Automation and Molecular Diagnostics: a New Era in Clinical Microbiology

JASON V. EVANS

LEARNING OBJECTIVES

1. Describe the 3 categories of nucleic-acid–based testing.
2. Describe the currently available and developing technology for the clinical microbiology laboratory, naming specific examples and the pros and cons of their implementation and use.
3. Describe the impact this newly implemented and future technology may have on clinical microbiology workflow, personnel training, and the education of future laboratory professionals.

ABSTRACT

The environment of the clinical microbiology laboratory is rapidly changing. Testing methods, based on organism growth with an array of liquid and solid media, are being replaced by newer methods. These new methods enhance the rate of organism identification and increase the sensitivity and specificity by which identification occurs. Most of these new techniques are based on nucleic hybridization and polymerase-chain–reaction technology. The techniques can range from identification of single organisms or organism families to multiplexed-syndromic panels, which can concurrently examine for the presence of numerous suspect organisms based on the symptoms exhibited by the patient. In addition, the clinical microbiology laboratory now has access to a level of automation thus far only seen in the chemistry and hematology sections of the clinical laboratory. These transitions have been repeatedly shown to enhance the level of patient care when properly implemented into the laboratory workflow. Conversely, with the rapid encroachment of these new technologies comes potential downfalls, which include cost and challenges with training laboratory staff. Collectively, the clinical microbiology laboratory is coming into a new era of technology and patient care that will bring about dramatic changes to conventional testing and organism identification.

ABBREVIATIONS: BCID - blood culture identification, BD - Becton Dickinson, CLIA - Clinical Laboratory Improvement Amendments, CNS - central nervous system, FDA - Food and Drug Administration, FDA-ARGOS - Food and Drug Administration Database for Regulatory-Grade Microbial Sequences, GI - gastrointestinal, MALDI-TOF - matrix-assisted laser desorption/ionization time-of-flight, NGS - next-generation sequencing, PCR - polymerase chain reaction, POC - point-of-care, STI - sexually-transmitted infection, TLA - total laboratory automation, WASP - Walk-Away Specimen Processor.

INDEX TERMS: molecular diagnostics, syndromic panels, next-generation sequencing, total laboratory automation.

Clin Lab Sci 2019;32(4):156–165

INTRODUCTION

The clinical microbiology laboratory has experienced rapid advancements in technology and automation in recent years. Culture-based testing methods remain the "gold standard" for most laboratories, but there have been several technology advancements incorporated that decrease result turnaround time and subsequently enhance patient care. The previous article for this focus series discusses at length the biochemical and traditional testing that is still a large component of organism identification as well as the increased incorporation of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technology. For this article, these methods will be referred to as "conventional" testing. The term "conventional" encompasses all culture-based methods that require a pure isolated colony for testing to occur. Although not yet a conventional method, MALDI-TOF technology still relies on pure isolates and will only be briefly discussed.

In this review, we will focus on recent advances in molecular diagnostics that have entered the microbiology laboratory, which include single-target polymerase chain reaction (PCR) based- and nucleic acid hybridization-based assays to the newest syndromic panels that are rapidly receiving Food and Drug Administration (FDA) approval. We will also discuss how this technology is being incorporated into point-of-care (POC) testing, classified as waived by the Clinical Laboratory Improvement Amendments (CLIA), and the associated advantages and pitfalls. Lastly, we will look into the future of the clinical microbiology laboratory with the potential incorporation of next-generation sequencing (NGS), metagenomics for

***Jason V. Evans**, West Virginia University*

***Address for Correspondence: Jason V. Evans**, West Virginia University, jason.evans@hsc.wvu.edu*

organism identification, and advancements in microbiology total laboratory automation (TLA). With all of the recent advances, there are many corporations developing new testing platforms that are rapidly receiving FDA-approval or will receive FDA-approval in the near future. Literature searches render many comprehensive reviews that discuss these new technologies, give detailed comparisons among platforms, and make compelling arguments for the pros and cons of each. The scope of this review is limited and geared toward a general audience and, therefore, cannot acknowledge every advancement or piece of literature. Any mention of specific technology, scientific studies, or reviews is in no way an endorsement of one over another.

SINGLE-TARGET MOLECULAR DIAGNOSTICS

The development of PCR by Cary Mullis[1] in 1985 is arguably one of the biggest contributions to science and modern medicine. The principles behind this technique have allowed science to decipher the genomes of many species and serve as the basis for the field of molecular diagnostics.[2] Some of the first molecular tests to become FDA-approved and introduced into the clinical microbiology laboratory were single-target tests that use PCR-based amplification techniques. These assays were followed by technique modifications such as transcription-mediated amplification, loop-mediated isothermal amplification, and helicase-dependent amplification, to name a few.[3] These single-target molecular tests use signal amplification and hybridization techniques.[4] One of the first areas of the clinical laboratory to greatly benefit from these advancements was the virology section. Molecular testing for viral pathogens in patient samples made way for the elimination of tedious and contamination-prone viral cultures using mammalian-cell systems. These culture methods can take weeks for a positive result, if any viable virus can be propagated at all.[5] Culture-based assays also require the clinical microbiology laboratory to have a designated biosafety cabinet, incubators, and reagents specific for mammalian-cell culture.[6] The introduction of molecular diagnostics eliminated this need and allowed for determination of viral presence directly from patient specimens with results determined in hours instead of days or weeks.[5] Arguably one of the largest impacts molecular testing has had on patient and public health is detecting HIV. The rapidness and sensitivity of molecular tests allow for early detection of the virus so that proper treatment regimens may be started sooner and—from the public health standpoint—provide the patient with the knowledge of their HIV status, which helps in the prevention of the spread of the disease. [5] Another example is the introduction of PCR-based testing for influenza A and B.[7] These influenza tests give very rapid results to the ordering physician so that patients may be started on appropriate antiviral therapies. The influenza tests also provide valuable epidemiologic information about the prevalence of the virus during yearly influenza seasons. As can be seen on the FDA website,[8] there are multiple testing platforms and kits that allow for the direct detection of viral presence. Of the PCR-based technologies listed all have their specific pros and cons, mainly surrounding variability in sensitivity and specificity,[2] but they collectively improve patient care. Selection of the ideal platform is a laboratory-specific determination that must be based on technical expertise as well as space and budget constraints.

