Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Diagnostic Molecular Microbiology: A 2018 Snapshot

Marilynn Ransom Fairfax, MD, PhD, Martin H. Bluth, MD, PhD, Hossein Salimnia, PhD, D(ABMM)

INTRODUCTION

When Kary Mullis developed the polymerase chain reaction (PCR) in 1983, its potential benefits were obvious to clinical microbiologists: faster, cheaper, more accurate detection and enumeration of all organisms in a specimen, without waiting for a culture. The discipline of infectious disease also sought the opportunity for simultaneous antimicrobial susceptibility testing. These dreams have slowly matured into realities. Multiplex arrays are approved or in development for the diagnosis of respiratory and gastrointestinal infections direct from patient specimens with results obtained in under an hour. An array was cleared by the US Food and Drug Administration (FDA) in August, 2013 that can detect common bacterial and fungal agents of bloodstream infections, as well as several important antibiotic-resistant genes, within about an hour after the culture bottle turns positive. Approaches are being made to organism identification and susceptibility testing directly from a blood sample without the

KEYWORDS

- Molecular microbiology
- PCR
- Probe tests
- Rapid molecular diagnosis of infections
- MALDI-TOF
- Nuclear magnetic resonance
- Next gen sequencing
- Time lapsed imaging

KEY POINTS

- Molecular biological techniques have evolved expeditiously and in turn have been applied to the detection of infectious disease.
- Maturation of these technologies and their coupling with technological advancements have afforded clinical medicine additional tools toward expedient identification of infectious organisms at concentrations and sensitivities previously unattainable.
- These advancements have been adapted in select settings toward addressing clinical demands for more timely and effective patient management.

INTRODUCTION

When Kary Mullis developed the polymerase chain reaction (PCR) in 1983, its potential benefits were obvious to clinical microbiologists: faster, cheaper, more accurate detection and enumeration of all organisms in a specimen, without waiting for a culture. The discipline of infectious disease also sought the opportunity for simultaneous antimicrobial susceptibility testing. These dreams have slowly matured into realities. Multiplex arrays are approved or in development for the diagnosis of respiratory and gastrointestinal infections direct from patient specimens with results obtained in under an hour. An array was cleared by the US Food and Drug Administration (FDA) in August, 2013 that can detect common bacterial and fungal agents of bloodstream infections, as well as several important antibiotic-resistant genes, within about an hour after the culture bottle turns positive. Approaches are being made to organism identification and susceptibility testing directly from a blood sample without the

a Department of Pathology, Wayne State University School of Medicine, 540 East Canfield Street, Detroit, MI 48201, USA; b Clinical Microbiology Laboratories, DMC University Laboratories, 4201 St. Antoine Street, Detroit, MI 48201, USA; c Pathology Laboratories, Michigan Surgical Hospital, 21230 Dequindre Road, Warren, MI 48091, USA
* Corresponding author.
E-mail address: mfairfax@dmc.org

Clin Lab Med 38 (2018) 253–276
https://doi.org/10.1016/j.cll.2018.02.004
0272-2712/© 2018 Elsevier Inc. All rights reserved.
necessity for culture. Microbiology lines are available, starting with automated plate streakers and ending with molecular identification of organisms grown on solid media. Despite these molecular and technological advancements, humans must still view the culture plates, perhaps on a television screen, and select colonies to analyze.

Furthermore, although cost containment is of paramount importance in today’s medical marketplace, “cheaper” is an ambiguous target. Microbiology laboratories are diagnostic facilities that drive subsequent therapy. Increased laboratory costs for more rapid microbial identification have been shown to result in the earlier use of appropriate antibiotics, shorter durations of hospital stay, better outcomes, and decreasing overall health care costs.\textsuperscript{1–3}

The diagnosis of persistent human papilloma virus (HPV) infections followed by appropriate therapeutic interventions should decrease the incidence of cervical carcinomas, the cost of treatment, and the attributable morbidity and mortality.

New technologies have enabled microbiologic investigations that were not included in our original diagnostic approaches. Next-generation sequencing (NGS) can detect and quantify populations of organisms in patient specimens. This raises the possibility of distinguishing pathogenic organisms, present in high numbers, from colonizers that are generally presumed to be present in lower numbers. Certain colonic organism profiles seem to correlate with the development of cardiovascular disease.\textsuperscript{4} A patient’s colonic flora could be analyzed, and if the profile were unfavorable, the bacteria could be eradicated and replaced.

Tests in use in 2018 have evolved significantly from those cited in our 2013 review\textsuperscript{5} and will continue to do so. Thus, this article is a snapshot of rapidly changing diagnostic microbiology laboratory techniques and its clinical applications. Emphasis has been placed on tests with high market share in diagnostic microbiology and on those with technologies that are personally regarded by the authors as particularly interesting. The role of specimen processing in concentrating nucleic acid targets and removing inhibitors of amplification is largely neglected, despite its important role in the sensitivity of the assay. However, many new procedures are automated and include specimen processing as part of a hands-off procedure. Most techniques mentioned here involve real-time PCR (RT-PCR), unless otherwise specified. Because most RT-PCR platforms are closed systems, they decrease the incidence of amplicon contamination in the laboratory, and have allowed many nucleic acid amplification techniques to become commercially available. The authors have also attempted to select current citations to support salient points, and these selections are arbitrary. Failure to mention a publication, technique, or trade name should not be construed as denigrating that article, technique, or manufacturer.

**PROBE TECHNIQUES**

The first molecular diagnostic tests approved by the FDA were probe techniques. Many probe tests are still in wide use today because they fill important niches. Some involve novel detection methodologies.

**Hybridization Protection Assays**

Among the first FDA-approved molecular tests were the Gen-Probe ([San Diego, CA], which became a wholly owned subsidiary of Hologic [Bedford, MA] in 2012). Pace 2 probe hybridization protection techniques are used for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from patient specimens. They have been largely replaced by more sensitive amplification tests. A number of their AccuProbe culture confirmation tests remain available. Among the most useful are *Mycobacterium*
tuberculosis complex, Mycobacterium avium, Mycobacterium intracellulare (separately or together), Mycobacterium kansasii, and Mycobacterium gordoni. These and other tests available from the same manufacturer all use most of the same reagents and instrumentation, which facilitates the use of multiple assays in the same laboratory.

These tests succeed because they target ribosomal RNA (rRNA), which is present in up to $10^5$ copies per organism. In bacterial ribosomes, there are common sequences, as well as genus-specific and species-specific sequences. The culture confirmation tests remain viable because they are intended to detect organisms in visible colonies or in liquid medium with detectable growth. Thus, 2 amplification steps have already been performed by nature. Sensitivities reported in the Hologic/Gen-Probe package inserts range from 98% to 100%.

At development, these assays were novel; they were nonradioactive and performed totally in solution, with 1 sample transfer step and no nucleic acid extraction. The hybridization protection assays are based on the differential sensitivity of the acridinium ester, which is used to distinguish the relatively labile ester on nonhybridized probes from the more stable form in DNA–RNA hybrids. This detection system is also used in this manufacturer’s nucleic acid amplification tests (NAATs). All the AccuProbe assays are similar, but the nucleic acid release steps are variable, depending on the ease of disruption of the organism.

The mycobacterial probe tests are particularly valuable when used in conjunction with liquid medium or 7H11 thin-plate techniques used for the rapid detection and identification of mycobacteria required by the College of American Pathologists (Northfield, IL). Thin-plate colonies can be probed the day they are detected, and their morphology can be used as a guide to selection of the appropriate probe. This represents significant time and money savings, although it does not eliminate the need to grow M tuberculosis for susceptibility testing. Cultures in Middlebrook 7H9 broth must be AFB stained to confirm the presence of mycobacteria and then probed with all 4 probes. The probe techniques described are also used to detect amplification products in amplification assays developed by GenProbe/Hologic for sexually transmitted diseases and blood bank testing (discussed elsewhere in this article).

**Hybrid capture technique**

Since the 1940s, Pap smears have contributed to great advances in the prevention and diagnosis of cervical carcinoma. In the 1980s and 1990s, it was recognized that infection with HPV is necessary but not sufficient for the development of cervical carcinoma. More than 100 HPV genotypes exist, of which 13 are associated with a high risk of cervical carcinoma (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). More than 70% of sexually active women become infected with HPV. Most infections, even with high-risk organisms, resolve without apparent sequelae. Why others progress is still unknown. The Digene Hybrid Capture 2 (hc2; Qiagen, Gaithersburg, MD) is FDA approved for primary screening, and to determine whether women with atypical squamous cells of undetermined significance should be subjected to culposcopy. It may be used with the Cytom PreservCyt Solution for the Cytom ThinPrep Pap Test (Hologic). In the first step, the patient specimen is allowed to react with a pool of RNA probes designed to hybridize specifically to the DNA of high-risk HPV strains. Antibody to RNA/DNA hybrids coats the wells of a microtiter plate and captures any hybrids. After washing away unbound specimen and reagents, detector antihybrid antibodies, each conjugated with multiple molecules of alkaline phosphatase, bind to each capture target, thereby amplifying the signal. A colorless substrate for the alkaline phosphatase is added and chemiluminescence develops proportional to the amount of second antibody bound.
More sensitive tests, typically involving target amplification, have been devised, but most clinical outcomes data are available with hc2. The need for more sensitivity has been questioned, because hc2 has already been shown to be sensitive, but not highly specific, for the development of cervical intraepithelial neoplasia or overt malignancy. The presence of high-risk DNA below the detection limit of hc2 seems to be associated with a low risk for malignancy. However, new information suggests that infections acquired in the early years of sexual activity may reactivate with aging. If this is supported by further studies, more sensitive assays might be indicated. There is also a suggestion that unusual HPV strains may cause cervical carcinoma or precursor lesions in restricted populations. Thus, the strains included in the assay may need periodic review. Amplification techniques are now available which can distinguish HPV-16 and HPV-18 from the other high-risk organisms, which has facilitated targeted follow-up algorithms, and outcomes data are increasing so that this unique probe technique is becoming less common. The use of hybrid capture as the basis a detection system in amplification assays is still possible, and used in some assays; however, it is not considered a method with high sensitivity.

