Direct-from-Specimen Pathogen Identification

Evolution of Syndromic Panels

Marc Roger Couturier, PhD, D(ABMM)[a,*],
Jennifer Dien Bard, PhD, D(ABMM)[b]

KEYWORDS

• Syndromic panel • Respiratory panel • Sepsis panel • Gastroenteritis panel
• Meningitis panel • Encephalitis panel

KEY POINTS

- Syndromic panels should evolve to include additional pathogen targets.
- Syndromic panels should be flexible in design to better meet the user's needs.
- Syndromic panels should include host response markers to correlate with pathogen detection.
- Syndromic panels should have shorter run times, less complexity, and be placed near the point of care.
- Syndromic panels should be expanded to include more antimicrobial resistance determinants and phenotypic susceptibility results.

INTRODUCTION

Clinical laboratory testing for pathogens associated with a specific syndrome have historically relied on multiple different analytical approaches to maximize broad pathogen detection. This is especially true for organisms for which culture was not a feasible modality, and instead culture-independent methods such as antigen detection, direct staining, or targeted polymerase chain reaction (PCR) were the aggregate conventional methods. Since the late 2000s, commercial manufacturers of in vitro diagnostic products have invested significant resources into the development of

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[a] ARUP Laboratories, University of Utah, 500 Chipeta Way, Salt Lake City, UT 84108, USA;
[b] Microbiology and Virology Laboratories, Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, University of Southern California, 4650 Sunset Boulevard MS#32, Los Angeles, CA 90027, USA
* Corresponding author.
E-mail address: marc.couturier@aruplab.com

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multiplex molecular diagnostic assays that are capable of detecting a broad array of pathogens that collectively could cause a single clinical syndrome. This became known as the syndromic panel approach to pathogen testing, and this avenue of test design and development has carried through the recent decade with an ever-growing portfolio of offerings from various in vitro diagnostic manufacturers.

Syndromic testing has carried the field of clinical microbiology to a new frontier of diagnostic capabilities. Although many of the advantages of syndromic testing have been met with equal and opposite disadvantages and challenges, a common opinion is that molecular methodologies for many conventional infections are more accurate, rapid, and convenient than most traditional culture and direct detection techniques previously used by laboratories. But what is next and what should the future of these syndromic panels look like? Where are the gaps and what are the unmet needs? This article describes the current state-of-the-art with regards to commercially available (primary those cleared by the US Food and Drug Administration [FDA]) syndromic panels for respiratory tract infections, gastrointestinal (GI) pathogen detection, blood stream infections, and central nervous system (CNS) infections, while providing a provocative and speculative look into the future of syndromic panel testing for infectious diseases.

RESPIRATORY TRACT SYNDROMIC PANELS

Respiratory tract infection panels were the first example of syndromic testing to become commercially available to clinical laboratories approximately 10 or more years ago on a limited number of platforms that have since increased to include multiple different platforms and panel designs. These assays target upper respiratory tract (URT) pathogens from nasopharyngeal swabs or lower respiratory tract pathogens from aspirates or bronchial lavages. The lower respiratory tract panels (**Table 1**) have only recently been introduced to clinical care, and therefore they are not discussed in detail owing to the unclear nature of their future needs.

Upper Respiratory Tract Panels

URT syndromic testing was a welcomed replacement for insensitive and cumbersome viral cultures and stains as well as imperfect bacterial serology assays. Although PCR for influenza was available to laboratories previously, syndromic panels expanded the detection capacity to include a broad array of common viral targets and select bacteria not traditionally detected by culture. These assays vary in terms of breadth and scope of targets (**Table 2**). The platform ease of use and complexity is also variable. Extreme ends of the spectrum include the BioFire FilmArray Respiratory EZ panel (BioFire Diagnostics, Salt Lake City, UT, USA), which is Clinical Laboratory Improvement Amendments of 1988 waived and can be performed at near point of care, versus the Luminex NxTAG Respiratory Pathogen Panel (Luminex Corporation, Austin, TX USA), which is high complexity and requires molecular laboratory expertise and workspace.