Another area of microbiology testing that has benefitted from molecular advancements is the identification of sexually-transmitted infections (STIs).[2] STIs are noted public health issues[9] and some of the causative organisms are notoriously difficult or impossible to culture in standard laboratory settings (ie, *Chlamydia trachomatis* and *Trichomonas vaginalis*). Others, such as *Neisseria gonorrhoeae*, can cause asymptomatic infection—especially in women—leading to missed diagnoses. Using molecular-based testing for suspected STIs or STI screening, especially for individuals at higher risk or who engage in high-risk behaviors, greatly benefits public health because of more rapid testing and result reporting.[2,10]

These single-target assays have also been of great use to patient treatment and/or hospital management in terms of identification of drug-resistant organisms (methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, etc) and causes of nosocomial infections, such as *Clostridioides difficile*, which typically leads to patients being placed in isolation.[11] Recognition of these pathogens in a timely manner helps prevent the spread of these organisms throughout patient-care areas. Conversely, it also helps prevent unnecessary isolation procedures, which leads to reduced hospital costs.[12]

A summary of the number of available FDA-approved molecular tests for single organisms or organism groups (ie, same genus or subtypes) can be found in Table 1.

POINT-OF-CARE MOLECULAR DIAGNOSTICS

As seen with other methods employed in clinical microbiology laboratories (and other laboratory sections), once an assay or technique has been thoroughly vetted and validated the move to simplify the assay or technique and reduce laboratory hands-on time and overall costs is possible. The majority of POC testing in the past has been of the immunochromatography variety,[13] but the introduction of POC-molecular testing is coming to the forefront owing to "PCR-in-a-box" technology that uses a closed system, thus greatly reducing the potential for contamination and aberrant or incorrect results.[4] This type of testing has already been employed in the form of influenza A and B and Group A *Streptococcus* testing.[13] It is easy to see the utility of these types of tests in a POC setting, such as a walk-in clinic. Patients may receive their results while

Table 1. FDA-approved (nonpanel) molecular tests per organism/organism group

Organism	Number of Tests*	Organism	Number of Tests*
Adenovirus	3	Influenza and respiratory viruses	50
Bacillus anthracis	1	*Leishmania* spp.	1
Bordetella spp.	5	*Mycobacterium tuberculosis*	8
Candida spp.	6	*Mycobacterium* spp.	9
Clostridium difficile	17	*Mycoplasma genitalium*	1
Coxiella burnetii	1	*Mycoplasma pneumoniae*	2
Chlamydia trachomatis/Neisseria gonorrhoeae	47	Nonvariola orthopoxvirus	1
Cytomegalovirus	5	Norovirus	1
Dengue virus	1	*Plasmodium* spp.	1
Enterococcus spp.	6	*Rickettsia* spp.	1
Enterovirus	1	*Staphylococci*	19
Escherichia coli/Klebsiella pneumoniae/Pseudomonas aeruginosa	1	*Streptococci*	21
Francisella tularensis	1	Shiga toxin	1
Herpes simplex virus	18	*Trichomonas vaginalis*	7
Hepatitis virus	14	Variola	1
Human metapneumovirus	3	*Yersinia pestis*	1
Human papillomavirus	7		

*Modified from www.fda.gov nucleic-acid–based tests.

still in the examination room (in most instances), allowing rapid progression from diagnosis to prescribed treatment. A major benefit of this rapid testing is the minimization of an afflicted individual's contact with other people. Another benefit is that POC tests are designed to be foolproof by being simplistic in design and procedure; they are unlikely to give false results, thus allowing nonlaboratory personnel to run tests with success.[14] This, however, does give cause for concern and requires a watchful eye as more CLIA-waived tests come to the forefront because they often lack accredited laboratory professionals performing regular quality control and assurance.

SYNDROMIC PANELS

Syndromic panels are exciting new laboratory tools that are actively being used in many clinical microbiology laboratories. These multiplexed panels are a hot topic in clinical microbiology, and extensive reviews and studies have been published describing their design and clinical performance.[15-20] The highlights and capabilities of these systems will briefly be covered.

Multiplexed panels are referred to as "syndromic" because they are designed to test for a battery of organisms (viral, bacterial, fungal, and parasitic) that are commonly associated with a specific set of symptoms exhibited by the patient. A major advantage of these systems, which is attributed to their rapid introduction into the clinical laboratory, is that testing is performed directly from patient samples without the need for culturing isolated organisms. To date, panels for sepsis, respiratory tract infections, gastrointestinal (GI) tract infections, and central nervous system (CNS) (meningitis/encephalitis) infections are approved for clinical use.[15] BioFire Diagnostics's FilmArray (or FilmArray Torch) and Luminex Corporation's Verigene systems have panels for syndromes listed as previously mentioned. GenMark Diagnostics Inc's eSensor and ePlex systems offer a respiratory panel.