Peptide nucleic acid fluorescence in situ hybridization
Peptide nucleic acid fluorescence in situ hybridization (PNA-FISH; AdvanDx, Inc, Woburn, MA, now owned by OpGen) accelerated the diagnosis of sepsis. Common agents of sepsis can be identified in about an hour after the blood culture bottle has been gram stained. Although behaving like a standard FISH assay, the PNA-FISH probes consist of an uncharged peptide backbone to which the bases are attached. This is thought to allow the probes to enter the permeabilized bacterial cell more easily and then bind more tightly to the negatively charged rRNA target. Numerous publications confirm that the use of this technique for rapid identification of the common organisms growing in the blood culture bottles improves antibiotic stewardship and shortens length of stay. Depending on the patient, identification of a coagulase-negative *Staphylococcus* may facilitate discontinuation of antibiotics and early discharge.

Our high-throughput laboratory (>200 blood cultures per day) was an early adapter of the staphylococcal, enterococcal, and candida probes, although we do not currently use those for bacteria owing to the availability of more sensitive platforms with the ability to detect a great number of pathogens involved in bacteremia and sepsis. Multiple PNA-FISH assays received FDA clearance, including the assay for the detection of *Staphylococcus aureus/coagulase-negative Staphylococcus*, *Enterococcus fecalis/E faceium*, gram-negative traffic light (identification of *E coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and yeast traffic light (identification of *Candida albicans*, *C krusei*, *C tropicalis*, *C glabrata*, and *C parapsilosis*). Our laboratory has been involved in clinical trials of AdvanDx PNA-FISH products and our data showed high sensitivity and specificity of these products as well as the ease of use. Although the popularity of PNA-FISH as a diagnostic test in large laboratories is declining, the FISH technique is being used by Accelerate Diagnostics (Tucson, AZ) to facilitate organism identification in positive blood culture bottles.

Affirm VPIII microbial identification test The VPIII probe test (Becton Dickinson, Franklin Lakes, NJ) is intended for the diagnosis of vaginitis/vaginosis, conditions causing millions of physician visits annually. Affirm detects rRNA from *Gardnerella vaginalis*, used as an indicator for bacterial vaginosis (BV), *C albicans*, and *Trichomonas vaginalis*. The sensitivity has been adjusted to avoid giving positive results with low concentrations of *G vaginalis* and *C albicans*, which often colonize the normal vagina using a lateral flow immunochromatographic enzyme assay. This assay maintains low
sensitivity (requiring about 5000 copies of trichomonas nucleic acid to give a positive result), which may be ideal for Gardnerella but bad for Trichomonas species. It is formatted to be performed in a cassette superficially resembling the lateral flow tests used for the serologic detection of influenza or rotavirus antigens and was the first FDA-approved molecular test based on lateral flow. The methodology for lateral flow is as follows: after collection on a proprietary swab, the specimen is lysed to release the nucleic acids, buffered to stabilize the nucleic acids and establish stringency, and added to the cassette, which is incubated at the proper temperature for nucleic acid hybridization. The cassette contains 5 “beads,” each coupled to a capture probe: 1 for each of the 3 analytes, plus positive and negative controls. Next, enzyme-linked detector probes bind to specific sequences on the target organism’s rRNA. Unbound sample components and probes are washed away. A colorless substrate is converted to a blue product if sufficient target and detector probe have bound. A blue “bead” indicates a positive result.

Numerous publications have revealed that health care providers are significantly less accurate than VPIII for the diagnosis of significant candidiasis and T vaginalis. The Affirm test has highlighted the fact that multiple infections are common. The role of the VPIII in the diagnosis of BV is the subject of debate, however, because the use of G vaginalis alone as an indicator of BV is not optimal. In addition, there have been criticisms of the limit of detection (LOD) of the assay, particularly for T vaginalis, and of the inability of the assay to highlight the presence of drug-resistant Candida spp. Automated NAAT assays (including the MAX Vaginal Panel assay by Becton Dickinson) resolve many of these problems. Becton Dickinson now suggests that the Affirm assay may be appropriate for smaller laboratories, whereas larger laboratories may wish to transition to its NAAT assay.

The Becton Dickinson MAX Vaginal Panel is the first FDA cleared, microbiome-based, PCR assay that directly detects the 3 most common infectious causes of vaginitis: BV, vulvovaginal candidiasis, and trichomoniasis. The MAX Vaginal Panel performed on the Becton Dickinson Max instrument is fully automated and diagnoses BV using a unique algorithm that quantitates the presence of Lactobacillus spp (good bacteria) as compared with G vaginalis, Atopobium vaginae, BV-associated bacteria, and Megasphaerae (BV-associated bacteria). It separately determines the presence of T vaginalis, as well as the presence of the Candida group (albicans, tropicalis, parapsilosis, and dublinensis), and of C glabrata and C krusei, which are more antibiotic resistant.

** Advances in Sexually Transmitted Disease Testing **

Culture, immunoassay, and probe tests for both N gonorrhoeae also known as Gonococci and C trachomatis are less sensitive than NAATs, and NAATs have been the standard of care since the late 1990s, although certain jurisdictions still require culture for legal cases. In this text N gonorrhoeae and Gonococci are used interchangeably.

C trachomatis and gonococcal infections of the female genital tract present a diagnostic challenge because many patients with C trachomatis and Gonococci are asymptomatic. Sexually transmitted diseases are becoming more prevalent even in the United States and Europe. This has led to the implementation of widespread screening, using urine or self-collected swabs, collection procedures with increased patient acceptance. All of these tests are also available for physician-collected vaginal swabs and for male urethral swabs and urines.

Commercial qualitative NAATs for diagnosis of C trachomatis and Gonococci have been available for about 20 years. The first PCR assay, the semiautomated Amplicor C trachomatis/N gonorrhoeae test, was developed by Roche (Basel, Switzerland). It was closely followed by the Abbott LCX assay (Abbott Laboratories, Chicago, IL) for the
simultaneous detection of *C. trachomatis* and *N. gonorrhoeae*, which used ligase chain reaction technology to amplify the targets. In 2003, the Abbott LCX assay for *C. trachomatis/Gonococci* was withdrawn abruptly from the market. In the late 1990s, other amplification assays became available, a strand-displacement amplification assay from Becton Dickinson, the ProbeTec ET, and a transcription-mediated amplification test from GenProbe were introduced. Both of these evolved over the years and additional tests were also FDA approved. Other testing approaches include the Becton Dickinson ProbeTec *C. trachomatis/Gonococci Qx* assay performed on the Viper XTR, Hologic/Gen-Probe Aptima Combo 2 assay performed on both the Panther and Tigris instruments, the Abbott Real-Time *C. trachomatis/N. gonorrhoeae* assay, and the Roche Diagnostics Cobas *C. trachomatis/N. gonorrhoeae* assay run on the Cobas 4800 instrument. A current problem with all molecular diagnostic tests for *C. trachomatis/Gonococci* is the inability to test for antibiotic resistance. *N. gonorrhoeae* has inexorably developed resistance to each antibiotic regimen that became widely used, and the question of antimicrobial resistance in *C. trachomatis* has hardly been considered, although treatment failures are well-known. Because the transport systems for *C. trachomatis* and *Gonococci* are not compatible with culture and susceptibility testing, it seems inevitable that molecular susceptibility testing must be developed, but we are unaware of any such development.

**Hologic/Gen-Probe Aptima Combo 2 assay**
This assay is performed on either the Tigris or the Panther instruments. It targets a region of the 23S rRNA of *C. trachomatis* and one from the 16S rRNA from *Gonococci*. They are amplified via DNA intermediates by using transcription-mediated amplification, a technique that uses both a reverse transcriptase enzyme and a phage-derived RNA-dependent RNA polymerase. The detection of the amplicons is achieved using the hybridization protection assay described herein in the section on probe tests. Briefly, single-strand chemiluminescent DNA probes bind to their complementary target, protecting the labile acridinium ester. After hydrolysis of the esters on unbound probes, light emitted from the ester that had been protected within the target-probe hybrids is measured and reported as relative light units. Assay results are determined by a cutoff based on the total relative light units and the curve type.19,20

**Becton Dickinson ProbeTec *C. trachomatis/Gonococci Qx* assay on the Viper XTR**
This assay uses the isothermal DNA nick translation technique called strand-displacement amplification for the detection of target sequences in the patient sample. It targets a sequence in the cryptic *C. trachomatis* plasmid and a sequence of *Gonococci* genomic DNA, respectively. Extraction of DNA from clinical samples is based on pH-dependent binding of the DNA to ferric oxide particles followed by binding of the combination to paramagnetic beads. After removing the contaminants, the purified DNA is released and amplified by real-time strand-displacement amplification in the presence of a fluorescently labeled detector probe. The presence of the target DNA is determined by comparing the peak fluorescence with a cutoff value. Many articles have discussed the performance of this assay to detect the targets in clinical specimens, such as urine or vaginal/cervical samples. This assay is not evaluated for pregnant women as well as patients younger than 18 years of age.21–23

**The Abbott RealTime *C. trachomatis/N. gonorrhoeae* assay**
This RT-PCR assay targets 2 sequences within the cryptic plasmid of *C. trachomatis*. This plasmid is found in all serovars of *Chlamydia* to date. The dual targets allow detection of both the wild-type *C. trachomatis* and the variant, which has a deletion in one of the target sequences. The *Gonococci* target is found within the
organism’s Opa gene. Cheng and colleagues evaluated the Abbott RealTime C trachomatis/Gonococci assay in comparison with the Roche Cobas Amplicor C trachomatis/N gonorrhoeae assay. They showed high agreement between the 2 assay. However, their data demonstrated that the Abbott assay was more sensitive than the Roche assay for both C trachomatis and N gonorrhoeae with enhanced ability to detect dual infections.