Future target consideration

Emerging respiratory viruses such as enterovirus D68 and coronavirus family members have been identified recently in outbreaks affecting multiple countries.[1,2] These emerging and evolving viruses pose the most immediate opportunity for assays to provide more comprehensive detection of respiratory viruses. The FilmArray RP2Plus panel currently includes the Middle East respiratory syndrome coronavirus; however, the clinical performance is not fully understood owing to limited widespread circulation of the virus. This target is suppressed in patients who do not meet clinically compatible presentations. This opens up an interesting future opportunity for flexible testing

Table 1
Syndromic panels currently cleared by the US FDA for detection of lower respiratory tract pathogens associated with pneumonia

	FilmArray	Curetis
	Pneumonia Panel	**Hospitalized Pneumonia Panel**
Shared bacterial targets	*Staphylococcus aureus*[a]	*S aureus*
	Streptococcus pneumoniae[a]	*S pneumoniae*
	Proteus spp.[a]	*P spp.*
	Klebsiella aerogenes[a]	*K aerogenes*
	Klebsiella pneumoniae group[a]	*K pneumoniae*
	Klebsiella oxytoca[a]	*K oxytoca*
	Serretia marcescens[a]	*Serretia marcescens*
	Moraxella catarrhalis[a]	*M catarrhalis*
	Pseudomonas aeruginosa[a]	*P aeruginosa*
	Acinetobacter calcoaceticus-baumannii complex[a]	*Acinetobacter baumannii complex*
	Haemophilus influenzae[a]	*H influenzae*
	Mycoplasma pneumoniae	*M pneumoniae*
	Chlamydophila pneumoniae	*C pneumoniae*
	Legionella pneumoniae	*L pneumoniae*
Unique bacterial targets	*Streptococcus pyogenes*[a]	*Stenotrophomonas maltophila*
	Streptococcus agalactiae[a]	*Morganella morganii*
	Escherichia coli[a]	*Klebsiella variicola*
		Citrobacter freundii
Resistance determinants		*tem*
		shv
		ermB
	mecA/C	*mecA*
	MREJ	*mecC*
	ctx-M	*ctx-M*
	imp	*imp*
	kpc	*kpc*
	ndm	*ndm*
	vim	*vim*
	Oxa-48-like	
		oxa-23
		oxa-24/40
		oxa-48
		oxa-58
		sul1
		gyrA83
		gryA87
Viral or fungal targets	Adenovirus	*Pneumocystis jirovecii*
	Coronavirus	
	Human metapneumovirus	
	Human rhinovirus/enterovirus	
	Influenza A	
	Influenza B	
	Parainfluenza virus	
	Respiratory syncytial virus	

[a] Bacterial targets are reported semiquantitative.

Table 2
Syndromic panels currently cleared by the US FDA for detection of URT pathogens

	FilmArray		Verigene	Luminex			GenMark	
Target Organism	**RP**	**RP EZ**	**RP *Flex***	**xTAG RVP**	**xTAG RVP Fast v2**	**NxTAG RPP**	**XT-8 RVP**	**ePlex RP**
Viruses								
Respiratory syncytial virus	•	•			•			
Respiratory syncytial virus A			•	•		•	•	•
Respiratory syncytial virus B			•	•		•	•	•
Influenza A	•	•	•		•	•	•	•
Influenza A matrix				•	•			
H1 subtype	•	•	•	•	•	•	•	•
H3 subtype	•	•	•	•	•	•	•	•
H1-2009 subtype	•	•					•	•
Influenza B	•	•	•	•	•	•	•	•
Parainfluenza virus		•						
Parainfluenza 1	•		•	•		•	•	•
Parainfluenza 2	•		•	•		•	•	•
Parainfluenza 3	•		•	•		•	•	•
Parainfluenza 4	•		•			•		•
Human metapneumovirus	•	•		•	•	•	•	•
Adenovirus	•	•	•	•	•	•		•
Adenovirus C							•	

Adenovirus B/E								•	
Human rhinovirus/enterovirus		•					•		•
Human rhinovirus	•		•	•	•			•	
Coronavirus		•							•
Coronavirus HKU1	•						•		
Coronoavirus NL63	•						•		
Coronavirus 229E	•						•		
Coronavirus OC43	•						•		
MERS coronavirus	•[a]								
Human bocavirus							•		
Bacteria									
Mycoplasma pneumoniae	•	•					•		•
Chlamydia pneumoniae	•	•					•		•
Bordetella pertussis	•	•	•						
Bordetella parapertussis	•[b]								
Bordetella parapertussis/B bronchiseptica			•						
Bordetella holmesii			•						

•, Panel is cleared by the FDA to detect this pathogen.
[a] Available on RP2 PLUS (Not cleared by US FDA).
[b] Available on RP2.

options related to such pathogens. Because the positive predictive value of a test result will be significantly impaired in the absence of circulating virus, emerging respiratory pathogens (eg, Middle East respiratory syndrome coronavirus, enterovirus D68, severe acute respiratory syndrome) could be included in a syndromic panel, but the end-user could have the option of disabling this target in the absence of documented circulation.

