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Molecular Diagnostic Advances in Transplant Infectious Diseases

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Abstract

Purpose of Review The infectious complications of transplantation can have devastating consequences for patients. Early and accurate diagnosis is essential to good outcomes. This review describes recent advances in pathogen-directed diagnostic testing and discusses the role of new methods for transplant infectious diseases.

Recent Findings Several molecular assays have been introduced into clinical practice in recent years. When the results of rapid testing are linked to patient-specific interventions, improved outcomes can be realized. Syndromic testing along with metagenomic next-generation sequencing (mNGS) represents novel approaches to infection diagnosis. However, the optimal use of these tests for transplant patients along with an overall assessment of cost-effectiveness demands further study.

Summary Molecular diagnostics are revolutionizing transplant care. Clinicians need to be aware of the current diagnostic landscape and have a working knowledge of the nuances related to test performance, result interpretation, and cost.

Keywords Immunocompromised host · Molecular testing · Syndromic testing · PCR-based assays · Transplant infections, metagenomic next-generation sequencing (mNGS)

Introduction

Solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients are at risk for life-threatening infections. Making a rapid and accurate diagnosis of infection is essential to improve clinical outcomes in transplant medicine; however, culture-based testing is often too slow and/or insensitive to reliably guide treatment. Molecular diagnostics targeting organism-specific nucleic acids may circumvent many of the limitations of traditional methods. For example, culture-independent molecular diagnostics may be less impacted by prior antimicrobial therapy, potentially have a more rapid turnaround time (TAT), and can be designed to simultaneously detect large numbers of potential pathogens (i.e., syndromic testing). These are ideal characteristics for implementation in transplant patients.

Recent advances in molecular diagnostics include the availability of sensitive nucleic acid amplification tests (NAATs) that can be performed at the point-of-care, highly multiplexed PCR panels designed to simultaneously detect the most common causative agents of a specific clinical syndrome and the use of metagenomics to detect all potential pathogens [1, 2]. Unbiased metagenomics approaches are especially appealing in immunocompromised hosts where the differential diagnosis of potential pathogens is broad and environmental or commensal microorganisms can become pathogenic.

Molecular diagnostics can be more expensive than culture-based methods. Nonetheless, when results are actionable, laboratory costs can be offset through downstream benefits to the health system. Recent studies suggest that the implementation of rapid molecular diagnostic tests can lead to reductions in

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unnecessary antibiotic use and shorter hospital lengths of stay (LOS) and potentially impact mortality when paired with antimicrobial stewardship program (ASP) guidance [3, 4]. Rapid molecular testing can also contribute to early detections of pathogens of significant interest to public health as well as guide more timely initiation of infection prevention efforts. This review describes recent advances in pathogen-directed molecular diagnostic modalities, with a focus on new tests and clinical studies published in the last 5 years.

Bloodstream Infections

Bloodstream infections (BSIs) are a major cause of morbidity and mortality in immunocompromised patients [5, 6]. Shortening time to organism identification has the potential to promptly inform effective antimicrobial therapy and improve patient outcomes [7]. Recent diagnostic developments for BSI include availability of assays that more rapidly identify common bacteria and *Candida*. New methods can either be applied to blood culture aliquots taken from bottles with detectable organism growth or used directly on blood without the need for pre-amplification in culture.

Tests on Blood Culture Aliquots

Numerous multiplex panels designed to identify the most common bacterial and fungal causes of BSI are commercially available and FDA-approved (Table 1) [8•, 9, 10–14]. Current formats include a PCR panel with all organism targets combined or smaller modular combinations that are selected by laboratory staff based on Gram stain morphology. The spectrum of organisms detected by these assays varies slightly. One disadvantage of current testing is reduced sensitivity for detection of organisms contained in polymicrobial BSIs. Molecular methods may also detect nonviable microbial DNA known to contaminate culture media and laboratory reagents [15–17]. Thus, it is important for clinicians to match the molecular result with the Gram stain and to question NAAT-positive results from culture-negative samples.

Tests Applied Directly to Blood Specimens

There has been significant interest in direct detection of microorganisms in an attempt to reduce time to critical results. The T2Bacteria and T2*Candida* (T2 Biosystems, Lexington, MA) tests are currently the only FDA-approved assays available for direct detection from blood. T2 assays are designed to detect and identify the 5 most common *Candida* species [12••] or ESKAPEc (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*) [13] pathogens in approximately 3–5 h. ESKAPEc species are frequently multidrug-resistant [18], and knowing which *Candida* species is present is useful for predicting fluconazole susceptibility. The analytical sensitivity, or limit of detection (LOD), of these tests varies slightly by organism and ranges from 1 to 3 CFU/mL for T2*Candida* or 5–11 CFU/mL for T2Bacteria [14, 19]. Because T2 analyzes whole cells and not fragmented circulating DNA, specificity may be improved relative to standard NAAT methods.