Sepsis

The search for a sensitive and rapid mean of organism/s identification from patients suspected of being septic is a major need in health care. The Centers for Disease Control and Prevention reports that 1 of 3 patients who die in a hospital have sepsis.[21] When a patient is admitted for sepsis, empirical antibiotic therapy is often administered until a specific organism is identified and susceptibilities are given. Most blood culture detection systems require some incubation time, and identification relies on conventional testing. To this end, blood culture panels have been designed to identify organisms from positive blood culture bottles. While the laboratory must wait for blood cultures to have positive results, these panels will expedite the identification process—compared with conventional methods—by allowing identification within 1–2.5 hours, depending on the organism and system used.[15]

The BioFire FilmArray blood culture identification (BCID) system can identify 27 bacterial and yeast pathogens with one assay that takes approximately 1 hour to complete. The Verigene system has 2 separate panels, 1 for gram-positive bacteria and 1 for gram-negative bacteria, that can detect 15 and 14 organisms within 2–2.5 hours, respectively.[15,17] Within the panel runtime, both systems are able to provide information on the presence of antibiotic resistance genes within isolates.[15,17] While this is not indicative of active antibiotic resistance, it may offer some guidance in the use of antibiotics. True antibiotic sensitivities must be collected through conventional testing. Collectively, these systems allow tailored antibiotic administration earlier (instead of empirical) and subsequently deliver better clinical outcomes.[22-25]

There are non-FDA–approved panels in development that can test blood samples directly without prior incubation. One such system is the T2Candida Panel from T2 Biosystems. T2Candida Panel has been shown to have similar sensitivity for the identification of 5 separate *Candida* species in conventional blood cultures.[26,27] This is a promising development for patients suspected of being septic, especially if this system or others like it can be modified to accommodate testing for a variety of other microorganisms.

Although not a syndromic panel-type platform as previously mentioned, a hybridization-based technology named the Accelerate Pheno system has been recently developed for blood cultures by Accelerate Diagnostics Inc. This system, using fluorescent *in situ* hybridization technology and automated single-cell microscopy, can detect gram-positive and gram-negative organisms directly from blood cultures and determine their antibiotic sensitivities by using a continuous colony growth monitoring system.[15]

Respiratory Tract Infections

There is more variety among available respiratory tract infection panels compared with the other panels discussed. All FDA-approved panels are multiplexed, use unique molecular-based detection systems, and offer quick turnaround times of 1 to 8 hours.[15,18] They also use nasopharyngeal swabs as the specimen of choice. Respiratory pathogen panels are offered on the BioFire's FilmArray, Verigene/Luminex systems, and GenDiagnostic's eSensor and ePlex systems. Each array offers their own variety of viral and bacterial respiratory pathogens and tests for a select sampling of bacteria that are notoriously challenging to culture (eg, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Bordetella* spp.). Compared with conventional assays, multiplexed testing for respiratory pathogens decreases the time to identification. The decreased time lowered hospital admission rates, required fewer chest X-rays, decreased length of hospital stays, and decreased duration of antimicrobial treatment (de-escalation of antibiotics).[28,29] The latter outcome is very important for antimicrobial stewardship because patients who arrive with respiratory illness symptoms that do not point to a specific diagnosis may be administered antibiotics as a precaution. Rapid identification of a viral infection leads to elimination of unnecessary antibiotic therapy, if initially started, thereby preventing the potential development of or selection for drug-resistant organisms.

Within the past year, BioFire released a pneumonia panel that specifically targets organisms typically found in lower respiratory tract infections. In contrast with the respiratory tract infection panels previously discussed, this pneumonia panel uses sputum or bronchoalveolar lavage specimens. Collectively, this panel can simultaneously report results for 33 separate targets including 26 pathogens (18 bacterial and 8 viral) and 8 antimicrobial resistance genes within 1 hour of startup.[30] Given the recent FDA-approval for this panel, there have been no published clinical studies comparing the sensitivity and specificity of this panel to conventional culture, but there is a study currently in early stages.[31] This panel will likely serve to fill a gap in the clinical microbiology laboratory, as a recent study demonstrated the efficacy of using the BioFire FilmArray BCID panel to test for organisms causing ventilator-associated pneumonia,[32] which is a non-FDA–approved use of this panel.

Gastrointestinal Tract Infections

Another available panel that has achieved FDA-approval is designed to potentially identify the causative agent(s) of infectious diarrhea. This is a rapid way to detect the most likely suspected organisms, as identification through conventional testing can require days. The Verigene system can detect a total of 9 targets (7 bacteria and 2 viral) and is scalable from 1 to 32 samples with a run time of less than 2 hours. The Luminex MAGPIX system can detect 20 separate pathogens (14 bacterial, 3 viral, and 3 parasitic) and can run 24 samples at a time. This system is not scalable and takes around 5 hours to complete the assay. Lastly, the BioFire FilmArray system/FilmArray Torch can target 22 pathogens (13 bacterial, 5 viral, and 3 parasitic) and is scalable from 1–12 assays, which take about 1 hour to reach completion.[15]

Advantages seen with these panels are that they collectively demonstrate a high sensitivity and specificity to the selected targets, with only a few exceptions, and typically catch organisms that are missed with conventional testing.[33-36] These panels can also quickly deliver information about whether a patient should be put in isolation procedures. This is critical in preventing the spread of nosocomial infections as well as saving costs by removing patients from isolation procedures days before conventional testing would allow.

The big question that remains (as with all panels) is how these GI panels will perform in day-to-day clinical microbiology laboratories. One caveat with these types of panels is that once identification is made there is not

an isolated colony for additional susceptibility testing and, therefore, no organism to send to state public health laboratories, as is necessary with organisms such as *Salmonella* spp., *Shigella* spp., or *Escherichia coli* O157:H7.[37,38] Another issue reported is that many assays will return multiple-target (2 or more) positive results in around 16% of samples tested.[33] This indicates that precaution must be taken with interpreting results and that the overall patient condition and symptoms must be closely examined in conjunction with panel results. For example, in a scenario where 2 or more pathogens may be encountered, a positive result for *C difficile* may have a very different meaning for a patient with community-acquired diarrheal disease than a patient who has been hospitalized. In this scenario, *C difficile* may very well be part of the average GI flora.[15] Again, the entire clinical scenario must be considered with these situations and indiscriminate treatment must be resisted for every organism that gives a positive result.

