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New Technologies in Clinical Microbiology

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Rapid identification of microorganisms in the clinical microbiology laboratory can be of great value for selection of optimal patient management strategies for infections caused by bacteria, viruses, fungi, mycobacteria, and parasites. Rapid identification of microorganisms in clinical samples enables expedient de-escalation from broad-spectrum agents to targeted antimicrobial therapy. The switch to tailored therapy minimizes risks of antibiotics, namely, disruption of normal flora, toxic side effects, and selective pressure. There is a critical need for new technologies in clinical microbiology, particularly for bloodstream infections, in which associated mortality is among the highest of all infections. Just as importantly, there is a need for the clinical laboratory community to embrace the practices of evidence-based interventional laboratory medicine and collaborate in translational research projects to establish the clinical utility, cost benefit, and impact of new technologies.

The topic "new technologies" described here was part of a group session entitled Clinical Microbiology in the Year 2015, part of the 2011 Camp Clin Micro meeting held in Houston, TX. The discussion focused on new and emerging laboratory methods, specifically those related to identification of bloodstream infections (BSIs), which are among the most critical of any infections characterized in clinical microbiology.

Rapid identification of BSIs and the associated condition known as sepsis syndrome are of particular importance because of associated morbidity and mortality. Sepsis, a common sequela of BSIs, ranks among the top 10 causes of death in the United States, where over 600 patients die each day (11, 12, 17). Furthermore, the incidence of BSI is expected to increase by as much 10% annually in the coming years (1, 7, 8, 12, 17), adding an enormous social and economic burden to U.S. health care systems. Estimated costs exceed $17 billion dollars per year (1, 7, 12).

According to the literature, the risk of death from septic shock increases by over 7% with every hour that passes from the onset of shock until the start of targeted therapy (34). A number of other studies confirm the urgency of rapid identification of pathogens and its benefit to survival and costs and the effective targeting of antimicrobial therapy (13, 25, 29, 33, 45).

Unfortunately, when laboratory methods rely solely on cultivation of pathogens and traditional phenotypic methods of pathogen characterization, physicians are forced to deduce the presence of BSI based on clinical symptoms, which are often nonspecific. Subsequently, antibiotic therapy is initiated based on clinical and epidemiologic profiles (28) rather than on laboratory evidence. Typically, within 1 to 3 days a microscopic Gram stain category (Gram-positive bacteria, Gram-negative bacteria, or fungi) provides physicians with a general direction for antibiotic therapy. Definitive results that eliminate the need for broad-spectrum therapy and enable de-escalation and the tailoring of treatment to the most effective antibiotic regimen often require more than 3 to 5 days. This gap has been implicated as one reason for high mortality and the emergence of drug-resistant microbes. The gap is even more prolonged for organisms that are fastidious, slow growing, noncultivable, or present as part of polymicrobial infections. Rapid de-escalation to narrow-spectrum antimicrobial therapy is a key aim of Surviving Sepsis Campaign guidelines created to reduce mortality for sepsis and BSI (13, 29).

There is evidence that laboratory interventions that decrease reporting time can be effective. Efforts to demonstrate the benefit of rapid reporting of Gram stain results showed that mortality decreased by 17% when Gram stain reports from positive blood culture (BC) bottles were reported in less than 1 h from the instrument flag (4). Furthermore, work performed by Doern et al. and Barenfanger et al. indicated that more timely interpretation of data from antimicrobial susceptibility testing (AST) can greatly impact patient outcomes (3, 15).

Despite the evidence showing that the rapid administration of an effective antibiotic saves lives (13, 25, 29, 33, 45), tools in clinical microbiology are primarily based on techniques that evolved 30 to 40 years ago (18). There is an urgent need for new technologies in microbiology to circumvent the issue of lag time for reports from positive blood cultures and other cultures.

Several technological advances using simple molecular methods have become available in recent years and show potential for cost benefit, specifically, peptide nucleic acid fluorescent *in situ* hybridization (PNA-FISH) (21–24, 36, 40) and targeted real-time PCR such as GeneXpert assays (5, 48).

PNA-FISH has been the technology most studied in terms of the collaboration between pharmacy and medical intervention. In 2006, Forrest et al. demonstrated that PNA-FISH could be used to distinguish *Staphylococcus aureus* species from coagulase-negative staphylococci (CoNS). This study surveyed the impact on hospital costs, length of patient stay, and the use of vancomycin. The implementation of this strategy was linked to lower hospital costs (approximately $4,000 lower per patient), decreased length of stay (~2 days), and decreased use of vancomycin (23). A follow-up study performed by Ly et al. (36)

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showed a similar reduction in length of stay (2 days) for routine patients and a 7-day reduction for patients in intensive care units (ICUs). The median cost reduction was $19,441 per patient. Most dramatic was the overall reduction in mortality rate, which fell from 16.8% to 7.9%. Specifically in the ICU, mortality was reduced from 47.8% to 9.5%. These benefits emphasize the positive impact of rapid molecular testing in the clinical laboratory. More evidence-based studies to characterize the utility of these and other PNA-FISH assays, such as those for *Candida* species and Gram-negative rods, are warranted.

