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New Developments in Rapid Diagnostic Testing for Children

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KEYWORDS

• Rapid diagnostic assays • Group A streptococcus • Influenza • RSV
• Syndromic multiplex panels • Gastrointestinal panel • Respiratory panel
• Meningitis encephalitis panel

KEY POINTS

- Compared with traditional rapid antigen testing, new diagnostics assays for Group A Streptococcus, influenza, and respiratory syncytial virus combine rapid turnaround time with high sensitivity and specificity.
- There has been a surge in the availability of multiplex syndromic panels that test for a broad range of pathogens associated with a single clinical presentation.
- Molecular infectious disease testing that was previously performed in microbiology laboratories is now being developed in easy-to-use platforms, which are available at point of care.

It is an exciting time in the field of microbiology because of the expansion of rapid diagnostic tests. Pathogen testing previously was performed by traditional laboratory methods such as bacterial, fungal, and viral culture or ova and parasite (O&P) examination. Testing could have a slow time to results; some testing lacked sensitivity, and most testing was centralized in the laboratory. Newer advances in pathogen detection assays are now providing rapid results and have greater sensitivity and specificity, and many assays are moving out of the centralized laboratory and to the patient as point-of-care tests. In this review, the authors chose to highlight the currently available US Food and Drug Administration (FDA)–cleared molecular assays for various infectious syndromes relevant to the pediatric population. Although outcome studies in this area are sparse, rapid results may result in decreased time to optimal antimicrobial treatment and improved patient outcomes.

Disclosure Statement: E. McElvania is a consultant for Luminex Corporation. M.D. Gonzalez has no disclosures.
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Infect Dis Clin N Am 32 (2018) 19–34
https://doi.org/10.1016/j.idc.2017.11.006
id.theclinics.com

RAPID DIAGNOSTIC TESTING FOR GROUP A STREPTOCOCCUS

Group A streptococcus (GAS), *Streptococcus pyogenes*, is the most common bacterial cause of pharyngitis, which infrequently can result in serious conditions such as bacteremia, post–streptococcal glomerulonephritis, and rheumatic fever. Strep throat is most common in children 5 to 15 years of age, which is complicated by the fact that approximately 15% of children in this group are asymptomatic carriers of GAS.[1] Throat culture is the gold standard for diagnosis of GAS pharyngitis, but due to the 24- to 48-hour turnaround time, rapid antigen testing has been the standard of practice in emergency departments (EDs) and outpatient clinics for many years. Because rapid antigen assays have low sensitivity compared with culture, throat swabs that test negative by the GAS rapid antigen assay must be cultured when collected from children less than 18 years of age. Rapid antigen testing when combined with reflex throat culture brings the sensitivity of GAS detection to an acceptable level of greater than 95%.

To increase the sensitivity of GAS detection, second-generation antigen assays have been recently developed. These assays use a reading device to interpret the assay rather than the naked eye, which increases the sensitivity of antigen detection and reduces the subjective nature of reading low positive test results. In a study of 48 pediatric patients with pharyngitis, the Sofia Strep A + FID (Quidel Corp, San Diego, CA, USA) was 88.9% sensitive and 93.3% specific for detection of GAS compared with culture.[2]

Illumigene group A Streptococcus assay (Meridian Bioscience Inc, Cincinnati, OH, USA) uses isothermal amplification for detection of GAS and was found to be 100% sensitive and 95.9% specific compared with culture in a study of 437 throat swabs collected from primarily pediatric patients (98% of patients <18 years of age).[3] A second study of 361 pediatric patients reported the Illumigene assay to be 98.6% sensitive and 96.5% specific.[4] For comparison, traditional GAS rapid antigen tests were also run on all specimens in both studies and had a sensitivity of 73.3% and 55.2%, respectively, and specificity of 89.1% and 99.1%, respectively. The Simplexa Group A Strep Direct Assay (DiaSorin Molecular LLC, Cypress, CA, USA) was found to be 97.4% sensitive and 95.2% specific compared with culture in a study of 1352 specimens across 4 pediatric testing sites.[5]