Roche Cobas 4800 instrument and C trachomatis/N gonorrhoeae assay

This multiplex PCR assay simultaneously detects 2 independent DNA targets for C trachomatis, one in the cryptic plasmid and the other on the C trachomatis genome. This assay can detect infections caused by the wild-type C trachomatis, in addition to other Chlamydia strains that may have deletions in the cryptic plasmid, or those that might not carry the cryptic plasmid at all. DR-9, a direct repeat region, is the target of the N gonorrhoeae assay. The use of this target makes the assay highly specific to N gonorrhoeae. This target does not cross-react with sequences found in commensal Neisseria, a feature that hampers the use of some other Gonococci assays for oropharyngeal and rectal samples.

Chernesky and colleagues performed a direct comparison of 4 second-generation C trachomatis/Gonococci assays to obtain information about their relative sensitivities and specificities. They used both first void urine and self-collected vaginal swabs. The sensitivities for C trachomatis using self-collected swabs were: Aptima Combo 2 run on the Tigris, 98.1%, and on the Panther, 96.2% (Hologic/GenProbe): RealTime assay on the m2000, 98.0% (Abbott): ProbeTec Qx assay on the Viper, 90.6% (Becton Dickinson), and the Cobas assay tested on the Cobas 4800, 84.6% (Roche).

Other qualitative assays

Hologic-Gen-Probe manufactures several widely used qualitative, transcription-mediated amplification-based microbiology tests; included among these are a direct test for M tuberculosis and the Procleix Ultrio Plus assay (Ultrio), which has also been licensed to Novartis (Emoryville, CA). The first assay (without the Plus) has long been approved for detection of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in specimens from blood donors and from organ donors, both living and deceased, but is not intended for the diagnostic workup of the diseases in the general population. Testing a pool of samples from up to 16 blood donor units is approved for blood bank testing. Recently, the FDA approved the inclusion of hepatitis B virus in this assay, and it has been renamed Ultrio Plus. It contains several HIV targets that allow it to detect HIV-1 (several strains) and HIV-2. Because test results are generally negative and high sensitivity is necessary, the inclusion of an internal control is essential. Components of positive pools are retested individually and then by assays for each individual analyte.

Other published data contain a listing of more than 135 published RT-PCR assays developed for the detection of 32 species of bacteria. This list does not include multiplexed tests or assays for viruses. The publication acknowledged that the list is incomplete and commented that most are laboratory-developed tests, with only 35 (counted from the list) being commercially available, whether FDA approved or not. This finding emphasizes the need for more rapid commercial development and FDA approval methods.

Although cyber green, a nonspecific detector, is still used occasionally, 3 main molecular detection systems are used: dual hybridization (fluorescent resonance energy transfer), TaqMan, and molecular beacon.

The fluorescent resonance energy transfer detector consists of 2 different probes, complementary to adjacent sequences on the target amplicon, and each attached
to a different fluorophore. When activated by incident ultraviolet light, the first fluorophore emits energy at an unmonitored wavelength. If the second probe is bound adjacent to it, the energy is transferred to the second fluorophore, which then emits light at the wavelength monitored by the sensor. At the end of the PCR assay, a melting curve for the amplicon–probe complex can be generated. The melting temperature is characteristic of each amplicon–probe combination. If there is a mismatch between the probe and the amplicon, the melting temperature decreases. The melting temperature difference has been exploited in a test that distinguishes between herpes simplex 1 and 2 (Roche). The PCR primers bind sequences common to both viruses within the HSV DNA polymerase gene. The amplicon detector probes match the HSV-2 sequence, which differs from that of HSV-1 by 2 bp. Melting curve analysis reveals a reproducible melting temperature for HSV-2 that is about 10°C higher than that exhibited by HSV-1. Occasional mutant HSV strains have been detected that have intermediate melting temperatures. These tests can be reported as positive for HSV, but the type is not clear. Types 1 and 2 could be distinguished by sequencing the amplicons, by using an assay using a different target sequence, or by the melting temperature of the amplicon, which is said to be 0.9°C higher for the HSV-2 variant than the type 1 variant.

The MultiCode RTx system uses an unusual PCR amplification technique in which no detector probe is used and in which the fluorescence actually decreases as amplification progresses. It was developed by EraGen BioSciences, Inc (Madison, WI; acquired by Luminex Corporation [Austin, TX] in 2011). Their MultiCode-RTx herpes simplex virus 1 and 2 kit was FDA approved in 2011. MultiCode RTx assays use 2 unusual nucleotide bases iC (2-deoxy-5-methylisocytidine) and iG (2-deoxyisoguanosine) that base pair only to one another and that are efficiently incorporated into PCR products. iG is put at the 5' end of the RTx primers along with its linked fluorophore. The reaction mix contains iC covalently linked to a quencher. In the initial cycle amplification, the iG, with its attached fluorophore, appears at the 5' end of the primer and hence at the 5' end of the nascent amplicon strands. When these serve as templates for copying in the other direction, an iC, with its attached quencher is added to the 3' end of the new strand, opposite the iG and its fluorophore. The approximation of fluorophore and quencher decreases the fluorescence. In melting curve analysis, fluorescence increases as the amplicons melt, and the melting temperature allows determination of the nature of the analyte.

QUANTITATIVE TECHNIQUES

Quantitative PCR in clinical microbiology are confined mainly to NAATs used for HIV viral load testing, although there are also FDA-approved quantitative RT-PCR assays for HCV, hepatitis B virus, and recently for cytomegalovirus. The trend is to make the assays referable to a World Health Organization international standard. Quantitative techniques have advanced significantly since the early days when Alice Huang first demonstrated that quantitative PCR was possible (for a detailed discussion and review of the literature, see Fairfax and Salimnia).

RT-PCR is inherently semiquantitative. Theoretically, one can construct a standard curve of copy number versus cycle threshold—and determine the quantity of analyte in a patient specimen by referring to the curve. However, variations in extraction efficiency and the presence of inhibitors can introduce significant errors, particularly at low levels of analyte, when one is attempting to distinguishing “only a few” from “none.” One relatively straightforward method to overcome this problem includes the addition of a control target or quantitation standard into the patient sample before
extraction. The target and the quantitation standard are extracted, amplified, and detected together, controlling for the extraction and for any inhibitors present.

The ideal quantitation standard should be the same size and base composition as the target to be quantified, with the same primer binding sites. It should differ enough in sequence that the detector probe or probes for the target do not bind to it, and its own detector probe should fluoresce at a different wavelength from that of the target. Then, quantitation should be a simple mathematical calculation. However, at low target concentrations, the standard curve is no longer straight. Roche has incorporated complex mathematical calculations into its recent viral load assays to account for this divergence.

Conversations have recently begun about possible “cures” of HIV infection, and as highly active antiretroviral therapy and improved plasma HIV viral load techniques converge, discussions have ensued about how to evaluate residual virus in well-controlled or possibly cured individuals. The most sensitive assays for determination of low-level infection are likely to be assays for HIV DNA copies in single cells. How to determine which ones are replication competent is crucial to this discussion. Aside from circulating CD4 cells, what cells should be investigated is unclear at this time. This active area of investigation was recently reviewed by Strain and Richman, who discussed very low-level contamination, signal-to-noise ratios in PCR testing, single-cell PCR, and other techniques that are beyond the scope of this article.

With respect to HIV quantification in plasma, further considerations affect testing and result interpretation. The most obvious problem results from the increase in sensitivity as the assays improve. Patients who were told that their virus levels were “undetectable,” suddenly have quantifiable virus. Time-consuming correlations between viral load and prognosis have to be redone for each new, more sensitive assay. Sequence differences between the numerous organism strains and the inherent mutability of the organisms also make accurate quantification difficult, especially for RNA viruses, such as HIV and HCV. One must target a stable sequence. However, the viral RNA polymerase enzymes are error prone and lack proofreading activity. Changes in the genetic sequence, particularly in the primer or probe-binding sites, may reduce the detected viral load. Minority quasispecies also cause problems (discussed elsewhere in this article). It seems that more than 1 target will be necessary for future assays.

