI Panel led to a reduction in the number of additional stool tests (3.02 vs 0.58, $p = 0.001$), a trend toward shorter duration of antibiotics (2.12 days vs 1.54 day, $p = 0.06$), significantly fewer imaging studies (0.39 vs 0.18, $p = 0.0002$), and a reduction in the length of hospital stay after sample collection (3.9 days vs 3.4 days, $p = 0.04$). Reduced length of stay was more pronounced for the adult population (4.3 days vs 3.6 days, $p = 0.01$).

Recent studies have also shown that these tests have important advantages for infection control. In a recent retrospective study, the FilmArray GI Panel was used to test frozen stool samples that had previously been tested for rotavirus and *C. difficile* for infection control purposes [48]. The study showed that 22% of the samples contained

pathogens that should have required infection control measures, including norovirus, rotavirus, and *C. difficile*. Of these patients, 60% were under no or inadequate contact precautions, for a total of 109 patient days. Conversely 24.5% of the patients with negative results by the FilmArray GI Panel were unnecessarily placed under contact precautions for a total of 181 patient days. This study illustrates that without a rapid comprehensive test result, contract precautions are not rationally applied, leading to both an increased risk of nosocomial infections and unnecessary costs associated with inappropriately applied contact precautions. These are important factors when considering the care of patients in hospitals and in long-term care facilities.

Use of the Luminex xTAG gastrointestinal pathogen panel (GPP) has been compared with conventional laboratory testing for hospitalized patients and was found to be cost-effective because the increased cost of the laboratory testing was more than offset by the cost saving for elimination of unneeded contact precautions. Importantly, the cost savings was directly related to the time to test result [49].

The National Institute for Health Care Excellence (NICE) in the UK recently published a very comprehensive health economics assessment of molecular multiplex panels [50]. They reported that there was considerable uncertainty in their models; however, the Luminex GGP Panel was determined to be cost-effective for use in community-acquired and traveler diarrhea and both the FilmArray GI Panel and the Luminex GGP were found to be cost-effective for use in inpatients with diarrhea. These models will be updated as new information (such as the recent study by Beal [47]) becomes available.

Future Directions

Further clinical utility studies will highlight the importance of the different pathogens detected by the multiplex panels. However, independent of this work, continuing improvements to these IVD systems are likely to increase their value in the clinical infectious disease setting.

Real-Time Pathogen-Specific Syndromic Epidemiology

In contrast to previous generations of infectious disease IVD platforms which used cell culture, microscopy, or immunoassay technology, the current generation of multiplex systems are all highly automated and computerized [4, 6–8, 51]. This opens the possibility of exporting the results of a patient test directly to an internet (or “cloud”) database. A pilot version of such a system has been achieved with development of FilmArray Trend [52]. Trend aggregates result from geographically dispersed clinical laboratories and displays the results on a website (www.syndromictrends.com) in close to real time, resulting in a form of infectious disease “weather map.”

The pilot project summarized the respiratory pathogen results for the FilmArray RP from >360,000 patient samples acquired over 4 years ending in July 2017 from 20

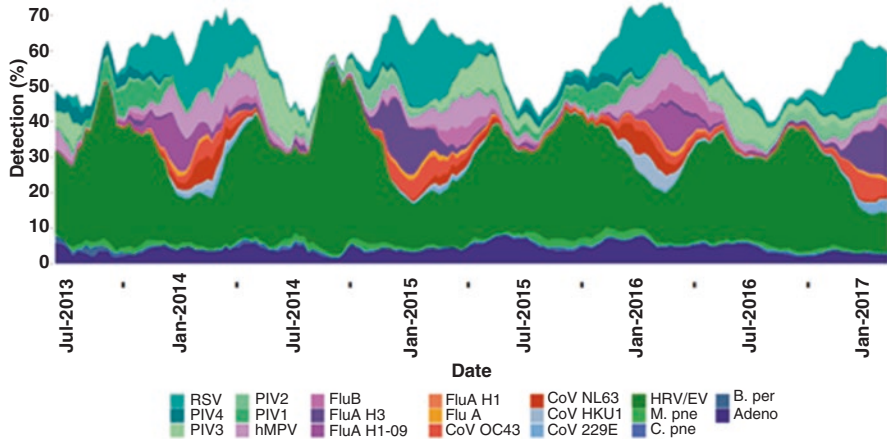


Fig. 3 Percent detection of each FilmArray RP pathogen in the FilmArray Trend RP dataset displayed as a stacked area graph for the July 2013 to July 2017 time period