Surveillance potential for novel pathogens

An alternative approach could be that certain targets could be tested but not immediately visible. The results could instead be communicated to the manufacturer through a cloud-based surveillance system. In this way, a sporadic false positive could be easily identified, but a community-level cluster of positives could be identified by the manufacturer and an investigation and communication with the end-user laboratories and treating physicians could ensue. Although this may be an Orwellian, intrusive system, a similar voluntary surveillance system already exists for BioFire users and could have value in future epidemiologic outbreak identification.[3] Rather than continuing to chase outbreaks after the fact, earlier crowd sharing of silent surveillance data could serve the entire community while not sounding an alarm on a sporadic false-positive result. This process would require a discussion of who pays for this extra effort and target detection—manufacturers, public health, or end-users.

Simple, rapid, and near point-of-care testing

The future of URT pathogen testing is ultimately coming to near point-of-care with extremely rapid turn around time. Although microbiology laboratories are hesitant to let go of their testing fiefdom, the logical progression of testing and rapid decision making lies in having answers immediately available and actionable. Examples of technologies that bring single or 2-target respiratory pathogen testing to near point of care for influenza have been embraced in many health care settings (despite imperfect performance characteristics) and have allowed for more efficient use of emergency rooms and decreased costs.[4,5] Comprehensive URT panels with even shorter turn around time than current assays could allow for improved management of admissions and patient cohorting, while possibly reducing early empiric (and often unnecessary) antibiotic administration.

GASTROINTESTINAL SYNDROMIC PANELS

GI pathogen detection has historically relied upon multiple classic complementary diagnostics modalities (eg, culture, fecal antigen detection, microscopy, single target PCR) to create a comprehensive pseudopanel of targets. A longstanding challenge with respect to GI pathogen detection includes multiple factors:

1. Limited capacities of the local laboratory and lack of usefulness for reference laboratory testing for acute GI illness
2. Physician ordering lapses stemming from a lack of understanding of test methods and detection capabilities
3. Preanalytical and analytical factors that decrease sensitivity of the available methods (eg, delayed transport or preservation of stool, inexperienced technologists, general unfamiliarity with less frequently encountered pathogens)

Syndromic panels have approached these challenges by compartmentalizing pathogens into a single large panel or multiple modular panels grouped by taxonomic relatedness (eg, bacteria, viruses, protozoa).

Modular Panels

Small modular panels can detect more common bacteria, viruses, or protozoa. These panels for the most part have targeted the highest prevalence or significance organism at the expense of less comprehensive detection of other pathogens. A panel capable of detecting *Salmonella*, *Shigella*, *Campylobacter jejuni/coli*, and Shiga-like toxin genes of Shiga-toxigenic *Escherichia coli* was essentially a replacement for conventional stool culture. However, clinical concerns about failing to detect less frequently encountered pathogens such as *Vibrio*, *Yersinia entrocolitica*, and enterotoxigenic *E coli* drove the creation of expanded bacterial panels which could be considered for specific patient populations or patients who initially test negative for the more common bacterial pathogens (**Table 3**).

Similarly, standalone parasitic panels primarily targeting the most common protozoal pathogens can be viewed as replacements for stool ova and parasite examinations, with only a rare subset of patients requiring a full ova and parasite examination to rule out helminth infections or less common protozoal pathogens (eg, *Cystoisospora*, *Balantioides*). The most common configuration of testing includes *Giardia*, *Cryptosporidium*, and *Entamoeba histolytica* (see **Table 3**).

Modular panels for enteric viral pathogens have not been widely commercialize to date, with only 1 product from BD (Beckton Dickinson, Franklin Lakes, NJ, USA) having clearance from the FDA (see **Table 3**). This may be understandable from a practical standpoint because conventional testing for GI viruses has not been universally embraced as a standard of care, with the exception of rotavirus antigen detection for neonates and PCR for noroviruses in outbreak settings.[6]

Table 3
Modular panels currently cleared by the US FDA for detection of GI pathogens currently cleared by the US FDA

Target Organism	BDMax	Prodesse
Bacterial		
Campylobacter	•	‡
Salmonella	•	‡
Shigella	•	‡
Shiga-like toxin 1 and 2 (STEC)	•	‡
Enterotoxigenic *Escherichia coli*	*	
Vibrio	*	
Yersinia enterocolitica	*	
Plesiomonas shigelloides	*	
Parasitic		
Giardia	+	
Cryptosporidium	+	
Entamoeba histolytica	+	
Viral		
Norovirus	††	
Adenovirus (40/41)	††	
Rotavirus	††	
Astrovirus	††	
Sapovirus	††	

-, enteric bacterial panel; *, extended bacterial panel; +, enteric parasite panel; ††, enteric viral panel; ‡, ProGastro SSCS.

Comprehensive Panel

Several commercial panels are capable of detecting bacteria, viruses, and protozoa in a single assay (Table 4). These panels vary by specific target in the case of bacteria and viruses; however, a core set of pathogen targets has been included in most commercial assays. These targets are thought to represent the most common enteric pathogens.