Because approval and release of diagnostic tests by different manufacturers are not coordinated, it is difficult to find articles comparing the performance of the latest offerings by different companies. Two current assays for HIV quantification in the United States are the Roche Cobas AmpliPrep Cobas TaqMan HIV viral load version 2.0 (CAP/CTM2) and the Abbott RealTime HIV-1 assay (ART HIV). The 2 assays have different lower limits of quantitation, 20 copies/mL for CAP/CTM2 and 40 copies/mL for ART HIV, which introduces difficulties in comparison. They have, however, been compared in 2 recent articles. Sire and colleagues extrapolated the ART HIV curves, and found that 10 of 17 specimens that were quantifiable by the CAP/CTM2 but not by the ART HIV could actually be quantified. The assays correlated well (r = 0.96), although CAP/CTM2 was more than 0.5 log10 higher than the ART HIV in 20% of 51 samples, whereas the ART HIV was more than 0.5 log10 higher in only 2 of them. Whose result is more accurate remains to be determined.

HIGHLY MULTIPLEXED POLYMERASE CHAIN REACTION PANELS

This section focuses only on highly multiplexed assays, roughly defined as those detecting more than 10 targets. Although many assays for individual etiology
agents of disease are available, testing for individual organisms is often uninformative. Numerous viral and some bacterial agents of respiratory disease cause similar symptoms. It is usually not obvious which agent or agents are infecting a given patient. Even at the peak of an influenza epidemic, an individual may be infected with respiratory syncytial virus instead or concurrently. Furthermore, mixed infections are more common than many had imagined.\textsuperscript{35,36} Some virus infections are treatable, and others have different isolation requirements if the patient is hospitalized.\textsuperscript{36} Thus, molecular panels that could detect multiple etiologic agents of diseases clearly are desirable. As of December 2017, 6 multiplex respiratory panels are FDA approved: Luminex xTAG RVP (xRVP) and RVP Fast (xRVPF) and Verigene respiratory pathogen flex (LUMINEX corporation); Film Array Respiratory Panel (FARP; BioFire Diagnostics [Now bioMerieux], Salt Lake City, UT), and the eSensor respiratory viral panel, ePlex Respiratory Pathogen Panel (GenMark Diagnostics, Carlsbad, CA). In comparison with other systems, the FARP detects the greatest number of pathogens, including 3 bacteria and 17 viral pathogens. One head-to-head comparison of all 4 test modalities was recently published.\textsuperscript{37} The various analytes differ in sensitivity and specificity, but this study found that the eSRP had 100\% sensitivity for all analytes compared. The xRVP was second, and more sensitive than the xRVPF, which was similar to the FARP. These results will require confirmation by other researchers; Babady and colleagues\textsuperscript{35} found the FARP to be more sensitive than the xRVPF for many analytes. All tests are capable of detecting mixed infections.

Ultimately, the final decision as to which test to implement in one’s laboratory may come down to questions of cost, hands-on time, complexity, time to result, and convenience. The FARP provides a result within about 1 hour, requiring only a few minutes of hands-on time, but can handle only 1 sample at a time. It seems ideally suited to a medium-sized laboratory where technologists without specialized molecular expertise can perform the tests. Some larger laboratories have bought multiple instruments or instruments with higher throughput (eg, Torch from BioFire) to facilitate throughput. But surge capacity may be lacking. The other systems test samples in batch mode with high throughput, but they require sample extraction before testing, and amplicon manipulation afterward, which may confine them to a “PCR laboratory.” Each handles 21 samples per run. None is suitable for 2 runs per shift, although staggered technologist start times could allow it with the xRVPF. The xRVP and eSRP require 7 to 8 hours for results.\textsuperscript{37}

A problem with panels is that they tend to maximize the number of analytes detected in their initial offering, based on instrument constraints. Therefore, adding something new seems to require that something old be removed. FDA reapproval requires significant financial outlays for any change, and new respiratory viruses keep appearing. Newly detected viruses since 2001 include human metapneumoviruses (2001), severe acute respiratory syndrome (2003), human bocavirus (2005), new influenza A viruses (H1N1, 2009; H7N9, 2013), and now, Middle East respiratory syndrome corona virus (2012), the latter 2 of which apparently have not (yet) appeared in the United States. MERS and H7N9 have not been reported in the United States between 2014 and 2017 (source: CDC https://www.cdc.gov/features/novelcoronavirus/index.html) but they apparently have a high mortality rate.\textsuperscript{38–40} The human metapneumoviruses and human bocavirus are included in some of the previously mentioned assays. It is not clear whether MERS corona virus can be detected by the coronavirus detector systems in these assays, but if so, it could not be distinguished from those that cause more common respiratory infections. Thus, keeping panels updated may prove difficult.
New multiplex panels have been developed and received FDA clearance for viral, parasitic, and bacterial agents of gastrointestinal diseases, and central nervous system infections. A BioFire assay for usual agents of bloodstream infections was FDA approved in August 2013. The Biofire gastrointestinal panel and meningitis panel also received FDA clearance on May 2014 and November 2015, respectively. In addition to the detection of bacterial pathogens, it detects several antibiotic resistance genes. Another system known as Verigene bloodstream tests (made by Nanosphere, now Luminex Corporation) provides bacterial identification and antibiotic resistance determination directly from positive blood culture bottles. The assay includes 2 cartridges, gram positive and gram negative. A Gram stain of positive blood culture bottle is needed to decide if a gram-positive or a gram-negative cartridge should be used. Luminex and BioFire also offer FDA-cleared gastrointestinal panels that detect and identify viral, bacterial, and toxins such as shiga1/2 directly from stool samples, whereas the blood culture panels should be run from positive culture bottles (which may take up to 5 days to become positive). Some can detect several antibiotic resistance genes as well as infecting bacteria.

**Prove-It Sepsis**

The Prove-It Sepsis assay can identify bacteria and fungi from positive blood culture in about 3.5 hours. The assay is approved in Europe, but not FDA cleared in the United States. Prove-it Sepsis runs on a strip with 8 wells, each of which can identify 2 sample. Each microarray identifies more than 60 bacteria, mecA, vanA, and vanB resistance markers and 13 fungi in a single assay. Tissari and colleagues, evaluated the clinical performance of this assay using 3300 patient samples and found. Based on their data, Prove-it Sepsis showed 95% sensitivity and 99% specificity.

**Quantitative Digital Polymerase Chain Reaction with Increased Accuracy at Low Copy Numbers**

Digital PCR (dPCR) is a quantitative method that provides a sensitive and reproducible way of measuring the amount of DNA or RNA present in a sample that is present in low copy numbers. It is similar to quantitative PCR in the reagents and amplification reaction. The method is simple and reproducible, and does not need quantitation standards or standard curves. Before amplification, the initial sample mix is partitioned into multiple individual wells or droplets, many of which end up containing no target sequence. After amplification, the number of positive and negative reactions is determined and the absolute number of target present in the initial reaction mix is calculated using Poisson statistics.

The dPCR is advantageous for applications requiring high sensitivity combined with accurate and reproducible enumeration of small numbers of targets. This method is obviously useful for quantification of viral loads, but can also be used for detection of rare alleles, determination of copy number variations, quantification of NGS libraries, as well as measurements of gene expression heterogeneity and multiplexing. There are currently no FDA-approved, commercially available dPCR systems in United States. However, several companies such as Raindrop Digital PCR System (Raindance Technologies, Lexington, MA), QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA), BioMark HD System, qdPCR 37K IFC (Fluidigm Corporation, South San Francisco, CA) and QuantStudio 3D Digital PCR System (Life Technologies, Carlsbad, CA) offer dPCR systems for research use. More information on dPCR, can be found in references.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Although “molecular diagnostics” is generally assumed to imply nucleic acid–based methods, mass spectrometry has been used in microbiology since the 1970s. At that time, mass spectroscopy was used almost exclusively for the identification of anaerobes by analysis of volatile or volatilized short-chain organic acids. New mass spectroscopy techniques provide a general tool for the identification of microorganisms growing in colonies on culture plates. This requires 2 to 5 minutes and has the potential to improve significantly the turnaround time for microbiology culture reports and to reduce labor costs, especially when coupled with other laboratory automation that is now becoming available. Up-front costs are high, but the cost per test is low and the rapidity of results and low cost per individual result can impact antibiotic usage and patient outcomes, shortening hospital stays and lowering total costs.\(^3\)

The new technology is matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Currently, there are 2 FDA-approved MALDI-TOF instruments available in the United States for rapid identification of microorganisms: one developed by Shimadzu Scientific Instruments (Columbia, MD) and licensed to BioMerieux (Durham, NC) and a second, manufactured by Bruker Daltonics (Billerica, MA), and licensed to both Siemens Healthcare Diagnostics (Tarrytown, NY) and to Becton Dickinson (Sparks, MD). The great benefit of quick, easy, and accurate identification of microorganisms has encouraged larger laboratories to implement these systems for routine organism identification despite their initial high instrument cost.

Microorganism identification by MALDI-TOF is based on the observation that each pathogenic organism has its own unique protein signature. Bacterial cells are fixed in a matrix and irradiated by a laser beam that releases and ionizes the proteins. These enter a vacuum column and move toward the detector based on charge and mass. The protein signature is checked for a match against a large and still growing database derived from different genera and species of microorganisms. Different instruments have different databases, and the government has limited laboratory access to databases containing the protein signature of potential agents of bioterrorism, although these are endemic in certain areas of the United States.