clinical laboratories in the United States (Fig. 3). Similar to social media-based disease reporting systems, the data is syndrome-based [53]. However, a unique feature of the Trend dataset is that analysis is pathogen-specific. The FilmArray RP patient test results demonstrate that a number of viruses (RSV, HMPV, PIV, and CoV) show seasonal occurrence that is similar to influenza and thus can be confused with influenza when a symptomatic diagnosis of influenza-like-illness is made. This has important implications for treatment of influenza and for determining the efficacy of influenza vaccination and thus emphasizes the value of multiplex testing for respiratory symptoms. The FilmArray Trend data also show that 7% of the FilmArray RP tests detect the presence of two or three pathogens in a single sample. The importance of this result for patient treatment is not currently clear. Clinical studies that focus on patients presenting with more than one pathogen are needed.

Data from the FilmArray GI panel are also available at the www.syndromic-trends.com website. Over time, additional FilmArray IVD panels will be added to Trend, thus enabling the tracking of the pathogens that those panels detect.

Moving Multiplex Testing Closer to Point of Care

In 2017 the US FDA cleared the FilmArray RP-EZ panel [54]. This panel has Clinical Laboratory Improvement Act (CLIA)-waived status and thus can be used in settings close to the patient including low-complexity outpatient settings. In other work, the time to result for the RP panel has been decreased from 63 min for RP v1.7 to 45 min for the RP2 panel [55]. Speed, ease of use, and a comprehensive test menu are all critical features if the possibilities of point-of-care syndromic testing are to be fully realized [56].

In the longer term, additional simplification of the test setup procedure as well as reductions in the time to result should further expand the number of outpatient settings able to use multiplex testing. The data from Farrar and Wittwer [57] on “extreme” PCR conditions (cycle times below 1 s) suggest that reductions in the time to result are limited by the instrument and not by the chemistry and that substantial improvements are still possible.

Technology Improvements and the Utility of Multiplex Nucleic Acid Testing

As noted earlier, the cost of multiplex diagnostic systems is not driven by that of the primers needed for each assay, so the number of assays in a test is limited by assay format and the ability to separate the signal for each analyte. The depth of multiplex achieved in PCR multiplexes for infectious disease has steadily increased from the earliest, manually performed multiplexes, compared to the those being developed for the automated systems of today. The point will soon be reached in which the feasible number of assays in a test begins to exceed the number of distinct pathogens that need to be tested for in any particular syndrome. However, there are situations (e.g., HIV drug resistance testing or HPV serotyping) where the ability to detect a limited number of point mutations would add great value to the test result.

There is also great interest in the direct detection of sepsis pathogens from blood because the hours saved by not having to culture the bacteria reduces the risk of incorrect antibiotic treatment. With a combination of direct enrichment of the bacteria from the blood and careful attention to removing endogenous bacteria from the test manufacturing process, it should be possible to achieve the necessary sensitivity to detect bacterial pathogens directly from blood, without the time and labor of culturing the sample.

An Infectious Disease Society of America policy paper from 2013 [58] made a number of recommendations for the key characteristics of future infectious disease IVD tests. The current generation of multiplex tests already meets many of these goals (direct testing from easily accessible sample types, able to rule out infection with high certainty, based on clinical syndromes). The technical improvements described above suggest that meeting many of the remaining goals (rapid testing, point-of-care syndromic testing, improved outbreak detection) will be accomplished in the next decade.

References

1. Buchan BW, Ledebner NA. Emerging technologies for the clinical microbiology laboratory. *Clin Microbiol Rev.* 2014;27(4):783–822. <https://doi.org/10.1128/CMR.00003-14>. PubMed PMID: 25278575; PubMed Central PMCID: PMC4187641.