Existing Gaps

Targets of unclear or unestablished significance

To date, GI panels have included definitive pathogens; however, some detect organisms with less definitively established pathogenicity. Such targets include enteroaggregative *E coli*, enteropathogenic *E coli*, and *Plesiomonas shigelloides.* Manufacturers should consider removing or masquing such targets or conducting additional clinical studies to better establish significance in specific test populations before integrating them into the panels.

Table 4
Syndromic, broad panels currently cleared by the US FDA for GI pathogen detection

Target Organism	FilmArray GI Panel	Verigene Enteric Pathogens Test	Luminex GI Pathogens Panel
Bacterial			
Campylobacter	•	•	•
Salmonella	•	•	•
Shigella	•	•	•
Shiga-like toxin 1 and 2	•	•[a]	•
Enterotoxigenic *Escherichia coli*	•		•
Enteropathogenic *E coli*	•		
Enteroaggregative *E coli*	•		
E coli O157	•		•
Vibrio	•	•	
Yersinia enterocolitica	•	•	
Plesiomonas shigelloides	•		
Clostridium difficile	•		•
Viral			
Norovirus GI and GII	•	•	•
Adenovirus 40/41	•		•
Rotavirus	•	•	•
Astrovirus	•		
Sapovirus	•		
Parasitic			
Giardia	•		•
Cryptosporidium	•		•
Cyclospora cayetanensis	•		
Entamoeba histolytica	•		•

•, Panel is cleared by the FDA to detect this pathogen.
[a] Verigene detects and reports each shiga-like toxin gene separately.

Targets with clinical relevance but not universally included

A glaring hole in the design of most commercial assays is the notable exclusion of *Cyclospora cayetanensis*. This pathogen is of significant importance in the Americas and in the recent decade has become a quasiseasonal illness in the United States owing to imported produce serving as a vehicle for multistate and nationwide outbreaks. One study has already demonstrated the value of detecting *C cayetanensis* directly from stool specimens during an outbreak.[7] Despite this prevalence and clinical/epidemiologic significance, the target has been excluded from multiple panels in favor of *E histolytica* (see **Tables 3** and **4**). Although *E histolytica* is a virulent protozoal pathogen, its incidence is very low in developed countries. In this author's laboratory, *Cyclospora* is the most frequently detected protozoal pathogen and *E histolytica* ranks fifth of the 5 protozoa detected by a syndromic panel. Furthermore, some commercial assays are not able to specifically identify *E histolytica* from the nonpathogenic *Entamoeba dispar*. This limitation is essentially the same problem that has plagued ova and parasite examinations for a century. Improving the species-level specificity for *E histolytica* and inclusion of *Cyclospora* in future products is imperative.

Clostridioides difficile testing without clinical indication

Testing for *C difficile* has been an area of clinical diagnosis that has evolved significantly over the past decade. Although the nuances and opinions with regard to this infection are beyond the scope of this work, there are some practical issues that arise as a result of panel tests that include this target. Testing for *C difficile* in children less than 24 months of age has been traditionally discouraged because children in this age group are often asymptomatically colonized.[8] Furthermore, testing for *C difficile* in otherwise healthy adult populations without previous use of antibiotics or hospitalization or hospital exposure has not historically been advocated during primary clinical evaluations.[8] Inclusion of this target can increase the risk for further overdiagnosis of *C difficile*.

Host response targeting

Another future area of consideration for aiding in the interpretation of colonization versus infectious state could include the integration of mucosal inflammatory marker expression, for example, proinflammatory cytokines. Markers of acute inflammation could serve as an adjunct metric to aid in interpreting the detection of potential colonizers like *C difficile* or organisms capable of shedding for week or month after convalescence (eg, *Salmonella* and norovirus). This would be an area still in need of significant research because molecular profiling for inflammatory bowel disease and other similar conditions is not yet a standard of care.

Demographic and region-specific testing

Demographic or region-specific assays (or convenient end-user customization) should be an area of future consideration for manufacturers. Future GI syndromic testing must be customizable to fit the needs of the individual laboratory or region while not overtesting for targets that have low to no prevalence in the region or demographic. For example, although including *Cyclospora* in a syndromic panel may be very relevant for the Americas and tropical/subtropical countries, it may be a completely unnecessary target in other regions (eg, Canada, Northern and Eastern Europe). Although rare infections may be encountered in these regions, it may not be cost effective to test all specimens for this target and in the absence of true infections, the positive predictive value of this target will be poor. Considering demographic-driven testing, a community hospital with primarily uncomplicated patients would have a different pathogen list to consider for clinical care than a large academic medical center that supports a transplant center, human immunodeficiency virus clinic, and travel/tropical medicine clinic.