MALDI-TOF can identify bacteria, both aerobic and anaerobic, mycobacteria, and fungi. The analysis of organisms with tougher cell walls, such as gram-positive bacteria, yeasts, and fungi, requires slight modifications of procedure.\(^4\) Hundreds of abstracts and articles attest to its ability to rapidly and accurately identify bacteria and fungi to the genus and species level.

MALDI-TOF for the identification of etiologic agents of sepsis generally cannot be performed until the pathogen has first been grown in blood culture bottles and subcultured onto standard solid media. These steps are time consuming and lead to the use of broad-spectrum, empiric antibiotics. To increase speed of identification, protocols have been developed to use MALDI-TOF directly from newly positive blood culture bottles,\(^4\) although it should not be used in samples containing multiple organisms. Data are also accumulating illustrating the ability of MALDI-TOF to identify some antibiotic-resistant organisms.\(^5\) However, more work is needed in this area before MALDI-TOF could become a valid alternative to routine antibiotic susceptibility testing.

Laser-induced breakdown spectroscopy

Rapid advances in the development of laser-induced breakdown spectroscopy (LIBS) have transformed it from an elemental analysis technique to one that can be used directly for the identification of complex materials, including clinical microbiology
specimens. A growing number of recent articles have illustrated the ability of LIBS to rapidly detect and accurately identify various biological, biomedical, or clinical samples. These analyses are sensitive, specific, and rapid, requiring no sample preparation. LIBS can identify microorganisms based on a determination of their elemental compositions. LIBS uses a strong laser pulse to atomize the content of bacterial cells. Light from these “high-temperature sparks” is collected. Analysis of the peaks in the atomic emission spectrum allows the identification and relative quantification of the atoms present in each sample. The “spectral fingerprint” of the peaks and their ratios is compared with a database of LIBS spectra derived from different genera and species of microorganisms to allow identification of bacterial genus and species. As yet, there is no FDA-cleared LIBS system for rapid identification of pathogen microorganisms.51,52

**Raman spectroscopy**

Interest has also recently been generated in the possibility of using Raman spectroscopy for the rapid identification of a variety of bacteria. Raman spectroscopy uses the interaction of light with molecules to measure functional group vibrations. When photons from an incident, focused laser interact with a molecule, they cause the molecule to transition to an excited vibrational state, accompanied by a corresponding energy loss in the photon; this energy loss results in the light undergoing a frequency shift, and hence a change in color, which is measured. The spectrum is unique for each bacterial species. It can be checked against a database for rapid identification of the pathogen. Raman spectroscopy is distinct from other current techniques because of its ease, low cost, and high speed. It provides information on the chemical composition and the structure of biomolecules within the microorganisms. Thus, slight changes in the chemical makeup of organisms can be determined by Raman spectroscopy and used to differentiate genera, species, or even strains. A study by Chouthai and colleagues,53 showed reliable, rapid, and accurate identification of *Candida* species using Raman spectroscopy. Detection of pathogens is possible from complex matrices, such as soil, food, and body fluids. Further, spectroscopic analysis may allow determination of the effect of antibiotics on bacteria.53–55

**Multiplexed Automated Digital Microscopy (Time Lapse Imaging): Accelerate Pheno System**

The automated Accelerate Pheno technique (AXDX; Accelerate Diagnostics) received FDA approval in February 2017. It performs both bacterial identification and antibiotic susceptibility testing directly from the positive blood culture bottle, without requiring the isolation of purified colonies, cutting almost 40 hours off the time to identification and antibiotic susceptibility testing. The techniques use digital microscopy and allow identification of 1 to 4 bacterial pathogens from a positive blood culture bottle in around 1 hour and antibiotic susceptibility testing about 6 hours later. It succeeds where other techniques fail because it analyzes single cells. Organism identification is performed using multiplexed automated digital microscopy and FISH probes (described elsewhere in this article). Antibiotic susceptibility testing is performed by observing the growth of individual, live, immobilized bacterial cells in the presence of antimicrobial agents. Phenotypic antibiotic susceptibility testing results with a panel of standard antibiotics require about 7 hours after the blood culture bottle turns positive. By guiding appropriate antibiotic therapy this microscopic technique can reduce mortality and morbidity. AXDX identifies and performs antibiotic susceptibility testing on 6 gram-positive and 8 gram-negative bacterial genera/species and 2 *Candida* species. In this system,
bacterial cells are first purified from blood culture growth medium by gel electrofiltration followed by cell immobilization via electrokinetic concentration before FISH and antibiotic susceptibility testing.

Our laboratory has evaluated the performance (accuracy) of Accelerate system. In 2015 and 2016, we tested 300 clinical samples on this system and compared the result with our standard method, which currently consists of a combination of phenotypic, biochemical, and/or MALDI-TOF techniques for pathogen identification combined with antibiotic susceptibility testing via the Becton Dickinson Phenix automated microbiology system. The data revealed high sensitivity (93.8%) and specificity (99.7%) for identification of pathogens as well as high categorical (94.8%) and essential agreement (96.3%) for antibiotic susceptibility testing results. The system was robust and on average provided identification in 1.4 and antibiotic susceptibility testing results in 6.7 hours after bottle positivity.

Marschal and colleagues compared the performance of AXDX with conventional culture-based methods. AXDX correctly identified 88.7% (102 of 115) of all blood stream infections (BSI) episodes and 97.1% (102 of 105) of 28 isolates that are covered by the system’s identification panel. The AXDX generated an antibiotic susceptibility testing result for 91.3% (95 of 104) samples in which it identified a gram-negative pathogen. They found the overall category agreement of 96.4%, for sensitive, intermediate, and resistant interpretation between AXDX and culture-based antibiotic susceptibility testing, with the rate for minor discrepancies 1.4% (change between sensitive or resistant and intermediate), major discrepancies 2.3% and 33 very major discrepancies 1.0%.

Resonant mass measurement for determination of antibiotic susceptibility in bacteria

LifeScale biosensor rapid antimicrobial susceptibility LifeScale (Affinity Biosensors, Santa Barbara, CA) uses resonant mass measurement to enumerate and determine the mass of bacteria exposed to antibiotics, allowing it to determine antibiotic susceptibility within approximately 3 hours. A beam suspended at 1 end resonates at a specific frequency. If a mass is added to the beam, the resonant frequency decreases an amount related to the added mass. The beam is suspended in a vacuum and the bacteria, in growth medium, pass through a microchannel in the beam. This technology has been developed into the Life Scale Biosensor, which can make these determinations in a standard 96-well plate, including the minimum inhibitory concentration plates prepared by Sensititre (ThermoScientific, Waltham, MA). Individual bacteria can be enumerated and their individual masses rapidly determined (in femtograms $10^{-15}$ g) can be determined rapidly as they pass through the microchannel in the beam. Two posters were presented at the 2016 ASM Microbe to demonstrate proof of concept. Antibiotic susceptibility was determined using multiple gram-negative organisms that are generally used in clinical laboratories as antibiotic susceptibility testing control organisms. Resonant mass measurement and standard laboratory antibiotic susceptibility testing results were compared. More than two-thirds of these antibiotics demonstrated 100% essential agreement with the standard results after 3 hours or less, whereas the standard techniques required 16 to 24 hours. Two antibiotics were in less than 90% categorical agreement and required a longer incubation time. The results indicate that a rapid antibiotic susceptibility testing based on resonant mass measurement produces reliable antibiotic susceptibility testing results on gram-negative strains. Ongoing investigations are planned to extend these results to gram-positive strains and to validate the method on clinical samples from positive blood cultures.
**Colorimetric sensor array: use of volatile organic compounds and color active indicator array for rapid bacterial identification**  
The SpecID (Specific Technologies, Mt. Lakes, CA) blood culture system provides organism detection and identification while the etiologic agent of sepsis is growing in a modified blood culture bottle and does so more rapidly than detection alone can occur in similar comparator blood culture bottles. When bacteria grow, they produce characteristic volatile organic compounds (VOC) that accumulate in the head gasses above the culture medium in the bottle; the spectrum of VOC produced is characteristic of a given genus and species of bacteria. An inexpensive, disposable array can be substituted for the top of a bottle or built into a proprietary bottle. This array consists of chemical indicators that change color differently when exposed to the VOC mixtures, allowing for the determination of the VOCs released. The first instrument using this technique was shown in Amsterdam at European Society of Clinical Microbiology and Infectious Diseases 2016. In a proof-of-principle paper, Lim and colleagues \(^60\) demonstrated that the SpecID fingerprint can detect and identification the VOC fingerprints of organisms in pure culture with a sensitivity and specificity of 91% and 99.4%, respectively. It could distinguish between strains of *S. aureus*, suggesting that it may be used for epidemiologic purposes as well. Furthermore, detection was rapid, with organism identification with the arrays detecting growth almost 2 hours faster than the standard system. Whether it will work in mixed infections remains to be determined.