2. Zhang H, Morrison S, Tang YW. Multiplex polymerase chain reaction tests for detection of pathogens associated with gastroenteritis. *Clin Lab Med.* 2015;35(2):461–86. <https://doi.org/10.1016/j.cll.2015.02.006>.
3. Salez N, Vabret A, Leruez-Ville M, Andreoletti L, Carrat F, Renois F, et al. Evaluation of four commercial multiplex molecular tests for the diagnosis of acute respiratory infections. *PLoS One.* 2015;10(6):e0130378. <https://doi.org/10.1371/journal.pone.0130378>. PubMed PMID: 26107509; PubMed Central PMCID: PMC4481272.
4. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One.* 2011;6(10):e26047. Epub 2011/11/01. <https://doi.org/10.1371/journal.pone.0026047> PONE-D-11-14480 [pii]. PubMed PMID: 22039434; PubMed Central PMCID: PMC3198457.
5. Pierce VM, Hodinka RL. Comparison of the GenMark Diagnostics eSensor respiratory viral panel to real-time PCR for detection of respiratory viruses in children. *J Clin Microbiol.* 2012;50(11):3458–65. <https://doi.org/10.1128/JCM.01384-12>. PubMed PMID: 22875893; PubMed Central PMCID: PMC3486226.
6. GenMarkDx. The ePlex® Respiratory Pathogen Panel (RP)* 2016 [April 30, 2017]. Available from: <https://genmarkdx.com/wp-content/uploads/2016/09/GNMK-IMC-1046-E-ePlex-RP-Data-Sheet-US.pdf>.
7. Gadsby NJ, Hardie A, Claas EC, Templeton KE. Comparison of the Luminex respiratory virus panel fast assay with in-house real-time PCR for respiratory viral infection diagnosis. *J Clin Microbiol.* 2010;48(6):2213–2216. Epub 2010/04/02. [pii] <https://doi.org/10.1128/JCM.02446-09>. PubMed PMID: 20357215; PubMed Central PMCID: PMC2884497.
8. Lee CK, Lee HK, Ng CW, Chiu L, Tang JW, Loh TP, et al. Comparison of Luminex NxTAG respiratory pathogen panel and xTAG respiratory viral panel FAST version 2 for the detection of respiratory viruses. *Ann Lab Med.* 2017;37(3):267–71. <https://doi.org/10.3343/alm.2017.37.3.267>.
9. Butt SA, Maceira VP, McCallen ME, Stellrecht KA. Comparison of three commercial RT-PCR systems for the detection of respiratory viruses. *J Clin Virol.* 2014;61(3):406–10. <https://doi.org/10.1016/j.jcv.2014.08.010>.
10. Tang YW, Gonsalves S, Sun JY, Stiles J, Gilhuley KA, Mikhlina A, et al. Clinical evaluation of the Luminex NxTAG respiratory pathogen panel. *J Clin Microbiol.* 2016;54(7):1912–4. <https://doi.org/10.1128/JCM.00482-16>. PubMed PMID: 27122378; PubMed Central PMCID: PMC4922127.
11. Dunbar SA. Bead-based suspension arrays for the detection and identification of respiratory viruses. In: Tang Y, Stratton CW, editors. *Advanced techniques in diagnostic microbiology*. 2nd ed: Springer New York, Heidelberg Dordrecht London; 2013. p. 813–34.
12. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science.* 1997;277(5329):1078–81.
13. Umek RM, Lin SW, Vielmetter J, Terbruggen RH, Irvine B, Yu CJ, et al. Electronic detection of nucleic acids: a versatile platform for molecular diagnostics. *J Mol Diagn.* 2001;3(2):74–84. [https://doi.org/10.1016/S1525-1578\(10\)60655-1](https://doi.org/10.1016/S1525-1578(10)60655-1). PubMed PMID: 11333303; PubMed Central PMCID: PMC1907350.
14. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol.* 1990;28(3):495–503. Epub 1990/03/01. PubMed PMID: 1691208; PubMed Central PMCID: PMC269651.
15. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chem.* 2003;49(6 Pt 1):853–60.
16. Seiner DR, Colburn HA, Baird C, Bartholomew RA, Straub T, Viciry K, et al. Evaluation of the FilmArray(R) system for detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*. *J Appl Microbiol.* 2013;114(4):992–1000. Epub 2013/01/03. <https://doi.org/10.1111/jam.12107>. PubMed PMID: 23279070; PubMed Central PMCID: PMC3617465.