Molecular tests have also been developed that allow rapid, point-of-care detection of GAS. These assays are highly sensitive and specific and may not require reflex to culture if testing is negative. Many of these tests are also Clinical Laboratory Improvement Amendments (CLIA) waived, which allows molecular testing to be performed by nonlaboratory personnel, such as physicians and nurses in an outpatient or ED setting. The Cobas Liat Strep A Assay (Roche Diagnostics, Indianapolis, IN, USA) is a waived, polymerase chain reaction (PCR)-based assay found to be 100% sensitive and 98.3% specific for GAS detection compared with an in-house developed molecular assay using 198 throat swabs from adults and children.[6] Another point-of-care molecular test is the Alere i strep A (Alere Inc, Waltham, MA, USA). In a study conducted across 10 US medical centers, 481 primarily pediatric specimens were tested by nonlaboratory personnel and found the Alere i strep A assay to be 98.7% sensitive and 98.5% specific compared with bacterial culture.[7]

In the past few years, there has been an explosion in the field of GAS assays. These new assays are rapid, have increased sensitivity over traditional rapid antigen tests, and in many cases, can be performed at point of care. Because molecular testing is more sensitive than culture, clinicians must be cognizant to only test patients with signs and symptoms of pharyngitis to prevent detecting GAS colonization. Molecular

testing does not distinguish between live or dead organisms, so molecular tests cannot be used to determine clearance of GAS following treatment. Testing for GAS by molecular methods can result in the missed opportunity to detect less common causes of bacterial pharyngitis such as Lancefield groups C and G β-hemolytic streptococci, which are identified by throat culture. In an effort to identify additional bacterial causes of pharyngitis, some assays such as the Simplexa Group A Strep Assay detect Lancefield groups C and G β-hemolytic streptococci in addition to GAS. A summary of FDA-cleared rapid second-generation and molecular GAS assays can be found in **Table 1**.

RAPID DIAGNOSTIC TESTING FOR RESPIRATORY VIRUSES

Millions of children are infected each year with influenza and respiratory syncytial virus (RSV), with even more falling ill with a variety of other respiratory viruses. Respiratory viral infections can span the clinical spectrum from no symptoms to death in rare cases. Children under the age of 5, and especially those under 2 years of age, are at high risk for complications from influenza, resulting in thousands of hospitalizations and approximately 100 to 150 deaths per year.[8] RSV is the leading cause of bronchiolitis and pneumonia in children under 1 year of age, causing 60,000 hospitalizations in children each year.[9]

Traditionally, viral culture was the gold standard for respiratory virus detection, but because of the long turnaround time, rapid antigen testing became the mainstay for influenza and RSV testing in the ED and outpatient settings. Just like rapid antigen testing for GAS, testing for influenza and RSV is rapid and inexpensive, but lacks sensitivity at 50% to 70%. For this reason, the Centers for Disease Control and Prevention (CDC) has discouraged the use of rapid antigen testing for influenza and RSV and promoted the use of molecular detection of these viruses. If rapid antigen testing must be used, the CDC recommends using these assays only when the prevalence of influenza or RSV in the community is greater than 10%, which raises the positive predictive value of the assay. Even when the prevalence is high, clinicians must interpret rapid antigen test results with caution, because a negative result does not exclude infection in a symptomatic patient.[10]

Like GAS testing, second-generation rapid antigen assays using a reading device have been developed to increase the sensitivity of influenza and RSV detection. A study of 240 pediatric specimens tested on 3 second-generation influenza platforms, the Veritor System Flu A + B (BD Diagnostics, Sparks, MD, USA), Sofia Influenza A + B FIA (Quidel Corp, San Diego, CA, USA), and BinaxNOW Influenza A&B (Alere Scarborough, Inc, Scarborough, ME, USA), found testing to be in agreement 93.8%, 94.2%, and 95.8% of the time for influenza A and 98.1%, 79.2%, and 80.8% of the time for influenza B, respectively, compared with real-time PCR.[11]

Assays using molecular methods of influenza and RSV detection have drastically increased the sensitivity of viral detection over previous methods, including rapid antigen testing and viral culture. The increased speed of detection is partially due to removal of an external extraction step before molecular testing, allowing testing platforms such as the Simplexa Flu A/B & RSV Direct (DiaSorin Molecular LLC, Cypress, CA, USA), Solana Influenza A + B (Quidel Corp, San Diego, CA, USA), and Xpert Xpress Flu/RSV (Cepheid, Sunnyvale, CA, USA) assays to provide results in 30 to 60 minutes. Because the sensitivity and specificity of molecular tests are high, testing can be performed year round regardless of the prevalence of influenza or RSV.