Shrestha and colleagues \(^61\) studied the ability of the SpecID arrays to identify a panel of important yeast pathogens. BacT/Alert bottles modified to contain the arrays were compared with standard bottles, all of which were inoculated with 10 mL of blood either unspiked or spiked with different quantities of the yeast species. Bottles containing no yeast were reported as negative, and the yeast in all positive bottles were detected correctly, regardless of a wide range of inoculum sizes. Growth by colorimetric sensor array was detected 6.8 hours faster than BacT/Alert system. The mean sensitivity for species-level identification by colorimetric sensor array was 74% at the time of growth detection, and increased with time, reaching almost 95% at 4 hours after growth detection. \(^60,61\)

**Cultureless systems for rapid detection and identification of pathogens directly from patient’s specimen**

*Magicplex sepsis real-time test (SeeGene)* Magicplex screens for more than 90 pathogens that cover more than 90% of sepsis-causing pathogens as well as 3 drug resistance markers for methicillin and vancomycin (mecA, vanA, and vanB) from whole blood sample. Also, this test is able to further identify the pathogens that are detected in the previous screening step with an additional 30 minutes. There are 73 gram-positive, 12 gram-negative, 6 fungi, and 3 antibiotic resistant genes that can also be discriminated. The Magicplex Sepsis Test requires 1 mL of a patient’s whole blood and provides test results within 3 hours (after extraction). The microbial DNA enrichment is based on Molzym’s MoYsis technology (Bremen, Germany) enabling up to 40,000-fold DNA enrichment over conventional technologies. Automated DNA isolation is performed with Seegene’s Seeprep12 (Seoul, Korea) instrument based on Nordiag’s “Arrow” technology/system. The pathogens identified by MagicPlex Sepsis assay are Streptococci (*S. agalactiae, S. pyogenes, S. pneumoniae*), Enterococci (*E. faecalis, E. gallinarum, E. faecium*), Staphylococci (*S. epidermidis, S. haemolyticus, S aureus*), *P. aeruginosa, A. baumannii, S. maltophilia, S. marcescens, B. fragilis, S. typhi, K. pneumoniae, K. oxytoca, P. mirabilis, E. coli, E. cloacae, E. aerogenes, C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei, and A. fumigatus*. The MagicPlex is compatible with AB7500 RT-CR (Lifetime
Technologies, Hanoi, Japan), CFX96 Real-time PCR (Bio-Rad), and SmartCycler II RT-PCR.

In a clinical validation of MagicPlex, Serra and colleagues demonstrated the detection of Candida DNA in pediatric patients in which the culture result was negative. However, the assay also had some false-negative results. In this study at least 1 mL of blood sample was inoculated into a VersaTREK REDOX1 bottle, which was incubated at 37°C for 3 hours or longer before DNA extraction. Carrara and colleagues showed the result of a comparative study of MagicPlex and standard blood culture on 267 patients. From 98 positive results, 11% were positive by both system, 11% only by MagicPlex, and 15% only by blood culture. Sensitivity and specificity were 65% and 92%, respectively, for the Magicplex Sepsis Test and 71% and 88%, respectively for blood culture. Denina and colleagues in a study of 89 pediatric patients, found that Magicplex allowed a 143% increase in the detection of septic episodes. However Ziegler and coworkers found that the test detected many organisms suspected to be contaminants and investigated increasing the cut-off value for positive. They concluded that MagicPlex shows a high specificity but changes in design are needed to increase pathogen detection. For viability in clinical laboratories, technical improvements are also required to further automate the process. This product is IVD CE marked and is not available in the United States.

LightCycler SeptiFast test MGRADE SeptiFast (Roche Molecular Systems), provides rapid identification to the species level of 25 common etiologic agents of BSI (bacterial and fungal) in less than 6 hours directly from 1.5 mL of whole blood without prior culture. The assay targets a multicopy region (internal transcribed spacer) to increase sensitivity (the detection limit is approximately 300 colony-forming units [CFU]/mL). Targets include 8 gram-negative bacteria or related groups, 6 gram positives, and 8 fungi including Aspergillus fumigatus. The test is designed to be run on the LightCycler2.0 Instrument manufactured by Roche, which combines rapid amplification with melting point analysis for rapid results. A related assay also can detect the mecA gene detection from S aureus, in a subsequent run using the LightCycler SeptiFast MecA Test MGRADE. SeptiFast is CE marked but not available in the United States. Markota and colleagues demonstrated a sensitivity of 87.5%, a specificity of 92.6%, and a negative predictive value 97.8% in comparison with standard blood culture. This system can be used as a supplement to the standard blood culture.

SepsiTest-UMD SepsiTest-UMD (Molzym) can use 1 to 10 mL of whole blood or tissue samples for detection of bacterial pathogens and yeast. After sample preparation and DNA concentration, the PCR assay amplifies 16S and 18S ribosomal sequences. Primers for Sanger sequencing are included in the assay. If positives are detected, they are identified by basic local alignment search tool analysis using an online data base. Additional kits are available for other fluids and swabs, but many are for research use only. Disqué and colleagues compared the SepsiTest with blood culture and found a sensitivity of 60%, a specificity of 98%, a negative predictive value of 91%, and a positive predictive value of 86%. The system is not fully automated, and the results are not available quickly. The test is approved for in vitro diagnostics in Europe but not in the United States.

T2 biosystems sepsis solution The T2 biosystem is recognized in the US market for their FDA-approved assay for the diagnosis of candidemia directly from a blood sample of a patient in about 5 hours. T2-weighted MRI is a small instrument that uses PCR, followed by hybridization on probe-decorated nanoparticle microclusters.
Hybridization will induce changes in magnetic resonance signals, resulting in rapid identification of candida. One of the important features of this technique is direct use of blood samples by the instrument without any prior processing steps. It also benefits from high sensitivity that enables the system to detect a few CFU per milliliter of the target in whole blood. In a report from Mylanakis and colleagues, the performance of T2 system for rapid detection of Candida species directly from the bloods of patients with suspected candidemia was evaluated. The report indicates that T2-weighted MRI demonstrated an overall specificity per assay of 99.4% (95% confidence interval [CI], 99.1%–99.6%) with a mean time to a negative result of 4.2 ± 0.9 hours. Subanalysis yielded a specificity of 98.9% (95% CI, 98.3%–99.4%) for C albicans/Candida tropicalis, 99.3% (95% CI, 98.7%–99.6%) for Candida parapsilosis, and 99.9% (95% CI, 99.7%–100.0%) for Candida krusei/Candida glabrata. The overall sensitivity was found to be 91.1% (95% CI, 86.9%–94.2%) with a mean time of 4.4 ± 1.0 hours for detection and species identification. The subgroup analysis showed a sensitivity of 92.3% (95% CI, 85.4%–96.6%) for C albicans/C tropicalis, 94.2% (95% CI, 84.1%–98.8%) for C parapsilosis, and 88.1% (95% CI, 80.2%–93.7%) for C krusei/C glabrata. The LOD was 1 CFU/mL for C tropicalis and C krusei, 2 CFU/mL for C albicans and C glabrata, and 3 CFU/mL for C parapsilosis. The negative predictive value was estimated to range from 99.5% to 99.0% in a study population with 5% and 10% prevalence of candidemia, respectively. The T2 bacterial panel is another assay for detection of bacterial pathogens directly from the blood of patients with suspected bacteremia/sepsis. It can detect Escherichia coli, K pneumoniae, Pseudomonas aeruginosa, A baumannii, S aureus, and Enterococcus faecium and is expected to be FDA cleared by 2018. The usefulness of this system in the clinical setting and its potential impact on patient outcome await data from clinical studies.

Qvella FAST ID BSI panel The Qvella blood pathogen detection system (FAST ID BSI Panel) has been developed to identify bacterial and fungal pathogens directly from blood samples in less than 1 hour. It uses the rRNA as the target in a multiplex PCR for bacterial identification. The system benefits also from a tailored electric field for the lysis of the bacterial cells present in the blood sample, followed by a concentration step to obtain a highly concentrated and purified bacterial genomic materials. The purified nucleic acids are used in the multiplex real time reverse transcriptase PCR for rapid detection and identification of microorganisms directly from blood.

The FAST technology is implemented in the FAST ID BSI Panel, which is an integrated and closed device designed to fully automate the isolation, concentration, and lysis, as well as amplification and detection of nucleic acids from pathogens from whole blood samples. To perform a test, a whole blood tube is inserted into a FAST ID BSI Panel and it is placed into the FAST analyzer. The following processes occur automatically during the processing of a FAST ID BSI Panel by the analyzer: Target cell isolation and concentration, target cell electrical lysis and treatment, and amplification and detection of bacterial and fungal ribosomal targets in a spatially multiplexed array. This assay can detect the majority of sepsis-causing species. The system has been shown to be able to identify polymicrobial infections in spiked samples.

Khine and colleagues presented data at the 27th European Society of Clinical Microbiology and Infectious Diseases regarding the lower LOD of the Qvella FAST system. The LOD was determined to be approximately 1 CFU/mL for the various cell types. This low LOD is consistent with pathogen concentrations typically found in infected whole blood samples. For this dataset, detection was made at 100% of the time for Klebsiella pneumoniae, Pseudomonas aeruginosa, E faecium, C albicans,
and *C. glabrata* down to 1 CFU/mL. For *S. aureus*, detection was made at 100% of the time at or greater than 2 CFU/mL and greater than 80% of the time for 1 CFU/mL. However, less than 100% detection of *S. aureus* was at least partly attributable to the inherent difficulty and accuracy associated with spiking certain gram-positive bacteria at very low concentrations. The Qvella blood identification system is designed to detect more than 90% of pathogens isolated from patients with sepsis. Clinical trial of this system is expected to start in the second quarter of 2018.