17. Xu M, Qin X, Astion ML, Rutledge JC, Simpson J, Jerome KR, et al. Implementation of filmarray respiratory viral panel in a core laboratory improves testing turnaround time and patient care. *Am J Clin Pathol*. 2013;139(1):118–123. Epub 2012/12/29. doi:139/1/118 [pii] <https://doi.org/10.1309/AJCPH7X3NLYZPHBW>.
18. Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR, et al. Impact of a rapid respiratory panel test on patient outcomes. *Arch Pathol Lab Med*. 2015;139(5):636–41. <https://doi.org/10.5858/arpa.2014-0257-OA>.
19. Subramony A, Zachariah P, Kronos A, Whittier S, Saiman L. Impact of multiplex polymerase chain reaction testing for respiratory pathogens on healthcare resource utilization for pediatric inpatients. *J Pediatr*. 2016;173:196–201 e2. <https://doi.org/10.1016/j.jpeds.2016.02.050>. PubMed PMID: 27039227; PubMed Central PMCID: PMC4542417.
20. Brendish NJ, Malachira AK, Armstrong L, Houghton R, Aitken S, Nyimbili E, et al. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. *Lancet Respir Med*. 2017;5(5):401–11. [https://doi.org/10.1016/S2213-2600\(17\)30120-0](https://doi.org/10.1016/S2213-2600(17)30120-0).
21. Rappo U, Schuetz AN, Jenkins SG, Calfee DP, Walsh TJ, Wells MT, et al. Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients. *J Clin Microbiol*. 2016;54(8):2096–2103. <https://doi.org/10.1128/JCM.00549-16>. PubMed PMID: 27225406; PubMed Central PMCID: PMC4963510.
22. Green DA, Hitoalij L, Kotansky B, Campbell SM, Peaper DR. Clinical utility of on-demand multiplex respiratory pathogen testing among adult outpatients. *J Clin Microbiol*. 2016;54(12):2950–2955. <https://doi.org/10.1128/JCM.01579-16>. PubMed PMID: 27654334; PubMed Central PMCID: PMC45121384.
23. Veesenmeyer AF, Olson JA, Hersh AL, Stockmann C, Korgenski K, Thorell EA, et al. A retrospective study of the impact of rapid diagnostic testing on time to pathogen identification and antibiotic use for children with positive blood cultures. *Infect Dis Ther*. 2016;5(4):555–70. <https://doi.org/10.1007/s40121-016-0136-8>. PubMed PMID: 27832502; PubMed Central PMCID: PMC45125139.
24. Koncelik DL, Hernandez J. The impact of implementation of rapid QuickFISH testing for detection of coagulase-negative staphylococci at a community-based hospital. *Am J Clin Pathol*. 2016;145(1):69–74. <https://doi.org/10.1093/ajcp/aqv005>.
25. Forrest GN, Mehta S, Weekes E, Lincalis DP, Johnson JK, Venezia RA. Impact of rapid in situ hybridization testing on coagulase-negative staphylococci positive blood cultures. *J Antimicrob Chemother*. 2006;58(1):154–8. <https://doi.org/10.1093/jac/dkl146>.
26. Forrest GN, Roghmann MC, Toombs LS, Johnson JK, Weekes E, Lincalis DP, et al. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. *Antimicrob Agents Chemother*. 2008;52(10):3558–63. <https://doi.org/10.1128/AAC.00283-08>. PubMed PMID: 18663022; PubMed Central PMCID: PMC2565911.
27. French K, Evans J, Tanner H, Gossain S, Hussain A. The clinical impact of rapid, direct MALDI-ToF identification of bacteria from positive blood cultures. *PLoS One*. 2016;11(12):e0169332. <https://doi.org/10.1371/journal.pone.0169332>. PubMed PMID: 28036369; PubMed Central PMCID: PMC45201237.
28. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Land GA, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. *Arch Pathol Lab Med*. 2013;137(9):1247–54. <https://doi.org/10.5858/arpa.2012-0651-OA>.
29. Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. *Clin Infect Dis*. 2015;61(7):1071–80. <https://doi.org/10.1093/cid/civ447>. PubMed PMID: 26197846; PubMed Central PMCID: PMC4560903.
30. MacVane SH, Hurst JM, Boger MS, Gnann JW Jr. Impact of a rapid multiplex polymerase chain reaction blood culture identification technology on outcomes in patients with vancomycin-resistant Enterococcal bacteremia. *Infect Dis (Lond)*. 2016;48(10):732–7. Epub 2016 May 19. <https://doi.org/10.1080/23744235.2016.1185533>.