An exciting new development in the field of influenza and RSV detection has been the development of rapid, point-of-care molecular testing. These assays combine the quick

Table 1
Second-generation and molecular group A streptococcus testing, US Food and Drug Administration approved

Assay	Targets	Methodology	Turnaround Time, min	CLIA Waived	Pediatric References
BD Veritor System	Group A streptococci	Immunochromatographic assay	5	Yes	—
Quidel Sofia Strep A + FIA	Group A streptococci	Immunofluorescence-based lateral flow	5	Yes	Roper et al,[2] 2017
Alere i Strep A	Group A streptococci	Isothermal nucleic acid amplification	8	Yes	Cohen et al,[7] 2015
Roche cobas LiatStrep A Assay	Group A streptococci	Real-time PCR	15	Yes	Uhl & Patel,[6] 2016
Cepheid Xpert Xpress Strep A Assay	Group A streptococci	Real-time PCR	30	No	—
Quidel Solana Strep Complete Assay	Group A, C, and G streptococci	Isothermal helicase-dependent amplification	25	No	—
Meridian illumigene Group A Strep	Group A streptococci	Loop-mediated isothermal amplification	<60	No	Henson et al,[3] 2013; Felsenstein et al,[4] 2014
DiaSorin Simplexa Group A Strep	Group A, C, and G streptococci	Real-time PCR	60	No	Tabb & Batterman,[5] 2016

turnaround time of rapid antigen testing with the high sensitivity and specificity associated with molecular testing. Several of these assays are CLIA waived and can be performed at point of care by non-laboratory-trained individuals. Although molecular assays cost more than traditional rapid antigen assays, they provide accurate test results while patients are being seen in clinic or in the ED, allowing treatment decisions to be made while the patient is in house. A study of 545 specimens (85% collected from children) found the Alere i Influenza A&B assay to be 99.3% sensitive and 98.1% specific for influenza A, and 97.6% sensitive and 100% specific for influenza B, compared with viral culture and real-time reverse transcription (RT)-PCR used for discrepant analysis.[12]

A study of 2 CLIA-waived, point-of-care, molecular assays, Cobas Liat Influenza A/B (Roche Diagnostics, Indianapolis, IN, USA) and Alere i Influenza A&B (Alere Scarborough, Inc, Scarborough, ME, USA), tested 129 respiratory specimens (41% pediatric specimens). They found the Alere i to be 71.3% sensitive for influenza A and 93.3% sensitive for influenza B with 100% specificity for both viruses.[13] The Cobas Liat had 100% sensitivity and specificity for influenza A and B. The low sensitivity of the Alere i was thought to be due to specimens below the limit of detection (LOD) for the assay, and since this study, the assay has been revised to address this issue. A summary of FDA-cleared rapid second-generation and molecular influenza and combined influenza and RSV assays can be found in **Table 2**.

SYNDROMIC MULTIPLEX RESPIRATORY PANELS

Although influenza and RSV are the most common respiratory pathogens, there are many other respiratory viruses that cause significant disease, especially in immunosuppressed patients. The advent of syndromic multiplex assays allows for rapid identification of a large number of respiratory pathogens, both bacterial and viral, from respiratory specimens (**Table 3**). A study of 300 respiratory specimens (49% from pediatric patients) were tested on 4 multiplex respiratory panels, FilmArray RP, GenMark Dx eSensor (GenMark Diagnostics, Inc, Carlsbad, CA, USA), Luminex xTAG RVPv1 (Luminex, Austin, TX, USA), and the Luminex xTAG (bioMérieux, Durham, NC, USA) RVP fast.[14] The overall sensitivity was 84.5% for the FilmArray RP, 98.3% for the eSensor RVP (Luminex, Austin, TX, USA), 92.7% for the xTAG RVPv1, and 84.4% for the RVP fast. The specificity was greater than 99% for all assays. It should be noted that all assays used have been updated since the time of this study. Recently, the first CLIA-waived respiratory panel, FilmArray Respiratory Panel EZ (bioMérieux, Durham, NC, USA), has come to the market, allowing respiratory panels to be performed in outpatient settings. More information on rapid influenza and respiratory panel testing can be found in recent *Clinics in Laboratory Medicine* articles by Peaper and Landry[15] and Buller.[16]