T2*Candida* has been commercially available longer than the bacterial panel and therefore has more published data in support of analytic performance and clinical utility. In the T2 clinical trial supporting FDA clearance, sensitivity and specificity relative to blood culture were 91.1% and 99.4%, respectively [19]. T2*Candida*-positive/blood culture–negative samples were more likely to have come from patients receiving antifungal therapy and may be a marker of invasive candidiasis (IC) at a site distant to the bloodstream [19]. Additionally, a positive T2*Candida* in the first 5 days after a positive blood culture for *Candida* was associated with a 37-fold higher risk of developing complicated IC [20]. Alternatively, a negative T2*Candida* result may allow discontinuation of empiric antifungals in patients at low-to-moderate risk of IC [21, 22•]. Using the test to stop or withhold treatment was shown in a decision model to reduce the number of unnecessarily treated patients without missing a significant number of IC cases, which suggests the test may have a useful role for antifungal stewardship [23].

Although the T2Bacteria targets a group of pathogens important for transplant recipients, it only detects about 48–52% of BSIs overall [13, 14]. Like T2*Candida*, the bacterial panel may detect organisms not recovered in culture. A prospective trial from 11 US hospitals found that approximately 10% of blood culture–negative patients had positive T2Bacteria results, with 60% of these discrepancies adjudicated as "possible" or "probable" BSIs [14]. Positive T2Bacteria results may be useful for early modification of treatment based on institutional antibiograms. Alternatively, negative results may allow de-escalation of antibiotics in selected situations (e.g., stopping anti-pseudomonal coverage if the panel is negative for *Pseudomonas* spp.). Additional studies are needed to define best use and the overall impact of the T2Bacteria panel on antimicrobial use and patient outcomes.

Panel-based approaches are limited to the most common causes of a given infectious disease. Therefore, an all-inclusive unbiased approach to organism detection is potentially useful when more rapid targeted testing is negative. Metagenomic next-generation sequencing (mNGS) is a technology in which all of the microbial nucleic acids in a sample are amplified and analyzed without the need to guess in advance which pathogens may present [2, 24••, 25]. However, the complexity of mNGS testing from a clinical laboratory and bioinformatics perspective has largely limited testing to reference laboratories and a few boutique commercial laboratories.

Table 1 FDA-Approved assays for bloodstream infections

Platform	Method	Assay run time (h)	Number of organisms	AMR genes	Sensitivity	Specificity
FDA-approved assays from blood culture aliquots						
BioFire FilmArray [8•]	Multiplex PCR	1	B: 18 GP: 8 GN: 10 V: 0 F: 5	mecA vanA/B KPC	88.9–100%	85.4–100%
Verigene Blood culture [8•]	Multiplex Microarray	2–2.5	B: 20 GP: 12 GN: 8 V: 0 F: 0	mecA vanA vanB CTX-M IMP KPC NDM OXA VIM	72.7–100%	98.2–100%
GenMark ePlex [9]	Multiplex PCR	1.5	B: 41 GP: 20 GN: 21 V: 0 F: 15	mecA mecC vanA vanB CTX-M IMP KPC NDM OXA-23 OXA-48 VIM	97%	99%
Accelerate Pheno System [10, 11]	FISH with real-time microscopy	ID:1.4 AMR: 6.6	B: 27 GP: 15 GN: 12 V: 0 F: 2	18 drugs	ID: 80–96% AMR: 91–95%	98.9–100%
FDA-approved assays direct from blood						
T2*Candida* [12••]	T2 magnetic resonance	3–5	B: 0 V: 0 F: 5	N	91.1	99.4%
T2Bacteria [13, 14]	T2 magnetic resonance	4.4–5.5	B: 6 V: 0 F: 0	N	83	98%

B bacteria, *GP* Gram-positive, *GN* Gram-negative, *V* viruses, *F* fungi, *ID* identification, *AMR genes* antimicrobial resistance genes

One application of mNGS for BSIs has been to analyze circulating cell-free (cf)DNA directly in plasma, a specimen type thought to contain microbial nucleic acids released from organisms and/or lysed cells. The potential benefit of plasma testing is that it is less invasive than biopsy or endoscopy. Karius is a company focused on cfDNA for infectious diseases (Karius Inc., Redwood City, CA) [2, 24••]. This cfDNA test has been shown to detect pathogens as early as 2–5 days before bacteremia onset and to remain detectable for 3 days longer than blood culture [1], with a positive and negative agreement compared with traditional methods of 80% and 84%, respectively [26]. Additionally, a recent clinical and analytical validation of the test was performed using 2000 patient samples [24••]. Microbial cfDNA was detected in association with numerous infection types. However, differentiation of invasive pathogens from microbial DNA translocation from the gut or environmental/reagent contamination was problematic and will require clinicians to interpret the results with caution [24••].