In addition to PNA-FISH, a real-time PCR method is available for rapid testing of blood culture bottles. The benefit of the Xpert MRSA/SA BC assay (for methicillin-resistant *S. aureus*/*S. aureus* blood culture test) was assessed, and clinical improvements, such as decreased length of stay and health care costs, were documented in a set of 156 patients. In this study, the mean time to switch from empirical vancomycin to cefazolin or nafcillin in patients with methicillin-susceptible *S. aureus* bacteremia was 1.7 days shorter post-PCR ($P = 0.002$). In the post-PCR methicillin-susceptible and methicillin-resistant *S. aureus* groups, the mean length of stay was 6.2 days shorter ($P = 0.07$), and the mean hospital costs were $21,387 less ($P = 0.02$).

Both PNA-FISH and real-time PCR methods are easy to perform and can be readily adapted into the laboratory work flow. These technologies can complement existing practices such as rapid reporting of Gram stain results (3) for better patient management. Both PNA-FISH and real-time PCR hold individual merit, but limitations exist because of the limited menu of test organisms. In addition, each method requires time to gather supplemental information, such as Gram stain, to permit cost-effective assay/kit selection. The technologies would benefit from the addition of a multiple-target approach.

Multiplex and broad-range molecular diagnostics hold the promise of decreased time to results and increased sensitivity compared to growth-based blood culture systems but have not yet been fully exploited. To date, there are no existing clinical laboratory methods with equivalent analytical sensitivity comparable to that of routine blood cultures. Methods that allow for the simultaneous detection of multiple targets could provide a huge advantage for molecular-based methods by providing identification and resistance information concurrently. These methods are currently accurate for a small subset of microorganisms but show promise in terms of clinical and fiscal benefits. Broad-range strategies that aim to characterize bacteria and yeast and other microbes and that do not require prior knowledge of the genetic target are considered most desirable.

There are thousands of pathogens known to cause disease in humans, and standard PCR assays have been developed to detect the most commonly occurring pathogens. To meet the analytical needs of broad-range microbial identification, two additional strategies are currently in development for clinical laboratories: pyrosequencing and multitarget PCRs.

Pyrosequencing methods, while accurate (30, 42), make large demands on the technician's time to assess and compare sequences. The cost of the instrument is relatively high, but utility has been documented (31). The technology's use has been mostly limited to identification of microbial isolates and research for the identification of pathogens in blood culture bottles.

Real-time PCR methods are undergoing evaluation, but concordance with routine methods needs improvement. The Roche Molecular System LightCycler SeptiFast (47) is available and has been evaluated for rapid detection of specific organisms in blood culture bottles. The performance of SeptiFast for positive blood cultures is evolving. The concordance of SeptiFast and BC for both positive and negative samples was only 86.0% (46), and to date no clinical utility studies have been published.

Recently, SepsiTest from Molzym was evaluated. The SepsiTest uses a universal PCR from the 16S rRNA gene, with subsequent identification of bacteria from positive samples by sequence analysis of amplicons. Compared to blood culture (BC), the diagnostic sensitivity and specificity of the PCR were 87.0 and 85.8%, respectively ($n = 342$) (46).

Promising new broad-based techniques have merit, based on their ability to identify organisms difficult to culture or newly emerging strains, as well as their capacity to track disease transmission. Two techniques revolve around mass spectrometry (MS) technology: PCR combined with electrospray ionization-mass spectrometry (PCR/ESI-MS) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS).

Both mass spectrometry methods leverage the high sensitivity of mass spectrometry with links to large microbial databases, which enable users to obtain species-specific spectra that can be used to reproducibly identify microorganisms. Use of mass spectrometry methods is challenged by the high costs of instruments, yet there is potential for these technologies to supplant the foundation of clinical microbiology, replacing most biochemical testing as we know it. Both of these approaches require the purchase of a dedicated mass spectrometer and software package, with PCR/ESI-MS approximately two to three times the cost of MALDI-TOF MS. Expected differences occur for the day-to-day cost of consumables, which varies between high-cost PCR/ESI-MS and lower-cost MALDI-TOF MS. PCR/ESI-MS requires DNA extraction reagents used for PCR, such as buffers, enzymes, and primers, which result in higher cost per sample. MALDI-TOF MS requires only the medium to culture the organism and a small quantity of matrix (typically, less than 1 μg/sample), the cost of which is negligible in comparison to PCR/ESI-MS, for which additional costs provide additional capabilities. Overall, both methods show promise for both routine and, in some cases, epidemiological use in hospital settings. These two techniques, in a direct comparison, show no statistically significant differences in their performance. This report emphasizes the key differences between the two techniques, both of which will fill unique niches in the clinical microbiology laboratory.