A flexible end-user design already exists in the Verigene (Luminex Corp., Austin, TX, USA) RP Flex assay.

Detection of antimicrobial resistance

One challenge that is unmet by the current GI syndromic testing is the detection of antimicrobial resistance to primary empiric antibiotics. This is a daunting technical challenge in a specimen such as stool that contains copious genera of commensal microbiota; however, even a few targets for primary resistance could be invaluable to clinical care for some enteric bacterial pathogens. Organisms such as *Shigella*, *Salmonella*, and *Campylobacter* are currently cultured after syndromic testing in efforts to maintain public health outbreak tracking, but also to provide primary antimicrobial susceptibility testing for selected drug classes in cases of severe infection.[9,10] These organisms often are not culturable after primary syndromic testing, so having markers for macrolide, quinolone, and/or tetracycline resistance could be extremely helpful for treating clinicians and epidemiologic reporting.[11] Clearly this is a technically challenging request and one that may be more idealistic than realistic given current technologies, but should nonetheless be a future target goal for testing.

Gastrointestinal Panel Future Needs Summary

Future syndromic testing for GI pathogens should consider

1. Customizable or regional/demographic-driven targets
2. Inclusion of only clinically relevant targets or those with well-established clinical significance
3. Inclusion of inflammatory markers to aid in identifying potential colonizers versus active infections
4. Inclusion of antimicrobial resistance determinants for outbreak associated bacterial pathogens

BLOODSTREAM SYNDROMIC PANELS

Expeditious identification of bloodstream pathogens through the use of molecular syndromic panels has dramatically altered the standard of care in many laboratories. There are currently 6 FDA-cleared multiplexed assays directly from positive blood cultures (**Table 5**)—4 bacterial panels coupled with resistance determinants, 1 fungal panel, and 1 panel that generates minimum inhibitory concentration in lieu of resistance markers. There are currently 2 direct from whole blood panels.

Future Directions and Considerations

Target wish list

Expansion of existing panels to include additional pathogens and more comprehensive resistance determinants is ongoing. The ePlex BCID-GP panel is currently the most comprehensive panel for gram-positive organisms and also includes pan–gram-negative and pan-*Candida* targets as a safeguard for Gram stain interpretation (see **Table 5**). Additional gram-positive organisms that may be considered are *Streptococcus mitis/oralis*, an important bloodstream infection (BSI) agent in patients with underlying hematologic and oncologic diseases, and *Corynebacterium jeikeium*. The inclusion of targets for *Mycobacterium* species, particularly the rapid-growing nontuberculosis *Mycobacterium*, would also be beneficial for certain patient demographics.

A relatively underappreciated pathogen group are anaerobes, accounting for approximately 20% of BSIs.[12] An up and coming gram-negative panel (BCID-GN) targets numerous obligate anaerobes, including *Fusobacterium necrophorum*, *F nucleatum*,

Table 5
Syndromic, broad panels currently cleared by the US FDA for bloodstream pathogen detection

	BioFire	Luminex		GenMark		Accelerate	T2 BioSystem	
Target Organism	**FilmArray BCID[a]**	**Verigene BC-GN[a]**	**Verigene BC-GP[a]**	**ePlex BCID-GP[a]**	**ePlex BCID-FP[a]**	**PhenoTest BC**	**T2Candida[b]**	**T2Bacteria[b]**
Gram-negative bacteria								
Acinetobacter species		•						
Acinetobacter baumannii	•					•		
Citrobacter species		•				•		
Enterobacteriaceae	•							
Enterobacter species		•				•		
Enterobacter cloacae complex	•							
Escherichia coli	•	•				•		•
Haemophilus infuenzae	•							
Klebsiella species						•		
Klebsiella oxytoca	•	•						
Klebsiella pneumoniae	•	•						•
Neisseria meningitidis	•							
Pan gram-negative				•				
Proteus species	•	•				•		
Pseudomonas aeruginosa	•	•				•		•
Serratia marcescens	•	•				•		
Gram-positive bacteria								
Bacillus cereus group				•				
Bacillus subtilis group				•				
Corynebacterium				•				
Cutibacterium acnes				•				

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Table 5
(*continued*)