Central Nervous System Infections

Patients with meningitis and encephalitis are in danger of facing devastating outcomes associated with high morbidity and mortality rates.[39] Upon presentation of symptoms, such as altered mental/neural states, headaches, or light sensitivity, there is an urgent need for rapid diagnosis of the infectious agent. Prior to 2015, there were several stand-alone PCR-based tests for potential viral agents that can cause these diseases but none for bacterial or fungal organisms.[15] Adding to the potential delay in treatment, conventional methods of identification for bacteria and fungi can potentially take days. Confounding this method even further is the fact that conventional culturing methods may come back with negative results (no growth) if a patient has been prescribed empirical antibiotic therapy immediately on arrival with the aforementioned symptoms.

BioFire's FilmArray meningitis/encephalitis panel was the first to offer multiplexed viral, bacterial, and fungal assays for the most encountered agents of CNS infections. This panel tests for 14 different pathogens (7 viral, 6 bacterial, 1 fungal), and results are returned in roughly 1 hour with only 2 minutes of hands-on time by the laboratory staff.[15] The advantages of this panel are similar to the others discussed in that it shows a higher sensitivity and specificity for the target organisms compared with conventional testing.[40-44] The panel can also detect bacterial and fungal organisms after antibiotic/antifungal therapies have begun.[15] In terms of antimicrobial stewardship, similar to other panels, confirmation of a viral pathogen that causes the CNS infection allows earlier discontinuation of antimicrobial therapy.[15]

The drawbacks to the panel reflect its utility in everyday clinical use. Situational awareness with the ordering physician in interpreting data is crucial. Many of the organisms that can be detected are becoming rarer thanks to immunizations (eg, *Haemophilus influenzae* or *Neisseria meningitidis*). It may also be difficult for this assay to supplant some current rapid testing already performed in the laboratory; such an example is testing of cerebrospinal fluid for *Cryptococcus neoformans* antigen.[45-47]

Syndromic Panel Summary

Overall, multiplexed nucleic acid detection panels offer several advantages for diagnosing infections in the clinical microbiology laboratory. Clinical data show that the panels are superior to conventional methods in both sensitivity and specificity.[22-25,28,29,33,36,40-44] The risk for contamination from the laboratory setting is also low because these assays employ closed systems; however, aberrant or discrepant results must be scrutinized, along with regular control testing, to rule out potential system contamination because there are studies indicating this can be a concern with certain panels.[40] Currently, there are studies using existing panels to test other sterile fluid samples (eg, synovial or pleural fluids) for the presence of infectious organisms, and the reports show some success with this approach (although not an FDA-approved panel use).[48,49] There are also FDA-approved panels used for military and bioterrorism purposes, which test for the most common organisms that could be encountered in biologic warfare.[8] These panels also have the major benefit of identifying infectious agents in 1–5 hours, depending on which panel/platform is used. This rapid turnaround time has major benefits to patient care in terms of the beginning (or stopping) treatments and isolation practices and, in some instances, promoting shorter hospital stays.[15,16,18]

Conversely, a major issue with these panels is implementation into clinical practice. The panels are associated with a high cost and the potential for nonreimbursement from the Centers for Medicare and Medicaid Services.[45] It can be argued that these costs per test can be offset by the money saved through more appropriate patient treatment, which leads to shorter hospital stays.[15] This is, of course, if the panels are used appropriately and lead to the successful identification of the infectious agent. Panels are also not typically customizable, so if the organism present is not on the list then it will not be detected. Despite the rapidness of these assays and the fact they detect organisms directly from patient samples, they leave no isolated colonies for subsequent susceptibility studies for which conventional methods are still the gold standard. Although some can detect antibiotic resistance genes within the patient sample, 2 major caveats exist. The first caveat is that the presence of a resistance gene does not necessarily dictate that an organism will display functional resistance to a particular antimicrobial.[50,51] Secondly, in the instance of coinfections, the presence of a resistance gene cannot be attributed to a specific organism.

For these panels to have optimal success, communication is key between the ordering physician and the laboratory. These panels may lose their diagnostic potential

very rapidly if they are viewed as a screening test in which one can probe to see what might be present with no supportive reasoning. For optimal implementation, it will require assessment of local epidemiologic factors, such as particular disease incidence rates, to determine which panels would be appropriate for use. In other words, this assay should not ideally be used to test for rarely encountered organisms as a first step in diagnosis. It is of high importance that proper workflow assessments be made so that these tests are used only when there would be a direct benefit to patient care and cost effectiveness.

NEXT-GENERATION SEQUENCING

One of the newest technologies developed is NGS, which is accompanied by subsequent metagenomics analyses. NGS is promising in that it can examine every organism in a patient specimen without requiring specific probes or primer sets.[52] NGS technology sequences small segments of the entire microbial genome and then compares the sequences with established databases for identification. Pyrosequencing, one of the first NGS technologies developed, allows entire sequencing of 16S ribosomal RNA as well as whole genomes of microbes.[52] Since then, other methods have been introduced that enhance NGS diagnostics.[52]

The massive amount of information collected from whole-genome sequencing or the tailored identification of every organism in a biofilm or the gut biome is staggering, but exciting, in terms of the diagnostic information that can be obtained and used for patient care. This technology can deliver significantly more information on an isolate or disease state than any current identification method in the clinical microbiology laboratory, including multiplexed nucleic-acid detection and MALDI-TOF. NGS techniques can identify and differentiate organism serotypes and antibiotic resistance genes, and they can provide additional genomic detail about an organism that may be useful.[52-57] The FDA is in the process of creating the Food and Drug Administration Database for Regulatory-Grade Microbial Sequences (FDA-ARGOS), which is a regulatory-grade database for microbial sequences that would be the reference standard to which NGS-generated diagnostic sequences would be compared.[58]