Tests for Antimicrobial Susceptibility

Rapid knowledge of antimicrobial drug resistance (AMR) is crucial for optimal selection of effective antibiotic therapy. AMR is an important obstacle for transplant patients, whose prolonged antibiotic use and increased exposure to nosocomial pathogens place them at an increased risk for infections with multidrug-resistant organisms. Genomic testing for AMR can be applied to a variety of sample types or cultured isolates. In addition, resistance gene detection can often be completed well before traditional phenotypic antibiotic susceptibility testing is complete. The four FDA-approved multiplex

platforms for blood culture aliquots detect a small number of resistance determinants (Table 1) [8•, 9, 10–14]; neither T2 nor the Karius test includes resistance markers.

The interpretation of genotypic AMR testing can be complicated, especially when the results of the genotype and classical phenotype do not match. Discrepancies occur when genes conferring AMR are present but not fully expressed or are not targeted in a given assay. Additionally, genotypic testing does not allow for minimum inhibitory concentration (MIC) determination, which is useful for optimization of antimicrobial dosing. To circumvent these limitations, there has been significant focus on the development of more rapid phenotypic susceptibility test methods which generate MIC values. The Accelerate PhenoTest BC (Accelerate Diagnostics, Tucson, AZ) is currently the only FDA-approved option for rapid phenotype, although multiple other systems are in development. The Accelerate platform uses molecular probes to identify 14 bacteria and 2 *Candida* spp. from positive blood culture aliquots [27] followed by rapid MIC results in 5–7 h using single-cell growth measurement in the presence of drug [10]. The assay reports 96 possible organism-drug combinations [10]. A multicenter clinical trial comparing patient outcomes associated with rapid BSI organism identification (ID) with limited molecular resistance detection vs. rapid ID plus phenotype was recently completed with results expected soon (ClinicalTrials.gov Identifier: NCT03218397).

Clinical Outcome Studies Focused on Rapid BSI Diagnostics

Numerous outcome studies have compared the impact of rapid BSI testing to routine microbiological methods. Overall, the availability of more rapid results appears to have a positive impact on time to optimal treatment, LOS, and mortality [28, 29]. These benefits, however, were only realized when rapid testing was paired with an ASP intervention [28, 29]. Recent surveys suggest that institutional ASPs are utilized in approximately 76% of HSCT and 71% of SOT centers in the USA [30••, 31]. Whether rapid testing with ASP guidance can have the same impact on outcomes specifically in transplant populations has not been definitely established. Rates of ineffective empiric treatment, and opportunities for antibiotic escalation or de-escalation based on rapid test results, may be different across immunocompromised and competent populations. Additional studies are required to assess the cost-effectiveness of newer, more expensive diagnostics such as mNGS as well as rapid phenotypic susceptibility testing in immunocompromised patients specifically.

Respiratory Infections

Pneumonia is one of the most common infectious complications following transplantation. Multiplex PCR panels for respiratory viruses have been available for some time and have well-established utility for diagnosis [32], prognosis [33], and infection control in the transplant setting. Multiple viruses in addition to influenza A/B are potential causes of pneumonia in transplant recipients. As a result, routine use of molecular diagnostics for the simultaneous assessment of multiple respiratory viruses is endorsed by a variety of expert and professional immunocompromised host guidelines [34–37]. There are numerous FDA-approved options for multiplexed respiratory virus testing with some differences in panel configuration and test characteristics (Table 2) [8•, 38–43]. The most notable performance variabilities are sensitivity differences for the adenovirus and influenza targets [44]. More recent developments in the respiratory diagnostic space include commercially available PCR panels for the causative agents of bacterial pneumonia, early evaluations of mNGS testing using lower respiratory tract specimens, and point-of-care molecular tests for influenza and RSV.

Bacterial Pneumonia Panels

Currently, there are two FDA-cleared pneumonia panels, which include common bacterial causes of pneumonia combined with selected drug resistance determinants (Table 2). The Unyvero assay (Curetis AG, Holzgerlingen, Germany) identifies 19 bacteria and 10 resistance markers for use with tracheal aspirate specimens. The spectrum of organisms covered is expected to span approximately 90% of the most common causes of pneumonia in hospitalized patients [45–48]. Similarly, the FilmArray Pneumonia Panel (BioFire Diagnostics, Salt Lake City, UT) detects 17 bacteria, 9 viruses, and 7 antibiotic resistance genes for use with sputum, endotracheal aspirate, and bronchoalveolar lavage (BAL) samples [49, 50]. The FilmArray (FA) reports semiquantitative results in genome copies per milliliter for 14 of the 17 bacteria when they present at levels $\geq 10^4$. Genome copy values are calculated based on an internal standard material that is present at a known concentration. These values have been shown to correlate with, but are higher than, corresponding quantitative culture units [51].