Rapid influenza testing is valuable for more than just convenience—it can affect patient outcomes. A study of influenza testing for pediatric patients presenting to the ED found that the use of rapid, multiplex PCR was the most cost-effective testing method (based on quality-adjusted life-years) compared with traditional PCR, direct-fluorescent antibody, and rapid antigen testing.[17] A meta-analysis of greater than 1500 pediatric patients found that rapid influenza detection in the ED decreased antibiotic usage, but the trend was not statistically significant.[18] The study did find that having rapid viral testing available did significantly decrease the rate of chest radiographs performed in the ED. Another pediatric study by Rogers and colleagues[19] found that implementation of the BioFire RP reduced antibiotic duration when test results were obtained in less than 4 hours. Also, if test results were positive for a respiratory virus, inpatient length of stay and time in isolation were decreased compared with before the BioFire RP was in use.

Table 2
Second-generation and molecular influenza and respiratory syncytial virus testing, US Food and Drug Administration approved

Assay	Targets Detected	Methodology	Turnaround Time, min	CLIA Waived	Pediatric References
BD Veritor System Flu A + B	Influenza A & B	Immunochromatographic assay	5–10	Yes	Dunn et al,[11] 2014
Quidel Sofia Influenza A + B FIA	Influenza A & B	Immunofluorescence-based lateral flow with reader	3–15	Yes	Dunn et al,[11] 2014
Alere i Influenza A & B 2	Influenza A & B	Isothermal nucleic acid amplification	<15	Yes	Bell et al,[12] 2014; Nolte et al,[13] 2016
Roche cobas Liat Influenza A/B and RSV	Influenza A & B and RSV	Real-time PCR	20	Yes	Nolte et al,[13] 2016
Cepheid Xpert Xpress Flu/RSV Assay	Influenza A & B and RSV	Real-time RT-PCR	30	No	—
DiaSorin Simplexa Flu A/B & RSV Direct	Influenza A & B and RSV	Real-time RT-PCR	60	No	—
Quidel Solana Influenza A + B Assay	Influenza A & B	RT-PCR followed by isothermal helicase-dependent amplification	45	No	—

Table 3
Multiplex respiratory panels, US Food and Drug Administration approved

Assay	Turnaround Time, h	Bacterial Targets	Viral Targets	Pediatric References
bioMérieux FilmArray Respiratory Panel	1	*Bordetella pertussis* *Chlamydophila pneumoniae* *Mycoplasma pneumoniae*	Influenza A, A/H1, A/H3, A/H1-2009 Influenza B RSV Parainfluenza virus 1, 2, 3, and 4 Human metapneumovirus Human rhinovirus/enterovirus[a] Adenovirus Coronavirus HKU1, NL63, 229E, and OC43	Popowitch et al,[14] 2013
bioMérieux FilmArray Respiratory Panel EZ (CLIA waived)	1	*B pertussis* *C pneumoniae* *M pneumoniae*	Influenza A, A/H1, A/H3, A/H1-2009 Influenza B RSV Parainfluenza virus Human metapneumovirus Human rhinovirus/enterovirus[a] Adenovirus Coronavirus	—
GenMark ePlex Respiratory Pathogen Panel (RP)	1.5	*C pneumoniae* *M pneumoniae*	Influenza A, A/H1, A/H3, A/H1-2009 Influenza B Respiratory syncytial virus A and B Parainfluenza virus 1, 2, 3, and 4 Human metapneumovirus, Human rhinovirus/enterovirus Adenovirus Coronavirus HKU1, NL63, 229E, and OC43	Popowitch et al,[14] 2013
Luminex Verigene Respiratory Pathogens Flex Test (RP Flex)	<2	*B pertussis* *Bordetella parapertussis/B bronchiseptica* *Bordetella holmesii*	Influenza A, A/H1, A/H3 Influenza B Respiratory syncytial virus A and B Human rhinovirus Parainfluenza virus 1, 2, 3, and 4 Human metapneumovirus, Adenovirus	—
Luminex NxTAG Respiratory Pathogen Panel	5	*C pneumoniae* *M pneumoniae*	Influenza A, A/H1, A/H3 Influenza B Respiratory syncytial virus A and B Human rhinovirus/enterovirus Parainfluenza virus 1, 2, 3, and 4 Human metapneumovirus, Adenovirus Coronavirus HKU1, NL63, 229E, and OC43 Human bocavirus	Popowitch et al,[14] 2013

[a] Unable to differentiate human rhinovirus and enterovirus.