Despite the powerful analytic capabilities of NGS, there are many caveats that act as a hindrance to its incorporation into clinical microbiology laboratories. First, the lack of FDA-approval is a significant hurdle; however, as just discussed, this should be alleviated in the near future once the FDA-ARGOS database is put into clinical use. Second, NGS in its current state is expensive and time-consuming compared with already available technology, such as multiplexed PCR testing.[59] Additionally, because NGS tests patient samples directly, therein lies the challenge of determining which organisms are colonizers/average flora or which are present in a pathologic state. Determination would be even more challenging in the setting of an immunocompromised patient, where the expected colonizer can very well be the source of disease. There is also the obstacle of how laboratories can implement and validate an instrument that can pick up any and all known pathogens, whereas current molecular testing uses preestablished groups of target organisms.[60] As technology improves and becomes more streamlined, associated costs become more justified, regulatory standards are set, and the ability to incorporate NGS in clinical laboratories will start to become more of a reality.[52,58-63]

TOTAL LABORATORY AUTOMATION

TLA and robotics have been a part of the clinical laboratory (especially chemistry and hematology) for decades; however, the microbiology laboratory has not progressed very much in this area. There is stand-alone automation in the microbiology laboratory, such as blood culture and identification systems (discussed in the previous focus series article); However, the idea of unified total automation, from specimen processing to release of patient results, is a new concept. A large reason for this is the variety of specimen types processed in the clinical microbiology laboratory. The variety has prevented a "one size fits all" approach to sample processing and distribution for culture, which would be a cornerstone for automation.

Liquid transport media has been suggested as a solution to specimen standardization[64] for total automation that also works well with the newest identification techniques in the microbiology laboratory, such as MALDI-TOF and multiplexed PCR.[65] The push for TLA is also driven by the often understaffed laboratory that is handling increased volumes of patient specimens sent in for testing.[65] Collectively, this makes automation very appealing because it can free up valuable time from what some may consider mundane or repetitive tasks such as media inoculation.

Currently, there are 2 systems for TLA in clinical microbiology: the Becton Dickinson (BD) Kiestra and the COPAN Diagnostics Inc Walk-Away Specimen Processor (WASP) Lab system. Both systems can store, label, and inoculate multiple types of media with samples and include a track system that will transport the inoculated plates to smart incubators.[66] Multiple studies have demonstrated that automating specimen inoculation alone can dramatically increase the quality and numbers of individually isolated colonies.[67-71] Additionally, automation may enhance the number of fastidious organisms isolated from urine samples.[72]

The aptly named smart incubators continuously monitor agar plate media for organism growth by using high-resolution imaging at regularly-timed intervals. These images can then be viewed by laboratorians at a workbench display where decisions can be made regarding further workup. The BD Kiestra is also able to deliver the

plates, upon request, to the workbenches should the laboratory scientist want to inspect them manually.[66]

The huge advantage of this type of automated analysis is that colony images can be shared with other laboratories, colleagues, and specialists around the globe. High-resolution imaging also leads to earlier single-colony detection, which may be sufficient for identification via MALDI-TOF systems.[66] A thorough review by Croxatto et al[66] describes in detail the similarities and differences in technical specifications between the BD Kiestra and COPAN WASPLab systems.

There are a number of obstacles in the way concerning the ubiquitous adoption of total automation in diagnostic laboratories, primarily the cost and size of the units. These automated laboratories are expensive and require space that may not be feasible to obtain with many current hospital infrastructures.[65] Another issue is that they are not inclusive of every culture and specimen type. Also, in the instance of a system or software crash or malfunction, backup processes and tests must still be completed. Overall, it is exciting to imagine this technology being incorporated into everyday clinical microbiology laboratories, but the reality is that it may not be ready for widespread use yet. It will take much deliberation to determine the best course of action for any particular laboratory or laboratory network to assess the feasibility of incorporating TLA.

DISCUSSION

Clinical microbiology laboratories are going through very exciting changes. New technology and vast improvements to existing technology are rapidly improving patient care and decreasing patient costs through reduction of the time it takes to identify infectious agents causing disease. Currently, diagnostic microbiology seems to be at a crossroad of conventional testing, implementation of advanced testing platforms, and full-scale automation. Admittedly, there are concerns surrounding this explosion of advanced technology. Laboratory directors and managers must scrutinize and decide what works best for their laboratory environment because a small rural hospital, if they still maintain a fully functioning microbiology laboratory at all, is not likely to see the same traffic as a larger hospital in an urban center. Therefore, the rural locale would likely not benefit as greatly by moving to a fully automated laboratory or incorporating the bulk of current and future advanced technology.

There is also concern that the new testing panels, with all of their advantages, will lure physicians into ordering tests that are unnecessary and potentially increase patient and laboratory costs.[15] On top of this, there is the issue of so much information being produced from these tests that interpretation of the actual meaning of the results could be made more difficult or altogether lost in translation. This could potentially lead to poorer patient outcomes as well as reversion to conventional methods to provide answers the panel could not provide. These are issues to take into consideration when incorporating new technology into existing laboratory workflows.

From an educational standpoint, these advances pose a conundrum in both the workforce and in the classroom. It has been estimated that the average age of a clinical microbiologist is over 50 years old.[65] This indicates that much of the current workforce has been taught, trained, and certified in conventional testing methodologies. The onus is placed on existing microbiologists to become competent and demonstrate expert judgement with technology rather than assume the "set it and forget it" style of newer technology platforms is error-free and, therefore, take all results at face value. In the classroom setting, the challenge lies with balancing the curriculum to match what is happening in the clinical laboratories. This leads to nearly double the amount of educational material because clinical laboratory educators are still currently held to the standard of teaching conventional methods as well as the principles behind the new technology that our graduates may encounter early on in their careers. Educators are now faced with the decision of whether to shift their curriculum further toward PCR-based and MALDI-TOF identification over conventional identification methods or to double their course content. Regardless of the path taken, the wheels of technology are spinning fast and watching the effects they have on clinical microbiology laboratories will be filled with a healthy mix of skepticism and wonder.