**NEXT-GENERATION SEQUENCING**

The advances in sequencing technology known as NGS have led to significant advances in basic sciences and clinical laboratory medicine, including microbiology. Currently available NGS techniques are based on using clonal amplicons for parallel multistrand sequencing. The combination of high-speed and high-throughput data analysis has made NGS an excellent tool to take clinical analysis of nucleic acid sequences to a new level. With NGS, it is possible to detect and quantify quasispecies of HIV, HCV, or hepatitis B virus circulating at low levels in the blood of infected patients and to learn more about their roles in the development of resistance and associated treatment failures. This was difficult or impossible by using methods mentioned previously. Despite increasing numbers of articles on the applications of NGS, this system has not found its way into routine clinical microbiology testing because of lack of FDA approval, high cost, and availability of alternative technologies. From the diagnostic microbiology viewpoint, NGS has significantly improved our ability to study the makeup of entire communities of microorganisms without culture. NGS can provide the genetic sequences of the members of entire microbial communities (microbiomes) and determine their relative frequencies. It could also be used to determine gene expression and metabolic pathway use in a microbial community, although this is currently beyond the scope of the clinical microbiology laboratory. Many studies of human microbiomes are focusing on the relationship between microbial communities and health and disease, including for example, the effect of colonic microorganisms on cardiovascular disease. NGS has also been applied to rapid investigations of outbreaks in hospital settings. We discuss herein 2 NGS-based diagnostic microbiology systems that are currently fairly advanced in development. They highlight the potential of NGS to improve detection and identification of pathogens in the clinical laboratory, especially those involving organisms that may be difficult or impossible to identify, or those situations that are otherwise technically demanding.

**Systems Based on Next-Generation Sequencing for the Identification of Pathogens from Clinical Specimens**

**Karius digital culture**

Karius (Redwood City, CA) has developed an NGS-based test to detect the presence in plasma of cell-free DNA, which is presumably derived from infecting pathogens. After amplification, human DNA is removed by a proprietary technique. NGS is performed on the total nucleic acids, enriching for the microbial sequences. These sequences are aligned with a pathogen sequence bank that consists not only of publicly available genetic information, but also of Karius’s internal data. Thus, it can potentially detect thousands of infecting organisms. The system is unique in that it can detect sequences from DNA viruses in addition to those from bacteria and fungi. Like standard organism detection assays, this system can detect bacteremia and fungemia.
In several studies Karius and collaborators have also shown that the system is able to diagnose infections with certain highly fastidious organisms or after antibiotics have rendered the infecting organism nonviable. The system could also detect the infecting organism in infections outside of the bloodstream when organismal DNA is released into the blood, including osteomyelitis, and deep infections that would otherwise require biopsies. In patients with known or suspected bacteremia, their system showed 80.0% positive agreement and 73.8% negative agreement (overall agreement of 76.7%) with standard blood culture. In comparison with the clinical diagnosis, their system showed 82.4% and 79.1% positive and negative agreement, respectively. 73–75

**PathoQuest iDTECT**

The iDTECT Blood (PathoQuest, Paris, France) also combines untargeted NGS with their sample preparation process and their genome sequence database, which is said to cover all clinically relevant human pathogens. It is the first CE-marked (October 2016), NGS-based method to detect bacteria and viruses in patients with known or suspected infections directly from patient blood thereby improving pathogen detection in biological samples. PathoQuest’s technology combines an NGS platform and a proprietary sample preparation process with a proprietary pathogen genome sequence database and automated analysis software covering all known clinically relevant human pathogens. Parize and colleagues, 76 using a prototype version of iDTECT, identified a relevant pathogen in more patients in a difficult-to-diagnose population than conventional testing.

**SUMMARY**

The application of molecular testing to the discipline of infectious disease diagnosis, prognosis and management has matured exponentially over the last few years. Future improvements will no doubt avail additional milestones in sensitivity, specificity, cost reduction, and turnaround time from specimen procurement to result for effective and expeditious patient management.

**REFERENCES**

1. Alexander BD, Ashley ED, Reller LB, et al. Cost savings with implementation of PNA FISH testing for identification of *Candida albicans* in blood cultures. Diagn Microbiol Infect Dis 2006;54:277–82.

2. Forrest GN, Roghmann MC, Toombs LS, et al. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. Antimicrob Agents Chemother 2008;52:3558–63.

3. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med 2013;137(9):1247–54. Accessed September 12, 2013.

4. Karlsson FH, Fak F, Nookaew I, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun 2012;3:1245. Accessed September 12, 2013.

5. Fairfax MR, Salimnia H. Diagnostic molecular microbiology: a 2013 snapshot. Clin Lab Med 2013;33:787–803.

6. AccuProbe *Mycobacterium tuberculosis* complex culture identification test. Package Insert. Hologic Gen-Probe. Revision 2011-02. Available at: http://www.genprobe.com/pdfs/pi/102896RevN.pdf. Accessed April 28, 2013.

7. Welch DF, Guruswamy AP, Sides SJ, et al. Timely culture for mycobacteria which utilizes a microcolony method. J Clin Microbiol 1993;31:2178–84.
8. Becton Dickinson. Bacte Myco/F Sputa. Package Insert. Revision 2010/2. Available at: http://www.bd.com/ds/technicalCenter/inserts/PP101JAA(2011002).pdf. Accessed April 20, 2013.

9. Lie AK, Kristensen G. Human papillomavirus E6/E7 mRNA testing as a predictive marker for cervical carcinoma. Expert Rev Mol Diagn 2008;8:405–15.

10. Qiagen. Digene Hybrid Capture 2 High-Risk HPV DNA Test. Ref 5199–1220. Package insert. Available at: http://www.qiagen.com/resources/Download.aspx?id5(0A98CB57-25B9-48A9-9C4F-4C66D1CDA47C) &lang5en&ver55. Accessed September 9, 2013.

11. Gravitt E, Rostich AF, Silver MI, et al. A cohort effect of the sexual revolution may be masking an increase in human papillomavirus detection at menopause in the United States. J Infect Dis 2013;207:274–80. Queried on line April 2, 2013.

12. Quiroga-Garza G, Zhou H, Mody DR. Unexpected high prevalence of HPV 90 infection in an underserved population: is it really a low-risk genotype? Arch Pathol Lab Med 2013;137(11):1569–73.

13. Salimnia H, Fairfax MR, Lephart P, et al. An international, prospective, multicenter evaluation of the combination of AdvanDx Staphylococcus QuickFISH BC with mecA XpressFISH for detection of methicillin-resistant Staphylococcus aureus isolates from positive blood cultures. J Clin Microbiol 2014;52(11):3928–32.

14. Della-Latta P, Salimnia H, Painter T, et al. Identification of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa in blood cultures: a multicenter performance evaluation of a three-color peptide nucleic acid fluorescence in situ hybridization assay. J Clin Microbiol 2011;49(6):2259–61.

15. Morgan M, Marlowe E, Della-Latta P, et al. Multicenter evaluation of a new shortened peptide nucleic acid fluorescence in situ hybridization procedure for species identification of select Gram-negative bacilli from blood cultures. J Clin Microbiol 2010;48(6):2268–70.

16. Abdelhamed AM, Zhang SX, Watkins T, et al. Multicenter evaluation of Candida QuickFISH BC for identification of Candida species directly from blood culture bottles. J Clin Microbiol 2015;53(5):1672–6.

17. Becton Dickinson. BD Affirm VPIII Microbial Identification Test. Package Insert. Revision of 2006/02 Queried on line April 25, 2013. Available at: http://www.bd.com/ds/technicalCenter/inserts/pkgInserts.asp#PF8.

18. BD Max vaginal panel package insert. Available at: http://www.bd.com/resource.aspx?IDX=32632. Accessed December 1, 2017.

19. Andrea SB, Chapin KC. Comparison of Aptima Trichomonas vaginalis transcription-mediated amplification assay and BD Affirm VPIII for detection of T. vaginalis in symptomatic women: performance parameters and epidemiologic implications. J Clin Microbiol 2011;49:866–9.

20. Gen-Probe. Aptima Combo 2 Assay. Package Insert. Version 2011-04. Queried on line: May 20, 2013. Available at: http://www.gen-probe.com/pdfs/pi/501799-EN-RevD.pdf.

21. Taylor SN, Van der Pol B, Lillis B, et al. Clinical evaluation of the BD ProbeTec Chlamydia trachomatis Qx amplified DNA assay on the BD Viper system with XTR technology. Sex Transm Dis 2011;38:603–9.

22. Becton Dickinson. ProbeTec Chlamydia trachomatis (CT) Qx Amplified DNA assay. Package insert. Version 2010/12. queried on line May 12, 2013. Available at: http://www.bd.com/ds/technicalCenter/inserts/8981498(201012).pdf.

23. Wolfe D, Hook EW, Meno L, et al. Evaluation of the BD Viper™ System in Extracted Mode to Detect Chlamydia trachomatis and Neisseria gonorrhoeae in
Male Swab and Urine Specimens Clinical Virology Symposium. Daytona Beach, FL, April 27, 2008.

24. Cheng A, Qian Q, Kirby JE. Evaluation of the Abbott RealTime CT/NG assay in comparison to the Roche Cobas Amplicor CT/NG assay. J Clin Microbiol 2011; 49:1294–300.

25. Chernesky M, Jang D, Gilchrist J, et al. Head-to-head comparison of second-generation nucleic acid amplification tests for detection of Chlamydia trachomatis and Neisseria gonorrhoeae on urine samples from female subjects and self-collected vaginal swabs. J Clin Microbiol 2014;52(7):2305–10.