DETECTION OF GASTROINTESTINAL PATHOGENS

Traditional testing for the array of the gastrointestinal pathogens, which includes bacteria, viruses, and parasites, has relied on a range of testing methodologies. The decision of the appropriate tests to order is complicated by the lack of symptoms/biomarkers to reliably differentiate between pathogen groups.[20] The identification of bacterial pathogens relies on stool culture, which can take days to result and has reduced sensitivity because of the fastidious nature of some pathogens, such as *Campylobacter* and *Shigella*. In an effort to increase the sensitivity of *Campylobacter* detection in stool specimens, antigen tests are available for rapid *Campylobacter* spp testing. Unfortunately, a large multicenter study evaluated 4 *Campylobacter* antigen assays and found that despite relatively high specificity (>95%), the positive predictive value was only 36% to 51%.[21] Based on these results, the use of *Campylobacter* antigen assays as stand-alone tests is not recommended.

Antigen testing has also been used for detection of viral and parasitic causes of gastroenteritis, including adenovirus 40/41, rotavirus, *Giardia lamblia*, and *Cryptosporidium*. Antigen testing offers a more rapid and relatively sensitive method for viral and parasitic pathogen detection relative to viral culture and O&P examination. Rapid antigen testing for parasites does not require multiple specimens to rule out infections, which is the practice for O&P examination. Readers are referred to a review of protozoal diagnostics for additional information.[22]

Detection of norovirus has always been difficult, because it cannot be cultured. For years, laboratory-developed molecular tests were the only method for norovirus testing. Recently, the first FDA-cleared molecular assay, Xpert Norovirus (Cepheid, Sunnyvale, CA, USA) became available for detection of norovirus genogroups GI and GII from stool specimens. In a multicenter study of approximately 1400 fresh and frozen stool specimens, this assay demonstrated high sensitivity (>98%) and specificity (>98%) for both norovirus genogroups.[23]

There is much overlap in symptoms of bacterial and viral causes of gastroenteritis, making them unable to be differentiated clinically. Often clinicians are not aware of which bacteria are included in their institution's standard stool culture, because this varies among laboratories. To solve these problems, multiplexed syndromic panels are now available for detection of numerous gastrointestinal pathogens. The FilmArray Gastrointestinal (bioMérieux, Durham, NC, USA) and xTAG Gastrointestinal (Luminex, Austin, TX, USA) panels detect bacterial, viral, and parasitic targets, whereas the Verigene Enteric Pathogens Panel (Luminex, Austin, TX, USA) detects both bacterial and viral pathogens. The ProGastro SSCS (Hologic Inc, Marlborough, MA, USA) only detects bacterial pathogens, whereas the BDMax Enteric system (BD, Franklin Lakes, NJ, USA) has a bacterial panel, extended bacterial panel, and a parasite panel. All assay targets are summarized in **Table 4**. Studies on each of these assays has been published, and all assays show high sensitivity and specificity for their respective targets.[24–31] In fact, these multiplex panels result in increased and unexpected detections that would not be identified by the ordering preference of the clinician. For example, in a study by Stockmann and colleagues,[32] they noted that for patients with only *Clostridium difficile* testing, the FilmArray Gastrointestinal pathogen panel identified an alternative pathogen in 29% of those patients.

Currently, there are few studies directly comparing these multiplex panels. Huang and colleagues[29] evaluated the performance for the shared analytes of the Verigene Enteric, FilmArray Gastrointestinal, and xTAG Gastrointestinal panels and found that the FilmArray and xTAG panels performed similarly except for reduced *Salmonella* detection with the later assay (79.2%) relative to the former assay (95.8%). The

Table 4
Multiplex gastrointestinal panels, US Food and Drug Administration approved