The technologic landscape of the clinical microbiology laboratory is rapidly evolving and does not appear to be slowing. In just a few decades, clinical microbiologists have witnessed the advances from solely culture-based manual-biochemical testing to automated-biochemical testing to the incorporation of MALDI-TOF organism identification. In addition to these impactful advances, PCR-based testing has already been deeply rooted into clinical microbiology testing. A key advancement associated with molecular testing is that it can be performed directly on patients' samples without the need for isolation of pure bacterial colonies, enhancing time to result, and—subsequently—patient care. Even now, current FDA-approved molecular assays continue to be improved in terms of sensitivity and number of targets in conjunction with the development of new assays. The excitement brought on by these changes is further compounded by the diagnostic potential for the next era of technologic progress, such as NGS and TLA. It will be thrilling to see what the future holds for the clinical microbiology laboratory.

REFERENCES

1. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harb Symp Quant Biol.* 1986;51 (Pt 1):263–273. doi: 10.1101/SQB.1986.051.01.032

2. Fairfax MR, Bluth MH, Salimnia H. Diagnostic molecular microbiology: A 2018 snapshot. *Clin Lab Med*. 2018;38(2):253–276. doi: 10.1016/j.cll.2018.02.004
3. Buchan BW, Ledeboer NA. Emerging technologies for the clinical microbiology laboratory. *Clin Microbiol Rev*. 2014; 27(4):783–822. doi: 10.1128/CMR.00003-14
4. Das S, Shibib DR, Vernon MO. The new frontier of diagnostics: molecular assays and their role in infection prevention and control. *Am J Infect Control*. 2017;45(2):158–169. doi: 10.1016/j.ajic.2016.08.005
5. Josko D. Molecular virology in the clinical laboratory. *Clin Lab Sci*. 2010;23(4):231–236. doi: 10.29074/ascls.23.4.231
6. Hematian A, Sadeghifard N, Mohebi R, et al. Traditional and modern cell culture in virus diagnosis. *Osong Public Health Res Perspect*. 2016;7(2):77–82. doi: 10.1016/j.phrp.2015.11.011
7. Wabe N, Li L, Lindeman R, et al. The impact of rapid molecular diagnostic testing for respiratory viruses on outcomes for emergency department patients. *Med J Aust*. 2019;210(7): 316–320. doi: 10.5694/mja2.50049
8. US Food and Drug Administration. Nucleic acid based tests: microbial tests. FDA.gov. 2019. Accessed July 12, 2019. https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests.
9. Low N, Broutet NJ. Sexually transmitted infections-research priorities for new challenges. *PLoS Med*. 2017;14(12): e1002481. doi: 10.1371/journal.pmed.1002481
10. Cristillo AD, Bristow CC, Peeling R, et al. Point-of-care sexually transmitted infection diagnostics: proceedings of the star sexually transmitted infection-clinical trial group programmatic meeting. *Sex Transm Dis*. 2017;44(4):211–218. doi: 10.1097/OLQ.0000000000000572
11. Josko D. Molecular bacteriology in the clinical laboratory. *Clin Lab Sci*. 2010;23(4):237–241. doi: 10.29074/ascls.23.4.237
12. Sewell B, Rees E, Thomas I, Ch'ng CL, Isaac M, Berry N. Cost and impact on patient length of stay of rapid molecular testing for clostridium difficile. *Infect Dis Ther*. 2014;3(2):281–293. doi: 10.1007/s40121-014-0034-x
13. Kozel TR, Burnham-Marusich AR. Point-of-care testing for infectious diseases: past, present, and future. *J Clin Microbiol*. 2017;55(8):2313–2320. doi: 10.1128/JCM.00476-17
14. US Food and Drug Administration. CLIA Categorizations. FDA.gov. 2018. Accessed July 12, 2019. https://www.fda.gov/medical-devices/ivd-regulatory-assistance/clia-categorizations.
15. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev*. 2017;31(1):e00024–17. doi: 10.1128/CMR.00024-17
16. Patel R. New developments in clinical bacteriology laboratories. *Mayo Clin Proc*. 2016;91(10):1448–1459. doi: 10.1016/j.mayocp.2016.06.020
17. Ward C, Stocker K, Begum J, Wade P, Ebrahimsa U, Goldenberg SD. Performance evaluation of the Verigene® (Nanosphere) and FilmArray® (BioFire®) molecular assays for identification of causative organisms in bacterial bloodstream infections. *Eur J Clin Microbiol Infect Dis*. 2015; 34(3):487–496. doi: 10.1007/s10096-014-2252-2
18. Hanson KE, Couturier MR. Multiplexed molecular diagnostics for respiratory, gastrointestinal, and central nervous system infections. *Clin Infect Dis*. 2016;63(10):1361–1367. doi: 10.1093/cid/ciw494
19. Huang RS, Johnson CL, Pritchard L, Hepler R, Ton TT, Dunn JJ. Performance of the Verigene® enteric pathogens test, Biofire FilmArray™ gastrointestinal panel and Luminex xTAG® gastrointestinal pathogen panel for detection of common enteric pathogens. *Diagn Microbiol Infect Dis*. 2016;86(4):336–339. doi: 10.1016/j.diagmicrobio.2016.09.013
20. Popowitch EB, O'Neill SS, Miller MB. Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. *J Clin Microbiol*. 2013;51(5):1528–1533. doi: 10.1128/JCM.03368-12
21. Centers for Disease Control and Prevention. Sepsis data and reports. CDC.gov. 2016. Accessed July 12, 2019. https://www.cdc.gov/sepsis/datareports/index.html.
22. Suzuki H, Hitomi S, Yaguchi Y, et al. Prospective intervention study with a microarray-based, multiplexed, automated molecular diagnosis instrument (Verigene system) for the rapid diagnosis of bloodstream infections, and its impact on the clinical outcomes. *J Infect Chemother*. 2015;21(12): 849–856. doi: 10.1016/j.jiac.2015.08.019
23. Beal SG, Thomas C, Dhiman N, et al. Antibiotic utilization improvement with the Nanosphere Verigene Gram-Positive Blood Culture assay. *Proc Bayl Univ Med Cent*. 2015; 28(2):139–143. doi: 10.1080/08998280.2015.11929214
24. Walker T, Dumadag S, Lee CJ, et al. Clinical impact of laboratory implementation of Verigene BC-GN microarray-based assay for detection of gram-negative bacteria in positive blood cultures. *J Clin Microbiol*. 2016;54(7):1789–1796. doi: 10.1128/JCM.00376-16
25. Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK. Rapid testing using the Verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. *Antimicrob Agents Chemother*. 2015;59(3):1588–1595. doi: 10.1128/AAC.04259-14
26. Pfaller MA, Wolk DM, Lowery TJ. T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis. *Future Microbiol*. 2016;11(1):103–117. doi: 10.2217/fmb.15.111
27. 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(6): 892–899. doi: 10.1093/cid/ciu959
28. Subramony A, Zachariah P, Krones A, Whittier S, Saiman L. Impact of multiplex polymerase chain reaction testing for respiratory pathogens on healthcare resource utilization for pediatric inpatients. *J Pediatr*. 2016;173:196–201.e2. doi: 10.1016/j.jpeds.2016.02.050
29. Rogers BB, Shankar P, Jerris RC, et al. Impact of a rapid respiratory panel test on patient outcomes. *Arch Pathol Lab Med*. 2015;139(5):636–641. doi: 10.5858/arpa.2014-0257-OA
30. bioMérieux. The BioFire® FilmArray® pneumonia (PA) panel: syndromic infectious disease testing for pneumonia. biofiredx.com. 2019. Accessed July 12, 2019. https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/.
31. National Institutes of Health; National Library of Medicine. Prospective clinical evaluation of the FilmArray® lower respiratory tract infection (LRTI) panel (LRTI). ClinicalTrials.gov. 2017. Accessed July 12, 2019. https://clinicaltrials.gov/ct2/show/NCT03361670.
32. Pulido MR, Moreno-Martínez P, González-Galán V, et al; MagicBullet Working Group. Application of BioFire FilmArray blood culture identification panel for rapid identification of the causative agents of ventilator-associated pneumonia. *Clin Microbiol Infect*. 2018;24(11):1213.e1–1213.e4. doi: 10.1016/j.cmi.2018.06.001
33. Spina A, Kerr KG, Cormican M, et al. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis. *Clin Microbiol Infect*. 2015;21(8):719–728. doi: 10.1016/j.cmi.2015.04.007
34. US Food and Drug Administration. 510(k) substantial equivalence determination decision summary. FDA.gov. 2014. Accessed July 12, 2019. https://www.accessdata.fda.gov/cdrh_docs/reviews/k140407.pdf.