31. MacVane SH, Nolte FS. Benefits of adding a rapid PCR-based blood culture identification panel to an established antimicrobial stewardship program. *J Clin Microbiol*. 2016;54(10):2455–2463. Epub 2016 Aug 3. <https://doi.org/10.1128/JCM.00996-16>. PubMed PMID: 27487951; PubMed Central PMCID: PMCPCMC5035429.
32. Messacar K, Hurst AL, Child J, Campbell K, Palmer C, Hamilton S, et al. Clinical impact and provider acceptability of real-time antimicrobial stewardship decision support for rapid diagnostics in children with positive blood culture results. *J Pediatric Infect Dis Soc*. 2016; <https://doi.org/10.1093/jpids/piw047>.
33. Messacar K, Breazeale G, Robinson CC, Dominguez SR. Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections. *Diagn Microbiol Infect Dis*. 2016;86(1):118–120. <https://doi.org/10.1016/j.diagmicrobio.2016.05.020>. PubMed PMID: 27342782; PubMed Central PMCID: PMCPCMC4993637.
34. Pardo J, Klinker KP, Borgert SJ, Butler BM, Giglio PG, Rand KH. Clinical and economic impact of antimicrobial stewardship interventions with the FilmArray blood culture identification panel. *Diagn Microbiol Infect Dis*. 2016;84(2):159–64. <https://doi.org/10.1016/j.diagmicrobio.2015.10.023>.
35. Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK. Rapid testing using the Verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. *Antimicrob Agents Chemother*. 2015;59(3):1588–95. <https://doi.org/10.1128/AAC.04259-14>. PubMed PMID: 25547353; PubMed Central PMCID: PMCPCMC4325795.
36. Sango A, McCarter YS, Johnson D, Ferreira J, Guzman N, Jankowski CA. Stewardship approach for optimizing antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for *Enterococcus* species. *J Clin Microbiol*. 2013;51(12):4008–11. <https://doi.org/10.1128/JCM.01951-13>. PubMed PMID: 24068006; PubMed Central PMCID: PMCPCMC3838051.
37. Suzuki H, Hitomi S, Yaguchi Y, Tamai K, Ueda A, Kamata K, et al. Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. *J Infect Chemother*. 2015;21(12):849–56. <https://doi.org/10.1016/j.jiac.2015.08.019>.
38. Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The effect of molecular rapid diagnostic testing on clinical outcomes in bloodstream infections: a systematic review and meta-analysis. *Clin Infect Dis*. 2017;64(1):15–23. <https://doi.org/10.1093/cid/ciw649>.
39. Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, et al. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis*. 2001;32(3):331–51. <https://doi.org/10.1086/318514>.
40. Polage CR, Stoddard GJ, Rolfs RT, Petti CA. Physician use of parasite tests in the United States from 1997 to 2006 and in a Utah *Cryptosporidium* outbreak in 2007. *J Clin Microbiol*. 2011;49(2):591–6. <https://doi.org/10.1128/JCM.01806-10>. PubMed PMID: 21106798; PubMed Central PMCID: PMCPCMC3043517.
41. Stockmann C, Rogatcheva M, Harrel B, Vaughn M, Crisp R, Poritz M, et al. How well does physician selection of microbiologic tests identify *Clostridium difficile* and other pathogens in paediatric diarrhoea? Insights using multiplex PCR-based detection. *Clin Microbiol Infect*. 2015;21(2):179 e9–15. <https://doi.org/10.1016/j.cmi.2014.07.011>. PubMed PMID: 25599941; PubMed Central PMCID: PMCPCMC4330102.
42. Hennessy TW, Marcus R, Deneen V, Reddy S, Vugia D, Townes J, et al. Survey of physician diagnostic practices for patients with acute diarrhea: clinical and public health implications. *Clin Infect Dis*. 2004;38(Suppl 3):S203–11. <https://doi.org/10.1086/381588>.
43. McNulty CA, Lassetter G, Verlander NQ, Yoxall H, Moore P, O'Brien SJ, et al. Management of suspected infectious diarrhoea by English GPs: are they right? *Br J Gen Pract*. 2014;64(618):e24–30. <https://doi.org/10.3399/bjgp14X676429>. PubMed PMID: 24567579; PubMed Central PMCID: PMCPCMC3876145.