There have yet to be any peer-reviewed publications evaluating the FDA-approved versions of current lower respiratory tract infection (LRTI) panels. However, a preclinical assessment of the Unyvero platform enrolled 49 patients with severe nosocomial pneumonia [45]. In all, 55.1% of subjects were PCR-positive, but only 8.2% were culture-positive. PCR-positive/culture-negative samples were more likely to have come from patients receiving antibiotics [45]. Additionally, several meeting abstracts have reported early experience with the FA system. Faron et al. observed a positive percent agreement (PPA) of 94.7% and 95.8% in BAL and sputum specimens, respectively, as compared with bacterial culture. This study pointed out that only 2 of 13 samples with a virus detected

Table 2 FDA-approved assays for respiratory infections

Platform	Assay run time (h)	Number of organisms	AMR genes	Sensitivity	Specificity
Luminex xTAG RVPv1 [8•]	8	V: 12 B: 0 F: 0	No	92.7%	99.8%
Luminex xTAG RVP Fast [8•]	6	V: 8 B: 0 F: 0	No	84.4%	99.9%
Luminex Magpix NxTAG RPP [8•]	4	B: 2 V: 18 F: 0	No	93%	93%
GenMark ePlex RPP [38, 39]	1.5	B: 2 V: 15 F: 0	No	85.1–95.1%	99.5–99.8%
BioFire FilmArray RP [8]	1	B: 2 V: 18	No	84.5%	100%
BioFire FilmArray PP [40, 41]	1	B: 18 V: 8 F: 0	mecA/C MREJ VIM OXA-48-like NDM KPC IMP CTX-M	BAL: 96.2% Sputum: 96.3%	BAL: 98.3% Sputum: 97.2%
Verigene RP *Flex* [42]	2-3	B: 3 V: 13 F: 0	No	79.2–100%	97.1–100%
GenMark eSensor RVP [8•]	7	B: 0 V: 14 F: 0	No	98.3%	99.2%
Unyvero Hospitalized Pneumonia [43]	5	B: 19 V: 0 F: 0	mecA TEM CTX-M OXA-48 OXA-58 VIM KPC NDM OXA-23 OXA-24	ID: 92.5% AST: 93%	ID: 97.4% AST: 98.8%

B bacteria, *V* viruses, *F* fungi, *AMR genes* antimicrobial resistance genes

by FA had a viral standard of care test ordered by the physician [49]. In theory, inclusion of viruses and bacteria in a single panel will minimize the chances that a significant pathogen is missed. Lannello et al. studied ICU patients with suspected ventilator-associated pneumonia [50]. The FA had a positivity rate of 79.4% compared with 65.8% with conventional culture [50]. Taken together, these initial observations suggest that PCR-based methods will detect more bacteria than culture. However, the inability of PCR to separate dead bacteria from viable ones will likely complicate the interpretation of LRTI panels in patients receiving antibiotics. Furthermore, neither semiquantitative PCR nor culture can separate commensals from invasive pathogens. This may be especially difficult in lung transplantation and intubated patients, where airway colonization is common. Ultimately, well-designed clinical trials are needed to understand the potential impact these new tests can have on informed antimicrobial usage and whether semiquantitative testing can help improve the PPV of bacterial detection.

Metagenomic Next-Generation Sequencing

mNGS also has potential clinical utility for diagnosing pneumonia. Despite extensive testing with available methods, current practice fails to identify a causative pathogen in a significant proportion of transplant-associated LRTI cases. The combination of RNA and DNA metagenomic sequencing theoretically allows detection of all types of organisms, and microbial RNA signatures may help differentiate active organism transcription from nonviable or latent infection. Measures of microbial diversity also may help distinguish colonization from active infection, with reduced diversity scores more likely to be observed in patients with active LRTI [52, 53].

A number of proof-of-concept retrospective mNGS studies have been conducted in immunocompromised patients. Langelier et al. applied this technology to the diagnoses of pneumonia in both the adult ICU [52] and HSCT settings [53]. In the ICU study, 45% (41/92) of subjects were immunocompromised, including 13 (14%) SOT recipients. Compared with conventional diagnostics, mNGS increased the rate of microbiological diagnosis for LRTIs (76% vs. 43%). Although the HSCT study was quite small (n = 22), mNGS confirmed all 6 pathogens identified by standard of care (1 bacterial, 5 viral), with 6 additional potential pathogens (2 bacterial, 4 viral) detected by mNGS only [53]. Young et al. used residual BAL specimens from 48 immunocompromised patients to compare mNGS yield to the current multi-test standard of care. The main limitation of mNGS assay in this report was failure to detect 5 of 6 PCR-positive *Pneumocystis jirovecii* cases [54]. Many mNGS assays involve chemical depletion steps to remove the high background of host DNA, which likely also has an effect on microbial DNA. Thus, well-designed DNA PCR is likely more sensitive than mNGS and is the test of choice when clinical suspicion for a given pathogen is high. In a separate study of 34 immunocompromised children, standard of care testing identified 17 likely pathogens and mNGS results were concordant in 11 of these. mNGS also detected additional potential pathogens in 11 of the 24 samples that tested negative by routine methods [55]. Interestingly, 14 samples contained low levels of *Aspergillus* RNA, even though only 1 patient was diagnosed with invasive pulmonary aspergillosis. These observations suggest that low-level airway colonization, or at least the presence of fungal nucleic acid, may be more common in an immunocompromised pediatric population than previously recognized [55]. In sum, available evidence suggests that mNGS may be a useful adjunct to the diagnosis of pneumonia, but currently, it cannot replace standard testing combining culture, antibiotic susceptibility testing, and targeted PCR.