35. Buss SN, Leber A, Chapin K, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. *J Clin Microbiol*. 2015;53(3):915–925. doi: 10.1128/JCM.02674-14
36. Khare R, Espy MJ, Cebelinski E, et al. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. *J Clin Microbiol*. 2014;52(10):3667–3673. doi: 10.1128/JCM.01637-14
37. Gebrehiwot SA, Rucinski SL, Schwab JJ, Patel R, Snippes P. "Reflexive culture"-a strategy for laboratories adopting molecular testing for enteric pathogens. Poster 188 presented at: ASM Microbe; 2016; Boston, MA.
38. Shane AL, Mody RK, Crump JA, et al. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. *Clin Infect Dis*. 2017;65(12):1963–1973. doi: 10.1093/cid/cix959
39. Giovane RA, Lavender PD. Central nervous system infections. *Prim Care*. 2018;45(3):505–518. doi: 10.1016/j.pop.2018.05.007
40. Leber AL, Everhart K, Balada-Llasat JM, et al. Multicenter evaluation of BioFire FilmArray meningitis/encephalitis panel for detection of bacteria, viruses, and yeast in cerebrospinal fluid specimens. *J Clin Microbiol*. 2016;54(9):2251–2261. doi: 10.1128/JCM.00730-16
41. Hanson KE, Slechta ES, Killpack JA, et al. Preclinical assessment of a fully automated multiplex PCR panel for detection of central nervous system pathogens. *J Clin Microbiol*. 2016;54(3):785–787. doi: 10.1128/JCM.02850-15
42. Launes C, Casas-Alba D, Fortuny C, Valero-Rello A, Cabrerizo M, Muñoz-Almagro C. Utility of FilmArray meningitis/encephalitis panel during outbreak of brainstem encephalitis caused by enterovirus in Catalonia in 2016. *J Clin Microbiol*. 2016;55(1):336–338. doi: 10.1128/JCM.01931-16
43. Messacar K, Breazeale G, Robinson CC, Dominguez SR. Potential clinical impact of the film array meningitis encephalitis panel in children with suspected central nervous system infections. *Diagn Microbiol Infect Dis*. 2016;86(1):118–120. doi: 10.1016/j.diagmicrobio.2016.05.020
44. Graf EH, Farquharson MV, Cárdenas AM. Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population. *Diagn Microbiol Infect Dis*. 2017;87(1):92–94. doi: 10.1016/j.diagmicrobio.2016.09.022
45. Schreckenberger PC, McAdam AJ. Point-counterpoint: large multiplex PCR panels should be first-line tests for detection of respiratory and intestinal pathogens. *J Clin Microbiol*. 2015;53(10):3110–3115. doi: 10.1128/JCM.00382-15
46. Doern CD, Lacey D, Huang R, Haag C. Evaluation and implementation of FilmArray version 1.7 for improved detection of adenovirus respiratory tract infection. *J Clin Microbiol*. 2013;51(12):4036–4039. doi: 10.1128/JCM.02546-13
47. Midgley CM, Watson JT, Nix WA, et al; EV-D68 Working Group. Severe respiratory illness associated with a nationwide outbreak of enterovirus D68 in the USA (2014): a descriptive epidemiological investigation. *Lancet Respir Med*. 2015;3(11):879–887. doi: 10.1016/S2213-2600(15)00335-5
48. Michos A, Palili A, Koutouzis EI, Sandu A, Lykopoulou L, Syriopoulou VP. Detection of bacterial pathogens in synovial and pleural fluid with the FilmArray blood culture identification system. *IDCases*. 2016;5:27–28. doi: 10.1016/j.idcr.2016.05.006
49. Vasoo S, Cunningham SA, Greenwood-Quaintance KE, et al. Evaluation of the FilmArray blood culture ID panel on biofilms dislodged from explanted arthroplasties for prosthetic joint infection diagnosis. *J Clin Microbiol*. 2015;53(8):2790–2792. doi: 10.1128/JCM.01333-15
50. Fluit AC, Visser MR, Schmitz FJ. Molecular detection of antimicrobial resistance. *Clin Microbiol Rev*. 2001;14(4):836–871. doi: 10.1128/CMR.14.4.836-871.2001
51. Milatovic D, Braveny I. Development of resistance during antibiotic therapy. *Eur J Clin Microbiol*. 1987;6(3):234–244. doi: 10.1007/BF02017607
52. Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Application of next generation sequencing in clinical microbiology and infection prevention. *J Biotechnol*. 2017;243:16–24. doi: 10.1016/j.jbiotec.2016.12.022
53. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA*. 2005;102(31):11070–11075. doi: 10.1073/pnas.0504978102
54. Qin J, Li R, Raes J, et al; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59–65. doi: 10.1038/nature08821
55. Graessler J, Qin Y, Zhong H, et al. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. *Pharmacogenomics J*. 2013;13(6):514–522. doi: 10.1038/tpj.2012.43
56. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472(7341):57–63. doi: 10.1038/nature09922
57. Price KE, Hampton TH, Gifford AH, et al. Unique microbial communities persist in individual cystic fibrosis patients throughout a clinical exacerbation. *Microbiome*. 2013;1(1):27. doi: 10.1186/2049-2618-1-27
58. US Food and Drug Administration. Database for reference grade microbial sequences (FDA-ARGOS). FDA.gov. 2018. Accessed July 12, 2019. https://www.fda.gov/medical-devices/science-and-research-medical-devices/database-reference-grade-microbial-sequences-fda-argos.
59. Rossen JWA, Friedrich AW, Moran-Gilad J; ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD). Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology. *Clin Microbiol Infect*. 2018;24(4):355–360. doi: 10.1016/j.cmi.2017.11.001
60. Goldberg B, Sichtig H, Geyer C, Ledeboer N, Weinstock GM. Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. *MBio*. 2015;6(6):e01888–e15. doi: 10.1128/mBio.01888-15
61. Long SW, Williams D, Valson C, et al. A genomic day in the life of a clinical microbiology laboratory. *J Clin Microbiol*. 2013;51(4):1272–1277. doi: 10.1128/JCM.03237-12
62. Fricke WF, Rasko DA. Bacterial genome sequencing in the clinic: bioinformatic challenges and solutions. *Nat Rev Genet*. 2014;15(1):49–55. doi: 10.1038/nrg3624
63. Greninger AL. The challenge of diagnostic metagenomics. *Expert Rev Mol Diagn*. 2018;18(7):605–615. doi: 10.1080/14737159.2018.1487292
64. Novak SM, Marlowe EM. Automation in the clinical microbiology laboratory. *Clin Lab Med*. 2013;33(3):567–588. doi: 10.1016/j.cll.2013.03.002
65. Ledeboer NA, Dallas SD. The automated clinical microbiology laboratory: fact or fantasy? *J Clin Microbiol*. 2014;52(9):3140–3146. doi: 10.1128/JCM.00686-14
66. Croxatto A, Prod'hom G, Faverjon F, Rochais Y, Greub G. Laboratory automation in clinical bacteriology: what system to choose? *Clin Microbiol Infect*. 2016;22(3):217–235. doi: 10.1016/j.cmi.2015.09.030
67. Burckhardt I. Laboratory automation in clinical microbiology. *Bioengineering (Basel)*. 2018;5(4):E102. doi: 10.3390/bioengineering5040102