Assay	Turnaround Time, h	Bacterial Targets	Viral Targets	Parasitic Targets	References
BDMax Enteric Bacterial Panel[a]	3	*Campylobacter* spp *Salmonella* spp *Shigella*/EIEC STEC	—	—	Harrington et al,[24] 2015
BDMax Extended Enteric Bacterial Panel[b]	3.5	ETEC *Plesiomonas shigelloides* *Vibrio* spp *Yersinia enterocolitica*	—	—	Simner et al,[26] 2017
BDMax Enteric Parasite Panel	4.5	—	—	*Cryptosporidium* *Entamoeba histolytica* *Giardia*	Madison-Antenucci et al,[25] 2016
bioMérieux FilmArray Gastrointestinal Panel[c]	1	*Campylobacter* spp *C difficile* *P shigelloides* *Salmonella* spp *Vibrio* spp (*cholerae*) EAEC EPEC ETEC STEC (*Escherichia coli* O157) *Shigella*/EIEC	Adenovirus 40/41 Astrovirus Norovirus Rotavirus Sapovirus	*Cryptosporidium* *Cyclospora cayetanensis* *E histolytica* *Giardia*	[27,29,32,34,36]

(*continued on next page*)

Table 4
(*continued*)

Assay	Turnaround Time, h	Bacterial Targets	Viral Targets	Parasitic Targets	References
Hologic Prodesse ProGastro SSCS Assay	4	*Campylobacter* spp *Salmonella* spp Shiga toxin 1 and 2 *Shigella* spp	—	—	Buchan et al,[28] 2013
Luminex Verigene Enteric Pathogens Test[d]	2	*Campylobacter* spp *Salmonella* spp *Shigella* spp *Vibrio* spp *Y enterocolitica* Shiga toxin 1 and 2 (*stx1* and *stx2*)	Norovirus Rotavirus	—	Huang et al,[29] 2016
Luminex xTAG Gastrointestinal Pathogen Panel[e]	5	*Campylobacter* spp *C difficile* *E coli* O157 ETEC STEC *Salmonella* spp *Shigella* spp *Vibrio cholera*	Adenovirus 40/41 Norovirus Rotavirus	*Cryptosporidium* *E histolytica* *Giardia*	[29–31,34]

Abbreviations: EAEC, enteroaggregative *E coli*; EPEC, enteropathogenic *E coli*; ETEC, enterotoxigenic *E coli*; STEC, shiga-toxin–like producing *E coli.*

[a] BDMax Enteric Bacterial Panel detects specific *Campylobacter* (*coli* and *jejuni*) species but only reports as a group.

[b] BDMax Extended Enteric Bacterial Panel detects specific *Vibrio* (*cholerae*, *parahaemolyticus*, and *vulnificus*) species but only reports as a group.

[c] FilmArray Gastrointestinal panel detects specific *Campylobacter* (*coli*, *jejuni*, and *upsaliensis*) and *Vibrio* (*cholerae*, *parahaemolyticus*, and *vulnificus*) species but only reports as a group. When STEC is detected, the assay then determines if it is an *E coli* O157 serotype.

[d] Verigene Enteric Pathogen Test detects specific *Campylobacter* (*coli*, *jejuni*, and *lari*) and *Vibrio* (*cholera* and *parahaemolyticus*) species but only reports as a group.

[e] xTAG Gastrointestinal Pathogen Panel detects specific *Campylobacter* (*coli*, *jejuni*, and *lari*) species but only reports as a group.

Verigene Enteric panel demonstrated similar specificity to the other assays but reduced sensitivity for detection of *Campylobacter* (83.3%), *Salmonella* (83.3%), norovirus (89%), and rotavirus (71.4%). In another study, the investigators evaluated the FilmArray Gastrointestinal and xTAG Gastrointestinal panels and found similar performance between both tests for shared analytes except the xTAG panel demonstrated lower specificity for norovirus in prospective and retrospective specimens.[33] Finally, in the study by Chhabra and colleagues,[34] the investigators specifically examined the analytical performance of the FilmArray Gastrointestinal and xTAG Gastrointestinal panels for detection of gastrointestinal viruses. The investigators noted that the FilmArray Gastrointestinal Panel demonstrated overall better analytical performance for viral detection relative to the xTAG panel.[34]