Clinical Outcome Studies

The cost-effectiveness and clinical impact of molecular syndromic testing for respiratory viral infections is still debated [56]. The last decade has seen transition from classical immunoassays and culture to traditional PCR and now more rapid multiplex methods. A recent systematic review by Vos et al. calculated a pooled sensitivity of 90.9% and specificity of 96.1% for rapid molecular detection of influenza, RSV, and other viruses [57]. Overall, highest test sensitivity was observed in children and a lower sensitivity was seen using larger panels as compared with single or double target assays. Significant heterogeneity was noted across studies. Despite differences in study methods, multiple individual reports have observed potential for decreased LOS, less unnecessary antibiotic use, and wiser antiviral therapy when rapid NAAT is performed [57].

Gastrointestinal Infections

Diarrhea is a nonspecific, yet common, clinical syndrome in SOT and HSCT patients. Differentiating infectious diarrhea from noninfectious etiologies is impossible based on clinical signs and symptoms alone. In addition, prompt recognition of gastrointestinal (GI) pathogens with potential for serious complications and cross-transmission (e.g., *Clostridioides difficile* and norovirus) is crucial in transplant recipients [58, 59]. The most common causative organisms of infectious colitis after transplantation are *C. difficile*, norovirus, and cytomegalovirus (CMV) [58, 60, 61]. Adenovirus is another important opportunistic pathogen. Laboratory-developed targeted PCRs for these organisms have been available for some time, but recent studies show that physician directed ordering often misses potential pathogens, given that the breadth of microorganisms that can cause diarrhea is quite broad and symptoms are not pathogen-specific [62]. Furthermore, traditional stool culture and stool ova and parasite (O&P) examinations have low yield [58, 60, 63].

Diagnostic Yield of Multiplex Testing

The advent of multiplex molecular assays for gastrointestinal (GI) infections has revolutionized the diagnosis of infectious diarrhea. Currently, there are three FDA-approved GI PCR panels (Table 3) [8•, 64]. Huang et al. compared all three assays using 152 stool samples and concluded that all have high sensitivity and specificity, with only some minor performance differences by platform [64]. Reporting of detected organisms can be customized on two of the platforms, and some laboratories have chosen to suppress *C. difficile* results, particularly for young children who may simply be colonized [8•, 65, 66].

In general, molecular testing can be expected to increase pathogen detection by threefold relative to standard methods [58]. In one pediatric study of 378 diarrhea samples, 72 additional viral infections and 100 additional bacterial infections were observed with use of the GI FA in comparison to physician-driven testing [62]. Overall, 20% of the specimens were positive for at least 2 pathogens and 35% of *C. difficile*–positive stools had additional detections [62]. The increased detection rates of multiplex GI panels also come with some interpretation challenges. As previously discussed, PCR may detect simple colonizers, and immunocompromised patients can shed certain organisms such as norovirus or respiratory viruses, for weeks or months. The significance of polymicrobial detections can lead to confusion about which organism or organisms are the cause of a patients illness [67].

Additionally, false positive molecular results have resulted in pseudo-outbreaks in immunocompromised populations [68, 69].

Negative PCR testing may also be useful because it can help to rule out infectious diarrhea and allow clinicians to consider noninfectious etiologies [70]. However, panels do not include all potential GI pathogens relevant to an immunocompromised population. For example, *Aeromonas* is not targeted on any FDA-approved multiplex panel and only the GI FA detects *Plesiomonas* [8•]. Even when pathogens are on the panel, only a few species or subtypes may be targeted. Adenovirus is an important example of a molecularly and antigenically diverse group of viruses where a variety of subtypes may cause GI disease [71].

The ease of panel testing has the potential to promote overuse, especially for patients with hospital onset diarrhea. Multiplex testing has been shown to have low yield when tested more than 3 days into hospitalization [66, 72]. In one observational study, repeat testing within 4 weeks after an initially negative panel also resulted in only a handful of tests turning positive, while about a half of initially positive results remained positive with repeat testing, even after symptoms had resolved [72]. These are important observations for transplant medicine, where nosocomial diarrhea is common and prolonged nucleic acid shedding is expected. In our opinion, cost-effective use of panel-based GI testing is probably optimal for the evaluation of new-onset diarrhea at the time of hospital admission or as a part of an outpatient evaluation of acute or persistent diarrhea. For transplant centers with modular testing capabilities, in the absence of obvious exposures or travel history, testing for enteric viruses and *C. difficilile* followed by endoscopic evaluation to rule out tissue-invasive CMV disease may be the highest yield approach.