	BioFire	Luminex		GenMark		Accelerate	T2 BioSystem	
Target Organism	**FilmArray BCID[a]**	**Verigene BC-GN[a]**	**Verigene BC-GP[a]**	**ePlex BCID-GP[a]**	**ePlex BCID-FP[a]**	**PhenoTest BC**	**T2Candida[b]**	**T2Bacteria[b]**
Enterococcus species	•			•				
Enterococcus faecium			•	•		•		•
Enterococcus faecalis			•	•		•		
Lactobacillus species				•				
Listeria species			•					
Listeria monocytogenes	•			•				
Micrococcus species				•				
Staphylococcus species	•		•	•		•		
Staphylococcus aureus	•		•	•		•		•
Staphylococcus lugdunensis			•	•		•		
Staphylococcus epidermidis			•	•				
S species	•		•	•		•		
Streptococcus agalactiae	•		•	•				
Streptococcus pyogenes	•		•	•				
Streptococcus anginosus			•	•				
Streptococcus pneumoniae	•		•	•				
Fungal								
Candida albicans	•				•	•	•	
Candida auris					•			
Candida dubliniensis					•			
Candida famata					•			

Candida glabrata	•				•	•	•
Candida guilliermondii					•		
Candida keyr					•		
Candida krusei	•				•		•
Candida lusitaniae					•		
Candida parapsilosis	•				•		•
Candida tropicalis	•				•		•
Cryptococcus gattii					•		
Cryptococcus neoformans					•		
Fusarium					•		
Pan *Candida*				•	•		
Rhodotorula					•		
Antimicrobial susceptibility testing							
Genotypic susceptibility							
CTX-M		•					
IMI		•					
KPC	•	•					
NDM		•					
OXA		•					
VIM		•					
mecA	•		•	•			
mecC				•			
vanA/B	•		•	•			
Phenotypic susceptibility							
Minimum inhibitory concentration*						•	

•, Panel is cleared by the FDA to detect this pathogen.
[a] Testing on positive blood cultures.
[b] Testing on whole blood.

and *Bacteriodes fragilis.* Likewise, the next generation of the FilmArray BCID (BCID2) will include *B fragilis.* Inclusion of additional obligate anaerobes (eg, *Clostridium* spp.) would further expand the breadth of coverage offered in these syndromic panels.

The inclusion of select agent targets such as *Brucella* spp, *Franciscella* spp., and *Burkholderia pseudomallei* may prevent unnecessary laboratory exposure. This request may be onerous for manufacturers to fulfill owing to restrictions associated with select agent testing. Last, the development of a fungal panel that includes comprehensive list of molds would significantly improve time to identification. The ePlex BCID-FP panel offers detection of *Fusarium* spp. (see **Table 5**), but some additional relevant targets to consider are *Scedosporium apiospermum* and *Lomentospora prolificans.*

Direct from whole blood testing

There is a crucial unmet need to bypass the primary incubation step entirely. Exciting new technologies are currently in development, and an excellent summary of these technologies are provided by Sinha and colleagues.[13] The first innovative step to this holy grail approach is the release of 2 FDA-cleared, whole blood assays (see **Table 5**) that target 5 bacteria and *Candida* species. This is a significant milestone in BSI diagnostics and provides insight for future panels. For starters, whole blood syndromic testing is an adjunct to conventional blood cultures and manufacturers must consider the pediatric population and associated blood volume limitations. A requirement of less than 1 mL of blood in children would be optimal. Second, the targets incorporated must be analogous to the aforementioned direct from positive blood culture panels. Preferably, a compendious list of bacterial, viral, and fungal pathogens should be offered, without compromising the sensitivity of the test. The ability to detect polymicrobial infections despite low pathogen load is paramount for appropriate antimicrobial coverage. Importantly, the breadth of targets and high sensitivity will allow for high confidence in a negative result to potentially decrease antimicrobial use. An overarching goal is to be as target agnostic as possible while maintaining rapidity.

There are a number of caveats to whole blood syndromic testing that needs to be rectified before widespread adoption. First, the increase risk for contamination exists, particularly if extensive manual manipulation is required. Emerging technologies are attempting to alleviate this risk by innovative approaches, including the use of small reaction mixture volumes to reduce the number of contaminating DNA.[14] Second, current BSI syndromic panels pricing is not conducive to testing on all patients with corresponding blood cultures. Manufacturers must be aware of the budgetary limitations instilled on laboratories that may prohibit adoption. The development of a diagnostic algorithm that integrates host response as a predicate of whole blood syndromic testing may be a potential strategy to distinguish low risk patients from septic patients. The SeptiCyte (Immunexpress Inc., Seattle, WA), a recently FDA-cleared quantitative reverse transcriptase PCR based assay, measures levels of biomarkers (CEACAM4, LAMP1, PLA2G7, and PLAC8) to distinguish between sepsis and noninfectious process in adult patients only.[15] A pilot study of 70 pediatric patients also demonstrated promising results.[16] A noteworthy limitation is the required 2.5 mL for testing and future development of host response assays should also be cognizant of blood volume constraints.