Although multiplex gastrointestinal panels offer more rapid results to clinicians, they can present a potential problem for public health surveillance efforts if bacterial pathogens are not cultured.[35] In addition, the lack of bacterial isolates could complicate treatment without antimicrobial susceptibility results. Another caveat to these molecular panel tests is that multiple pathogens can be present, as observed with 31.5% of specimens in a multicenter study of the FilmArray,[27] and 30.3% for the xTAG Gastrointestinal panel.[33] The clinical significance of multiple positive targets is currently unclear and can cause frustration for clinicians unsure which target or targets detected are responsible for their patient's symptoms. Finally, limited information is available on repeat multiplex testing. Park and colleagues[36] retrospectively evaluated patients with initially negative FilmArray Gastrointestinal results and found that 92.5% remained negative upon retesting within 4 weeks. Conversely, of patients with initially positive results, 53.8% remained positive for the same target within 4 weeks.[36] Continued asymptomatic shedding has been observed for gastrointestinal pathogens.[37,38] Taken together, these results show that molecular testing is not appropriate as a test of cure and that continued detection of targets can occur for an indeterminate amount of time regardless of patient symptoms. It is absolutely necessary to restrict testing to symptomatic individuals and carefully interpret any repeat positive results.

Several of the multiplex syndromic panels contain a target for *C difficile*. For pediatric patients, the American Academy of Pediatrics guidelines for *Clostridium difficile* infection (CDI) diagnosis discourages testing in those less than 1 years of age due to the high percentage of children in this age group who are asymptomatically colonized with *C difficile*.[39] In children aged 1 to 3 years, causes such as viruses should be considered before testing for *C difficile* for the same reason. As a discussion of *C difficile* testing is outside the scope of this article, the authors refer readers to a review of *C difficile* testing in pediatrics.[40]

RAPID DETECTION OF CENTRAL NERVOUS SYSTEM INFECTIONS

Traditional rapid diagnostic testing of cerebrospinal fluid (CSF) specimens uses cell count, protein, glucose, and Gram stain. However, this testing has limited analytical sensitivity and specificity in differentiating infectious versus noninfectious causes or in differentiating the bacterial versus viral versus fungal pathogens. The gold standard for identification of bacterial pathogens is CSF culture, whereas fungal causes are identified by culture and antigen testing (eg, Cyptococcal antigen testing). Bacterial and fungal cultures can take days to grow, test, and obtain a result. In addition, treatment with antimicrobial therapy before obtaining CSF can reduce microbial viability, leaving clinicians without a target for therapy. There exist bacterial antigen tests for CSF specimens that can provide rapid results, but such testing is not recommended by the Infectious Diseases Society of America.[41] Viral testing is now performed

using molecular-based methods, which surpass viral culture in sensitivity and turnaround time.

Molecular testing of CSF specimens for viral pathogens is frequently performed using laboratory developed tests (LDTs), which uses various methods for nucleic acid extraction, purification, oligonucleotide primer sets, and detection methods. Taken together, this can create interlaboratory variability of test performance, requiring clinicians to be aware of the relative performance of their institutional assays. Currently, there are 2 stand-alone FDA-cleared assays for detection of viral pathogens from CSF specimens, the Cepheid Xpert EV (Enterovirus) (Cepheid, Sunnyvale, CA, USA) and Simplexa HSV 1 and 2 Direct (**Table 5**). Both are qualitative assays performed directly off of CSF specimens. The Xpert EV (DiaSorin Molecular LLC, Cypress, CA, USA) detects an array of enterovirus serotypes, but not parechoviruses, and overall demonstrates high sensitivity (>97%) and specificity (100%).[42,43] Similarly, the Simplexa HSV 1 and 2 Direct has shown high sensitivity (96%) and specificity (97%), although depending on the comparator LDT, the LOD may be slightly higher than the evaluated LDT.[44,45]

There is only one FDA-cleared syndromic multiplex panel, the FilmArray Meningitis/Encephalitis panel (bioMérieux, Durham, NC, USA) (**Table 6**). It detects bacterial (n = 6), viral (n = 7), and fungal pathogens (n = 1) from CSF specimens. One large prospective study examined 1560 CSF specimens from adults and children using the FilmArray Meningitis/Encephalitis panel and found an 84.4% positive and greater than 99.9% negative agreement with the comparator methods.[46] Although it was noted that an additional 21 pathogens were detected using the FilmArray Meningitis/Encephalitis panel, there was also 22 unconfirmed/false positive detections. *Streptococcus pneumoniae* (n = 7) was the most frequent unconfirmed/false positive target, which the investigators proposed could be oral flora contamination during testing, necessitating the need for adherence to strict molecular testing procedures. In 2 pediatric specific studies examining the FilmArray Meningitis/Encephalitis panel, strong agreement was seen with conventional methods.[47,48] Although in 1 study, 2 herpes simplex virus (HSV)-1 detections were missed by the FilmArray Meningitis/Encephalitis panel that were likely near the LOD of the assay, HSV-1 was detected in both specimens by the standard-of-care LDT.[48] It should be noted that 2 studies found that this panel demonstrated reduced sensitivity for *Cryptococcus* detection relative to antigen testing.[49,50] Finally, to date, there is no clinical report of the performance of this assay for detecting relatively low incident pathogen *Listeria monocytogenes* and only 1 report for *Neisseria meningitidis*,[49] which was detected by the panel.