Impact on Infection Control and Public Health

One way to decrease hospital costs is to reduce the number of inpatient hospital days spent on isolation precautions. Although the use of the GI multiplex panels may increase the laboratory costs, faster comprehensive test results can translate into wiser isolation practices, as well as reduce unnecessary radiology examinations, antibiotics, and LOS [65]. One center calculated a cost savings of £66,765 by shortening average isolation time for 800 patients with diarrhea from 2202 to 1447 days using a rapid multiplex PCR platform [73]. Rand et al. also observed that 51.4% of patients with infectious diarrhea at their center were never placed on contact precaution isolation; however, this situation could also be rectified by stricter symptom-driven isolation practices [74]. Lastly, a panel-based approach to GI diagnostics has been shown to alert laboratories to the possibility of an outbreak. A recent example was a *Cyclospora* outbreak in Nebraska and Iowa [75]. Because *Cyclospora* detection requires use of specialized staining methods (e.g., modified acid-fast stain), it is often missed on routine O&P. Only after the outbreak was recognized by syndromic testing did clinicians start ordering the special stain [75].

Next-Generation Sequencing and the GI Microbiome

The advent of NGS technology has also facilitated our understanding of the gut microbiome composition and its role in several host functions including metabolism modulation, food digestion, and immune activation [76]. The gut microbiome composition is significantly altered by use of antimicrobial, immunosuppressive, or probiotic agents; myeloablative chemotherapy; and fecal microbiota transplantation [77, 78]. Distortion of the gut microbiome, also known as dysbiosis, has implications for disease progression, immune activation, and microbial translocation out of the gut. Loss of bacterial diversity and shifts in enteric bacterial communities from commensal to pathogenic bacteria impact graft vs. host disease, organ failure, onset of infection, and relapse/progression of disease after allo-HSCT [77, 79, 80]. Future efforts aimed at characterizing the degree of dysbiosis and its association with several clinical outcomes in transplant recipients represent an active area of research with potential diagnostic implications [81].

Table 3 FDA-approved assays for gastrointestinal infections

Platform	Assay run time (h)	Number of organisms	Sensitivity	Specificity
BioFire FilmArray [8•, 64]	1	B: 13 V: 5 P: 4	90.0–100%	97.9–100%
Luminex xTAG [8•, 64]	5	B: 8 V: 3 P: 3	79–100%	90.8–100%
Verigene Enteric Pathogens [8•, 64]	2	B: 7 V: 2 P: 0	71–95%	99–100%

B bacteria, *V* viruses, *P* parasites

Central Nervous System Infections

Central nervous system (CNS) infections including meningitis and encephalitis are life-threatening conditions, especially if diagnosed and/or treated late. Traditional testing includes Gram stain, cultures, targeted PCR, and various serologies. Despite extensive laboratory testing, approximately 62% of encephalitis cases go undiagnosed [82]. Cerebrospinal fluid (CSF) culture is notoriously insensitive, especially in patients who have received antibiotics prior to lumbar puncture. Furthermore, immunosuppressed patients may have misleading CSF cell counts due to underlying leukopenia; antibody-based testing may also be falsely negative [82, 83]. Given these limitations, combined with a broad differential diagnosis, panel-based NAAT represents an attractive option for the evaluation of suspected CNS infections.

The Meningitis/Encephalitis FilmArray (FA ME) panel is currently the only FDA-approved option for syndromic CNS testing, but other manufacturers have tests in development. The FA ME assay targets 6 bacteria, 7 viruses, and *Cryptococcus neoformans/gattii* [84], all of which can cause disease in immunocompromised patients. The clinical trial that supported FDA approval of the FA ME panel included 1560 residual CSF specimens from 11 different US institutions [84]. The standard of care comparator for this study was culture for bacteria and targeted singleplex PCR with DNA sequencing for viruses and yeast. Panel testing detected at least 1 pathogen in 136 of 1560 (8.7% specimens), but no confirmed cases of *Listeria* or *Neisseria* were included. Of the 43 FA ME detections that were negative by culture, only 21 (43%) were confirmed by singleplex PCR. These false positive detections were mostly *Streptococcus pneumoniae* and HSV-1, which accounted for 15.6% of all positive FA ME results in the trial [84].

Since FDA approval, numerous studies have reported benefits of a panel-based approach to suspected CNS infection, namely, reduced time to appropriate antimicrobial therapy, shorter LOS, decreased antibiotic use, and increased parechovirus detections in children [85, 86].