Antimicrobial susceptibility needs

Inarguably, the impact of timely susceptibility results is profound because it allows for antimicrobial optimization. New generations of BSI syndromic panels currently in development have expanded the list of resistance targets of epidemiologic and therapeutic importance including, *mecC* and *mcr-1* genes. However, targets to detect extended-spectrum beta-lactamase production remains limited to the $bla_{CTX\text{-}M}$ gene and would benefit from additional genes, including bla_{SHV} and bla_{TEM}.

In contrast with genotypic resistance detection offered by the majority of panels, there is a paucity of rapid phenotypic susceptibility platforms. Although both testing modalities have clinical usefulness, only panels that provide simultaneous identification and phenotypic susceptibility results can function as a standalone test. Expansion of the identification capabilities of the existing PhenoTest BC (Accelerate Diagnostics, Tucson, AZ, USA) would be beneficial, because the gram-positive targets are currently limited (see **Table 5**). Manufacturers need to also be cognizant of prioritizing the release of susceptibility testing options for novel antimicrobial agents and to accommodate clinical breakpoints changes.

Finally, the amalgamation of direct whole blood syndromic panel with phenotypic susceptibility testing is an ambitious request that may be realistically fulfilled in the near few years.

CENTRAL NERVOUS SYSTEM SYNDROMIC PANELS

Laboratory diagnostic tests are compulsory to confirm CNS infection. The diagnostic accuracy of conventional microbiological approaches, such as Gram stain and culture, is hampered by low sensitivity and/or slow turnaround time. New and emerging diagnostic approaches to aid in the diagnosis of meningitis and encephalitis may address the limitations of current laboratory practices.

There are currently only 3 FDA-cleared tests for the detection of pathogens directly from cerebrospinal fluid (CSF) specimens (**Table 6**). The first 2 are limited-target,

Table 6
Syndromic, broad panels currently cleared by the US FDA for meningitis/encephalitis pathogen detection

	BioFire	DiaSorin	Cepheid
Target Organism	FilmArray Meningitis/ Encephalitis	Simplexa HSV 1 and 2 Direct	Xpert EV
Bacterial			
Escherichia coli K1	•		
Haemophilus influenzae	•		
Listeria monocytogenes	•		
Neisseria meningitidis	•		
Streptococcus agalactiae	•		
Streptococcus pneumoniae	•		
Viral			
Cytomegalovirus	•		
Enterovirus	•		•
Herpes simplex virus-1	•	•	
Herpes simplex virus-2	•	•	
Human herpes virus-6	•		
Human parechovirus	•		
Varicella zoster virus	•		
Yeast			
Crytococcus neoformans/gattii	•		

•, Panel is cleared by the FDA to detect this pathogen.

qualitative tests that detects 1 to 2 viral pathogens. The final FDA-cleared test is the FilmArray Meningitis/Encephalitis panel, offering simultaneous detection of multiple pathogens from CSF samples. Testing is finite, and conventional culture remains of the utmost importance for the recovery of additional pathogens and for susceptibility testing when appropriate. Other key points about current syndromic testing for CNS infections include:

1. Increased pathogen detection compared with conventional laboratory approaches
2. Associated risks of contamination necessitate the need for strict adherence to molecular testing policies
3. Low viral load in CSF for certain viruses (eg, herpes simplex virus) may warrant additional testing from alternate sources (eg, blood)
4. Molecular testing may be suboptimal for certain pathogens (eg, cryptococcal antigen testing continues to be the diagnostic standard)
5. Detection of herpes viruses could represent either latent or active infection. Chromosomal integration is also a possibility in patients positive for human herpes virus-6 (inherited chromosomally integrated human herpes virus-6).
6. Corroboration of findings with clinical picture is paramount to ensure the most appropriate diagnosis and patient management.

Existing Gaps

Syndromic testing for CNS infections remains in its infancy. Continuous innovation and development are fundamental to the diagnostic advancement of CNS infections. A quick fix that current and future manufacturers may consider would be to decrease the required CSF volume to accommodate the copious numbers of tests often ordered.

Acute versus chronic central nervous system infections

It may be valuable to offer customizable panels for acute versus chronic CNS infections. Acute meningitis is often caused by viruses and bacteria included in the FilmArray Meningitis/Encephalitis panel. In contrast, chronic meningitis can result from infection with *Mycobacterium tuberculosis* and fungal pathogens including, *Aspergillus*, *Histoplasma*, *Blastomyces*, and *Coccidiodes*.[17] The inclusion of additional, uncultivable pathogens, may add further value to the panel (eg, *Bartonella* spp. and *Treponema pallidum*). This demographic consideration can be expanded geographically to include pathogens that may be applicable to certain regions.