26. Gen-Probe Procleix Ulitro Plus Assay. 502432-REG Rev.7, as submittted to the FDA. Queried on line May 20, 2013. Available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/UCM335285. pdf.

27. Maurin M. Real-time PCR as a diagnostic tool for bacterial diseases. Expert Rev Mol Diagn 2012;12:731–54.

28. Fairfax MR, Salimnia H. Quantitative PCR: an introduction. In: Grody WW, Strom C, Kiechle FL, et al, editors. Handbook of molecular diagnostics. London: Academic Press; 2010. p. 3–14.

29. Espy MJ, Uhl P, Mitchell S, et al. Diagnosis of herpes simplex virus in the clinical laboratory by LightCycler PCR. J Clin Microbiol 2000;38:795–9.

30. Issa NC, Espy MJ, Uhl P, et al. Sequencing and resolution of amplified herpes simplex virus with intermediate melting curves as genotype 1 or 2 by light cycle PCR assay. J Clin Microbiol 2005;43:1843–5.

31. Available at: http://www.luminexcorp.com/prod/groups/public/documents/lmnxcorp/342-multicode-tech.pdf. Accessed May 12, 2013.

32. Strain MC, Richmond DD. New assays for monitoring residual HIV burden in effectively treated individuals. Curr Opin HIV AIDS 2013;8:106–10.

33. Sire JM, Vray M, Merzouk M, et al. Comparative RNA quantification of HIV-1 group M and non-m with the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 v2.0 and Abbott Real-Time HIV-1 PCR assays. J Acquir Immune Defic Syndr 2011;56(3):239–43.

34. Wojewoda CM, Shalinger T, Harmon ML, et al. Comparison of Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 test version 2.0 (CAP/CTM v2.0) with other real-time PCR assays in HIV monitoring and follow-up of low-level viral loads. J Virol Methods 2013;187:1–5.

35. Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast-assay with the Idaho Technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. J Clin Microbiol 2012;50:2282–8.

36. McGrath EJ, Thomas R, Asmar B, et al. Detection of respiratory co-infections in pediatric patients using a small volume polymerase chain reaction array respiratory panel: more evidence for combined droplet and contact isolation. Am J Infect Control 2013;41:668–73. Queried on line April 1, 2013.

37. Popowich EB, O’Niel SS, Miller MM. Comparison of the Biofire FilmArray RP, Genmark eSensor RBP, Luminex xTAG RVPv1, and Luminex xTAG RVP Fast multiplex assays for detection of respiratory viruses. J Clin Microbiol 2013;51(5):1528–33. Accessed May 16, 2012.

38. Mahoney JB. Nucleic acid amplification-based diagnosis of respiratory virus infections. Expert Rev Anti Infect Ther 2010;8(11):1273–92. Accessed May 16, 2013.
39. de Groot RJ, Baker SC, Baric RS, et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV); announcement of the coronavirus study group. J Virol 2013;87:7790–2.
40. Liu D, Shi W, Shi Y, et al. Origin and diversity of novel avian influenza A H7N9 viruses causing human infection: phylogenetic, structural, and coalescent analyses. Lancet 2013;381(9881):1926–32.
41. Tissari P, Zumla A, Tarkka E, et al. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. Lancet 2010;375(9710):224–30.
42. Huggett JM, Whale A. Digital PCR as a novel technology and its potential implications for molecular diagnostics. Clin Chem 2013;59:1691–3.
43. Sedlak RH, Jerome KR. Viral diagnostics in the era of digital PCR. Diagn Microbiol Infect Dis 2013;75(1):1–4.
44. Laurie MT, Bertout JA, Taylor SD, et al. Simultaneous digital quantification and fluorescence-based size characterization of massively parallel sequencing libraries. Biotechniques 2013;55(2):61–7.
45. Whale AS, Cowen S, Foy CA, et al. Methods for applying accurate digital PCR analysis on low copy DNA samples. PLoS One 2013;8(3):e58177.
46. March-Rosselló GA, Muñoz-Moreno MF, García-Loygorri-Jordán de Urriés MC, et al. A differential centrifugation protocol and validation criterion for enhancing mass spectrometry (MALDI-TOF) results in microbial identification using blood culture growth bottles. Eur J Clin Microbiol Infect Dis 2013;32:699–704.
47. Mestas J, Felsenstein S, Bard JD. Direct identification of bacteria from positive BacT/ALERT blood culture bottles using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:3093–5.
48. Mitchell SL, Alby K. Performance of microbial identification by MALDI-TOF MS and susceptibility testing by VITEK 2 from positive blood cultures after minimal incubation on solid media. Eur J Clin Microbiol Infect Dis 2017;36(11):2201–6.
49. Ashton L, Lau K, Winder CL, et al. Raman spectroscopy: lighting up the future of microbial identification. Future Microbiol 2011;6(9):991–7.
50. Smith E, Dent G. A comprehensive but readable introduction to the field of Raman spectroscopy. In: Smith E, Dent G, editors. Modern Raman Spectroscopy, a
Practical Approach. West Sussex (United Kingdom): John Wiley and Sons; 2005. p. 1–21.

56. Lephart P, Kaye KS, Pogue JM, et al. Evaluation of accelerate Pheno™ system in a clinical setting: comparison of identification and antibiotic susceptibility test results of 224 prospective positive blood cultures to standard laboratory methods at Detroit Medical Center. European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, April 22–25, 2017.

57. Marschal M, Bachmaier J, Autenrieth I, et al. Evaluation of the accelerate Pheno system for Fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by gram-negative pathogens. J Clin Microbiol 2017;55(7):2116–26.

58. Schneider C, Babcock K, Harris P, et al. Rapid antimicrobial susceptibility tests by mass measurement on a 96 well plate. ASM microbe. Boston, June 16–20, 2016.

59. Babcock K, Schneider C, Harris P, et al. Phenotypic response of bacteria to antibiotics at single minute time scales. ASM microbe. Boston, June 16–19, 2016.

60. Lim SH, Mix S, Anikst V, et al. Bacterial culture detection and identification in blood agar plates with an optoelectronic nose. Analyst 2016;141:918.

61. Shrestha NK, Lim SH, Wilson D, et al. The combined rapid detection and species-level identification of yeasts in simulated blood culture using a colorimetric sensor array. PLoS One 2017;12(3):e0173130.

62. Serra J, Rosello E, Figueras C, et al. Clinical evaluation of the Magicplex sepsis real-time test (Seegeene) to detect Candida DNA in pediatric patients. Crit Care 2012;16(Suppl 3):P42.

63. Carrara L, Navarro F, Turbau M, et al. Molecular diagnosis of bloodstream infections with a new dual-priming oligonucleotide-based multiplex PCR assay. J Med Microbiol 2013;62:1673–9.

64. Denina M, Scolfaro c, Colombo S, et al. Magicplex sepsis real-time test to improve bloodstream infection diagnostics in children. Eur J Pediatr 2016;175(8):1107–11.

65. Ziegler I, Fagerstrom A, Stralin K, et al. Evaluation of a commercial multiplex PCR assay for detection of pathogen DNA in blood from patients with suspected sepsis. PLoS One 2016;11(12):e0167883.

66. Markota A, Seme K, Golle A, et al. SeptiFast real-time PCR for detection of bloodstream pathogens in patients with severe sepsis or septic shock. Coll Antropol 2014;38(3):829–33.

67. Disquè C, Kochem AJ, Mühl H, et al. Polymerase chain reaction detection of sepsis-inducing pathogens in blood using SeptiTest™. Crit Care 2008;12(Suppl 5):P10.

68. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, et al. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 2015;60:892–9.

69. Khine AA, Parmar V, Talebpour A, et al. Evaluating the analytical sensitivity of Qvella’s FAST(TM) ID system for early detection and identification of bloodstream infection in whole blood 27th ECCMID. Vienna, Austria, April 22–25, 2017.

70. Capobianchi MR, Giombini E, Rozera G. Next-generation sequencing technology in clinical virology. Clin Microbiol Infect 2013;19(1):15–22.

71. Song S, Jarvie T, Hattori M. Our second genome-human metagenome: how next-generation sequencer changes our life through microbiology. Adv Microb Physiol 2013;62:119–44.
72. Sherry NL, Porter JL, Seemann T, et al. Outbreak investigation using high-throughput genome sequencing within a diagnostic microbiology laboratory. J Clin Microbiol 2013;51:1396–401.

73. Abril MK, Barnett AS, Wegermann K, et al. Diagnosis of Capnocytophaga canimorsus sepsis by whole-genome next-generation sequencing. Open Forum Infect Dis 2016;3(3):ofw144.

74. Wanda L, Ruffin F, Hill-Rorie J, et al. Direct Detection and quantification of bacterial cell-free DNA in patients with bloodstream infection (BSI) using the Karius Plasma Next Generation Sequencing (NGS) Test. ID week October 4–8, 2017, San Diego.

75. Benamu E, Gajurel K, Anderson JN, et al. Performance of the Karius Plasma Next Generation Sequencing Test in Determining the Etiologic Diagnosis of Febrile Neutropenia: results from a pilot study. ID week October 4–8, 2017, San Diego.

76. Parize P, Muth E, Richaud C, et al. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. Clin Microbiol Infect 2017;23(8):574.e1-6.