Molecular testing for bacterial and fungal CSF pathogens does not replace traditional culture, because culture provides isolates for antimicrobial susceptibility testing and can detect pathogens not on the panel. Careful interpretation of the results from CSF multiplex panels needs to take into account the patient's clinical picture because false positive results can occur from contamination events. In particular, positive results for herpes viruses (eg, cytomegalovirus, HSV, human herpesvirus 6, and varicella zoster virus) could represent detection of latent or actively replicating virus.

Table 5
Singleplex detection of viral pathogens directly from cerebrospinal fluid specimens

Assay	Turnaround Time (h)	Targets Detected	Methodology
Cepheid Xpert EV	1	Enterovirus	Real-time PCR
DiaSorin Simplexa HSV 1 and 2 direct	1	HSV 1 and HSV 2[a]	Real-time PCR

[a] Simplexa HSV 1 and 2 Direct detects and differentiates between HSV1 and/or HSV2.

Table 6
Multiplex meningitis encephalitis panel, US Food and Drug Administration approved

Assay	Turnaround Time, h	Bacterial Targets	Viral Targets	Fungal Targets
bioMérieux FilmArray Meningitis/ Encephalitis Panel	1 h	*Escherichia coli* K1[a] *Haemophilus influenzae* *L monocytogenes* *N meningitidis* *Streptococcus agalactiae* (Group B) *S pneumoniae*	CMV Enterovirus HSV-1 HSV-2 HHV-6 Human parechovirus VZV	*Cryptococcus neoformans/gattii*[b]

Abbreviations: CMV, cytomegalovirus; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; HHV-6, human herpesvirus 6; VZV, varicella zoster virus.

[a] The FilmArray Meningitis/Encephalitis panel only detects *E coli* K1, which accounts for up to 80% of *E coli* causes of neonatal meningitis.

[b] *C neoformans* and *C gattii* are not differentiated by this assay.

DETECTION OF *KINGELLA KINGAE* FROM SEPTIC JOINTS

K kingae is a frequent colonizer of the oropharynx in young children 2 to 36 months of age, and its prevalence is increased in children who attend daycare.[51] In children colonized with *K kingae*, bacteria can translocate to the bloodstream, causing bacteremia and seeding of distal body sites, primarily joints and bones, where it causes infection. *K kingae* is a fastidious bacterium that rarely grows in culture from septic joints. To improve sensitivity of pathogen detection, excess joint fluid can be inoculated into blood culture bottles and incubated for increased recovery of *K kingae*.[52] Although there are no FDA-cleared molecular assays for detection of *K kingae*, testing is offered at reference laboratories and some hospitals have LDT PCR assays that are used clinically. Some pediatric orthopedic practices routinely test for *K kingae* in patients $\leq$4 years of age using either a *K kingae*–specific PCR assays or 16S ribosomal DNA sequencing directly from joint specimens.[53,54] Both inoculation of joint fluid into blood culture bottles and molecular detection assays have markedly improved the rate of *K kingae* detection from joint specimens compared with bacterial culture alone.

SUMMARY

New diagnostic assays for GAS, influenza, and RSV are pressing the boundaries of maintaining a rapid turnaround time and providing increased sensitivity and specificity of pathogen detection. Molecular testing is no longer confined to the walls of the laboratory but has been reimagined into easy-to-use platforms which can be used by nonlaboratory personnel at point of care. In addition, multiplex syndromic panels are allowing broad testing of pathogens associated with a single clinical presentation in a single assay. Together with clinicians, rapid and accurate pathogen detection in children may result in decreased time to optimal antimicrobial treatment and improved patient outcomes.

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