Shunt infections

An entire niche of CNS infections remains untouched. Cumulative incidence rates of shunt infections range from 10% to 22% per patient with 90% of infections occurring within 30 days of surgery.[18] No commercial molecular tests exist for the diagnosis of shunt infections and microbiology culture remains the primary approach. The microbiological demographic of shunt infections is attributed to skin flora and differ significantly from patients with acute meningitis and encephalitis. As such, the FilmArray Meningitis/Encephalitis panel is not appropriate for patients with indwelling devices and also not approved by the FDA for such testing. Laboratory-developed molecular tests that detect *Staphylococcus aureus* and *Cutibacterium acnes* from shunt specimens have yielded promising data, including increased detection in patients with prior antimicrobial exposure.[19] We envision future assays that target common shunt pathogens including, *S aureus*, *C acnes*, coagulase-negative staphylococci, gram-negative organisms, and *Candida* spp. A foreseen limitation would be the risk of skin flora contamination, which may impede the inclusion of coagulase-negative staphylococci target.

Testing in cases of mosquito exposure

Arbovirus infections are important causes of neuroinvasive diseases associated with endemic transmission or travel-related mosquito exposures. Molecular testing of CSF specimen may be inferior in cases of arbovirus infections because the window for viral detection is mere days from symptom onset compared with the measurement of IgM and IgG antibody responses, which remain elevated for weeks.[20] Thus, diagnosis is made primarily by serology. However, the superiority of serologic testing may be hampered in immunosuppressed patients, stressing the need for enhanced diagnostic tools.

Recent studies revealed promising results for detection of West Nile virus from whole blood and urine.[21,22] A retrospective study of 38 patients with West Nile virus confirmed superiority of molecular detection of West Nile virus in whole blood compared with CSF with a sensitivity of 86.8% and 16.6%, respectively.[21] Yet molecular testing of CSF specimens may still be valuable.[23] Potential syndromic panel for routine detection of arboviruses may integrate molecular testing from whole blood and CSF alongside serologic testing to improve diagnostic yield.

Syndromic testing paired with cytokine profiling

Owing to an active innate immune system in the CNS that rapidly responds to alterations in CNS homeostasis, the levels of biomarkers and cytokines released into the CSF may be diagnostic discriminators. Future syndromic panels may incorporate molecular target detection alongside markers of host responses. Studies on the role of biomarkers in predicting CNS infections and to differentiate bacterial from viral meningitis is ongoing. CSF lactate may be a promising marker to rule out bacterial meningitis with high sensitivity (93%–95%) and specificity (95%–96%),[24] albeit a significant decrease in sensitivity is noted with antibiotic exposure.[24]

A recent proof-of-concept study using the Luminex FlexMPA 3D technology determined that quantification of cytokine levels in the CSF can rule in or out an infectious process. Specifically, high levels of IP-10/CXCL10 were present in CNS infections and MDC/CCL22 levels were significantly higher in nonviral infections. Deciphering between oncologic process and autoimmune encephalitis may also be possible, as indicated by high levels of GRO/CXCL1, IL-7, and IL-8 in gliomas.[25] A potential steward of diagnostic testing would include screening for infectious processes by cytokine profiling followed by subsequent testing with syndromic panel. This method could potentially provide decipher between latent versus active viral infection or cases of chromosomally integrated human herpes virus-6 (iciHHV-6). Again, the integration of this algorithmic approach must maintain STAT testing capabilities to maximize the benefit of expeditious testing currently offered by syndromic panels. Extensive research is required to determine true clinical performance and usefulness of such tests.

Antimicrobial susceptibility

Inclusions of potential resistance markers and/or direct from specimen phenotypic susceptibility may be beneficial for some CNS pathogens including, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *E coli* K1. One example is to establish *H influenzae* antimicrobial profile as beta-lactamase positive, beta-lactamase negative ampicillin susceptible, and beta-lactamase positive ampicillin resistant by the detection of such genes as TEM-1, ROB-1, and PBP3.[26] In line with potential assays for shunt infections, inclusion of *mecA* and *mecC* gene for detection of methicillin resistance in *S aureus* is beneficial. An idealistic request is to develop direct from positive CSF specimen phenotypic susceptibility testing. Future development may consider

either a standalone identification and susceptibility testing platform or in collaboration with existing identification platforms to consolidate the susceptibility portion.

SUMMARY

There are numerous areas of opportunity to advance the role of syndromic panels in clinical care for respiratory, GI, blood stream infections, and CNS. Based on the primary points of this article, the future of syndromic testing should include:

1. Refining existing targets and inclusion of emerging pathogen targets
2. Expansion of specimen types and volumes required for testing
3. Integration of host response markers
4. Inclusion of additional antimicrobial resistance determinants and phenotypic susceptibility
5. Simple, fast, near point-of-care platforms

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