Impact of False positive and False negative Results

Panel-based testing also has some potential limitations. False positive results have been reported with the FA ME panel, which may cause clinicians to forgo further diagnostic efforts in search for other infectious etiologies [87]. False positives potentially arise from pre-analytical sample contamination with microbial DNA shed from the upper respiratory tract from the person collecting or handling the sample (e.g., *S. pneumoniae*). Appropriate containment and personal protective equipment is essential for specimen collection and processing. In addition, sensitive PCR assays can detect latent viruses in CSF leukocytes. All herpesviruses, for example, establish latency which means that ME FA detections could represent dormant virus, asymptomatic reactivation, or active infection that is causing disease.

There have been several case reports illustrating the impact of false positive and/or latent virus FA ME detections [87–89]. In one report, an initial false positive HSV-1 detection substantially delayed diagnosis of *Mycobacterium tuberculosis* meningitis [88]. In another case, a HHV-6 detection combined with a false negative *Cryptococcus* result significantly delayed diagnosis of cryptococcal meningitis [87]. Establishing the clinical significance of HHV-6 detection in the CSF of an immunocompromised host is difficult because asymptomatic reactivations are common, especially after allo-HSCT [90, 91]. Routine screening and/or prophylaxis for HHV-6 are not recommended in transplant recipients [90, 91]. CSF HHV-6 testing is best used in the evaluation of limbic encephalitis, the one clinical entity that has been clearly associated with HHV-6 after allo-HSCT. High levels of HHV-6 in blood and/or CSF should prompt consideration of chromosomal germline integration as opposed to invasive infection [90, 91]. It should also be emphasized that PCR testing for *Cryptococcus* detection is less sensitive than cryptococcal antigen (CrAg) testing. Newer CrAg lateral flow assays are easy, quick, cheap, and sensitive (IMMY, Norman, OK) [92]. Thus, CrAg testing remains the diagnostic standard for rapid diagnosis of cryptococcal meningitis.

Metagenomic Next-Generation Sequencing for CNS Infections

Selective case reports have highlighted the utility of unbiased mNGS for the etiologic diagnosis of subacute and chronic meningoencephalitis. In many of the published series to date, mNGS picked up pathogens known to cause CNS disease (that were not specifically looked for with routine tests) or rarely identified unusual/unexpected organisms [93–96]. More recently, a prospective multicenter study enrolled 204 hospitalized patients with idiopathic meningitis, encephalitis, or myelitis who had not received a definitive diagnosis at the time of enrollment. mNGS was performed on CSF and compared with conventional testing. Retrospective orthogonal confirmatory testing was used in an attempt to confirm detections made by mNGS only. Out of 58 infections, 19 (33%) were diagnosed by both conventional testing and mNGS, while 26/58 (45%) were detected by conventional testing (often serology) only. mNGS identified 13/58 (22%) infections that were not detected by conventional methods [97••]. Pathogens only detected by mNGS included St. Louis encephalitis virus, hepatitis E virus, and *Streptococcus agalactiae*. In all, mNGS results prompted a change in clinical management in 8/204 (3.9%) patients [97••].

Given the wide availability, relatively low cost, and potentially rapid TAT, targeted PCR will remain the recommended

frontline diagnostics for the foreseeable future. mNGS cannot replace cultures or serologic testing for entities like West Nile virus and syphilis. Timely application of mNGS may, however, guide earlier diagnosis of less common or emergent organisms, and it should be considered when the results of routine testing are negative and the clinical suspicion for infection remains high.

Molecular Diagnostics for Invasive Fungal Infections

Invasive fungal infection (IFI) disproportionally affects patients with SOT and HSCT and those with hematological malignancy receiving myelosuppressive or biological therapy [98, 99]. Invasive aspergillosis (IA) and candidemia (IC) are the most common fungal infections in transplant recipients [100, 101]. However, recent epidemiological studies have highlighted the rise of non-*Aspergillus* mold infections (i.e., *Mucorales*, *Fusarium* spp., *Scedosporium* spp.), while multidrug-resistant *Candida* spp. (e.g., *C. auris*) have emerged worldwide [102, 103]. The global emergence of antifungal resistance among *Candida* spp. and *Aspergillus* spp. poses a threat for effective therapy and infection control among transplant recipients [104•, 105]. Timely and accurate identification of pathogenic fungi, coupled with early initiation of antifungal therapy, represent the most critical factors for improving survival [106]. Conventional microbiological methods (e.g., culture and histopathology) are inherently insensitive, time-consuming and often require obtaining deep tissue samples through invasive procedures [107]. Conversely, culture-independent molecular methods enable rapid fungal identification and shorten the time to diagnosis [108, 109]. In recent years, newer fungal diagnostic tools have been introduced into clinical practice, and several others are undergoing clinical studies [110, 111].