68. Moreno-Camacho JL, Calva-Espinosa DY, Leal-Leyva YY, Elizalde-Olivas DC, Campos-Romero A, Alcántar-Fernández J. Transformation from a conventional clinical microbiology laboratory to full automation. *Lab Med*. 2017;49(1):e1–e8. doi: 10.1093/labmed/lmx079
69. Croxatto A, Dijkstra K, Prod'hom G, Greub G. Comparison of inoculation with the InoqulA and WASP automated systems with manual inoculation. *J Clin Microbiol*. 2015;53(7):2298–2307. doi: 10.1128/JCM.03076-14
70. Mischnik A, Trampe M, Zimmermann S. Evaluation of the impact of automated specimen inoculation, using Previ Isola, on the quality of and technical time for stool cultures. *Ann Lab Med*. 2015;35(1):82–88. doi: 10.3343/alm.2015.35.1.82
71. Froment P, Marchandin H, Vande Perre P, Lamy B. Automated versus manual sample inoculations in routine clinical microbiology: a performance evaluation of the fully automated InoqulA instrument. *J Clin Microbiol*. 2014;52(3):796–802. doi: 10.1128/JCM.02341-13
72. Lainhart W, Burnham CA. Enhanced recovery of fastidious organisms from urine culture in the setting of total laboratory automation. *J Clin Microbiol*. 2018;56(8):e00546–18. doi: 10.1128/JCM.00546-18