44. van den Brandhof WE, Bartelds AI, Koopmans MP, van Duynhoven YT. General practitioner practices in requesting laboratory tests for patients with gastroenteritis in the Netherlands, 2001–2002. *BMC Fam Pract.* 2006;7:56. <https://doi.org/10.1186/1471-2296-7-56>. PubMed PMID: 17014713; PubMed Central PMCID: PMCPMC1592493.
45. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol.* 2015;53(3):915–25. <https://doi.org/10.1128/JCM.02674-14>. PubMed PMID: 25588652; PubMed Central PMCID: PMCPMC4390666.
46. Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, et al. Spectrum of enteropathogens detected by the FilmArray GI panel in a multicentre study of community-acquired gastroenteritis. *Clin Microbiol Infect.* 2015;21(8):719–28. <https://doi.org/10.1016/j.cmi.2015.04.007>.
47. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KH. A gastrointestinal PCR panel improves clinical management and lowers health care costs. *J Clin Microbiol.* 2018;56(1) <https://doi.org/10.1128/JCM.01457-17>. PubMed PMID: 29093106; PubMed Central PMCID: PMCPMC5744222.
48. Rand KH, Tremblay EE, Hoidal M, Fisher LB, Grau KR, Karst SM. Multiplex gastrointestinal pathogen panels: implications for infection control. *Diagn Microbiol Infect Dis.* 2015;82(2):154–7. <https://doi.org/10.1016/j.diagmicrobio.2015.01.007>.
49. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in hospitalised patients. *J Infect.* 2015;70(5):504–11. <https://doi.org/10.1016/j.jinf.2014.11.009>.
50. National Institute for Health and Care Excellence. Integrated multiplex PCR tests for identifying gastrointestinal pathogens in people with suspected gastroenteritis (xTAG Gastrointestinal Pathogen Panel, FilmArray GI Panel and Faecal Pathogens B assay). *Diagnostics guidance [DG26]2017.*
51. Hazelton B, Gray T, Ho J, Ratnamohan VM, Dwyer DE, Kok J. Detection of influenza A and B with the Alere i Influenza A & B: a novel isothermal nucleic acid amplification assay. *Influenza Other Respir Viruses.* 2015;9(3):151–4. <https://doi.org/10.1111/irv.12303>. PubMed PMID: 25728758; PubMed Central PMCID: PMCPMC4415699.
52. Meyers L, Ginocchio CC, Faucett AN, Nolte FS, Gesteland PH, Leber A, et al. Automated Real-Time Collection of Pathogen-Specific Diagnostic Data: Syndromic Infectious Disease Epidemiology. *JMIR Public Health Surveill.* 2018;4(3):e59.
53. Simonsen L, Gog JR, Olson D, Viboud C. Infectious disease surveillance in the big data era: towards faster and locally relevant systems. *J Infect Dis.* 2016;214(suppl 4):S380–S5. <https://doi.org/10.1093/infdis/jiw376>.
54. FDA. FDA clearance for FilmArray Respiratory Panel EZ (RP EZ). 2016 Contract No.: K152579.
55. FDA. FDA clearance for FilmArray Respiratory Panel 2 (RP2). 2017 Contract No.: K170604.
56. Drancourt M, Michel-Lepage A, Boyer S, Raoult D. The point-of-care laboratory in clinical microbiology. *Clin Microbiol Rev.* 2016;29(3):429–47. <https://doi.org/10.1128/CMR.00090-15>. PubMed PMID: 27029593; PubMed Central PMCID: PMCPMC4861988.
57. Farrar JS, Wittwer CT. Extreme PCR: efficient and specific DNA amplification in 15–60 seconds. *Clin Chem.* 2015;61(1):145–53. <https://doi.org/10.1373/clinchem.2014.228304>.
58. Caliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC, et al. Better tests, better care: improved diagnostics for infectious diseases. *Clin Infect Dis.* 2013;57(Suppl 3):S139–70. <https://doi.org/10.1093/cid/cit578>. PubMed PMID: 24200831; PubMed Central PMCID: PMC3820169.