Molecular Diagnosis of Invasive Aspergillosis and Mucormycosis

Molecular methods for the detection and identification of filamentous fungi have been optimized and validated for a variety of different specimen types, but none have been FDA-approved in the USA. The European *Aspergillus* PCR initiative (EAPCRI) has been integral to the standardization of blood testing, and this work has been translated into reproducible test performance [112–114]. The optimal blood fraction (i.e., serum, plasma, or whole blood) for testing, however, remains undefined. Overall, *Aspergillus* blood PCR has moderate diagnostic accuracy (70–80% sensitivity) when used as stand-alone test for preemptive screening in high-risk hematology patients [111, 115, 116]. Clinical sensitivity and negative predictive value may be improved by combining *Aspergillus* PCR with fungal cell wall antigen tests [117–120]. Therefore, transplant centers adopting preemptive treatment strategies for IA should consider serial surveillance with both methods.

In contrast to blood testing, the optimal assay design specifications for BAL samples have not been established and considerable variation in *Aspergillus* PCR methods exist [121•]. Studies including neutropenic patients with lung infiltrates report a pooled sensitivity of 79–91% and specificity of 92–94% [122, 123] for *Aspergillus* PCR. The combination of *Aspergillus* PCR plus BAL-galactomannan (GM) seems to improve specificity and PPV [124, 125], but can be negatively impacted by anti-mold therapy [126]. Neither PCR nor GM can separate invasive airway pathogens from colonizers or environmental contamination [127]. Thus, these tests should be viewed as "detection tests" rather than diagnostic of invasive disease. Fungal diagnostics need to be interpreted cautiously in the context of host-risk factors and radiographic imaging. Lastly, molecular detection of azole resistance in *Aspergillus* spp. isolates directly from clinical samples is a field of active study [128]. Commercially available assays like AsperGenius® (PathoNostics BV, Maastricht, The Netherlands) [129] and laboratory-developed tests can detect *Aspergillus fumigatus* isolates harboring azole-resistance mutations.

Early diagnosis of invasive mucormycosis is a crucial step to mitigate its high morbidity and mortality in transplant recipients. Millon et al. described the use of a multi-species (*Mucor/Rhizopus*, *Rhizomucor*, and *Lichtheimia*) qPCR assay for detection of circulating mucorales DNA in serum, showing high sensitivity (90%) and earlier detection (by 3–68 days) compared with histopathological diagnosis [130]. Subsequent studies in critically ill burn patients and other immunocompromised hosts have corroborated the role of mucorales qPCR in serum for early diagnosis and treatment [131, 132•]. Preemptive detection of circulating mucorales DNA is an innovative approach that could potentially lead to a paradigm change in the care for transplant patients. However, larger studies are needed to evaluate the role of serum mucorales qPCR guiding early antifungal therapy and may be most useful at centers with a relatively high mucormycosis incidence or during ongoing outbreaks. Mucorales PCRs have also been described using BAL as well as fresh and formalin-fixed paraffin-embedded (FFPE) tissues with variable diagnostic accuracies [131, 133–135].

Panfungal Approaches

Histopathological evidence of invasive fungal elements in tissue remains the diagnostic goal standard for IFI. Unfortunately, histopathology cannot identify fungi to genus and/or species level, which is essential for empiric selection of antifungal therapy. Panfungal PCR followed by DNA

sequencing may help to establish a species-level identification. Common genomic targets for panfungal sequencing include the multicopy ribosomal RNA (rRNA) genomic locus (18S, internal transcribed spacers 1 and 2 (ITS1 and ITS2) and D1 and D2 regions of 28S rRNA) [136]. In the US, several reference laboratories and academic medical centers offer panfungal sequencing from body fluids and tissues. Diagnostic performance of these assays seems to depend on the amount of tissue, specimen type, and fungal burden available for testing [137••, 138, 139].

Detection of fungal cfDNA in plasma using mNGS technology may also have utility for detecting fungi [24••, 140]. Early experience with the Karius test is limited to small case series reports [1]. Results from ongoing larger clinical studies are anxiously awaited (DISCOVER trial, NCT02804464).

Conclusions

Molecular methods for infectious diseases continue to evolve. NAAT has clear benefits relative to conventional methods including improved analytical test performance and faster TAT. These are desirable attributes when caring for immunocompromised transplant patients. However, NAAT has limitations and transplant infectious disease clinicians need to be aware of the nuances of test interpretation. In addition, NAAT can not replace traditional culture; and serologies/antigen detection remain essential for several pathogens. Adoption and implementation of molecular diagnostic in the clinical laboratory also require a meticulous evaluation of workflow, cost, and opportunities for stewardship. Studies that assess the clinical impact of newer assays with measurable outcomes such as LOS, time-to-optimal treatment, and cost-effectiveness are needed.

Compliance with Ethical Standards

Conflict of Interest Brittany A. Young and Carlos A. Gomez declare that they have no conflict of interest.

Kimberly E. Hanson has served as a consultant for BioFire and T2 Diagnostics and received honoraria for this work.

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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