PCR/ESI-MS

PCR/ESI-MS instruments measure the mass/charge ratio (m/z) of amplicons, generated by multiplex PCRs that target several loci within bacterial or fungal genomes. The method targets both conserved and species-specific genetic regions to identify microbes based on amplicon base compositions relative to a known database of microorganisms (16). Using the

Abbott PLEX-ID or the Ibis T5000 instruments, PCR/ESI-MS is known for its ability to directly detect and identify bacteria and associated antibiotic resistance genes, viruses, fungi, and mycobacteria from clinical specimens and from isolates. Most recently, the method produced highly accurate results when used to identify pathogens directly from blood culture bottles (32). Genetic identification allows for discrimination to the strain type and allows identification of antimicrobial resistance genes. Through the use of multiple consensus primers, this method can detect target gene sequences despite silent mutations or genetic rearrangement, for example, in influenza virus (43).

PCR/ESI-MS offers extended utility for epidemiological surveillance and infection control. Testing can occur from bacterial isolates or directly from the clinical specimen. PCR/ESI-MS requires approximately 4 to 6 h for specimen testing. The method can identify mixtures of up to three to four microbes but requires the batching of six samples at a time. While protocols exist, errors in interpretation can occur when combinations of staphylococci and streptococci or of staphylococci and enterococci are found in the same sample (32). In these situations, one organism is preferentially amplified and identified. Misidentifications also occur when large differences in the bacterial densities of microbes are observed for mixed infections, such that only one of the two organisms is detected. Errors also occur for mixed infections for which the second identification is due to an incompletely populated database or genetically similar organisms belonging to the *Enterobacteriaceae* family. Future additions to the database with isolates from this family should resolve many of the issues for PCR/ESI-MS (32).

MALDI-TOF MS

Analysis of whole cells was first proposed in 1975 to study the biomarker profile of various bacterial species following pyrolysis-mass spectrometry for low-molecular-weight products (2). However, it was not until 1996 that the first MALDI-TOF MS experiment was successful in identifying bacteria directly from whole colonies based on protein biomarkers (10, 26). A great number of developments have been made over the last decade on whole-organism MALDI-TOF MS. The protein biomarkers that are measured in mass spectrometry of microorganisms are highly expressed proteins responsible for housekeeping functions, such as ribosomal, chaperone, and transcription/translation factor proteins.

In contrast to PCR/ESI-MS, MALDI-TOF MS relies on identification of protein profiles derived from highly conserved proteins and currently identified microbes, bacteria, fungi, and mycobacteria, to the species level. For MALDI-TOF, the protein profiles are generated from direct ionization of an intact colony or a bacterial protein extract after manual extraction. Identification occurs after a protein's spectral signature is correlated to a database of spectra collected from reference strains. MALDI-TOF MS, in its current form, requires subculture prior to identification. An advantage of MALDI-TOF MS is that it does not require batching.

MALDI-TOF MS is widely used because of its high accuracy, low consumable cost, and speed of analysis. A typical experiment consists of outgrowth of bacteria, colony selection and placement on a target, addition of matrix, and analysis with MALDI-TOF MS. Mass spectrometry identification is broadband such that the method can measure multiple analytes simultaneously, does not require prior knowledge about the organism, and is both fast and sensitive in that it does not require a prefractionation step. It generally measures all m/z between 2 and 20 kDa. Several reviews are available on this topic for a more in-depth overview (14, 19, 35).

Commercial systems exist that can integrate with traditional antimicrobial susceptibility systems. The MALDI Biotyper (Bruker Daltonics) has proven accuracy in the identification of bacteria (37), even for rare or fastidious bacteria, which are often a challenge to clinical laboratories using phenotypic identification evaluation due to their limited biochemical reactivity. Identification of yeast has also been reported (38). The method finds common use in Europe, where clinical microbiologists are using it to rapidly identify microbial colonies isolated from culture. Another MALDI-TOF system, the Vitek MS (bioMérieux), shows similar capabilities to the Bruker system. No statistically significant difference was identified between the two platforms for general bacteria (9).

A few reports show promise for the identification of microorganisms using MALDI-TOF MS without subculture (20, 39, 41, 44). Recent evaluations showed promise, but the methods were limited by the need for a large number of cells; adaptations showed successful identification for only approximately 80% of blood cultures. A promising new technology, the MALDI Sepsityper system (Bruker Daltonics), is under evaluation and aims to identify bacteria and yeast directly from positive blood culture bottles. If successful, the MALDI Sepsityper has the potential to reduce the time-to-identification for many different species.

It is important to note some of the current limitations associated with MALDI-TOF MS. The outgrowth of organisms from potentially contaminated material is still required in order to obtain isolated colonies of organisms as the technique's ability to resolve mixtures is lacking. Additionally, a high number of bacterial cells are required for identification, such that a whole intact colony is typically used for analysis, limiting the ability to rapidly identify microorganisms directly from biological fluids where the bacterial count is expected to be relatively low. Research is currently being done to mitigate some of these requirements. Finally, until accurate determinations of resistance factors can be made, parallel culture-based recovery of positive blood cultures will most certainly be required for the foreseeable future for antimicrobial susceptibility testing.

QUESTIONS DISCUSSED AND RECOMMENDATIONS: DOES MASS SPECTROMETRY ADD BENEFIT TO LABORATORIES IN TERMS OF TURNAROUND TIME AND/OR OUTCOMES?

General recommendations. (i) There is a need for creation of clinical laboratory consortiums or user groups to combine data from multisite investigations of new technology to meet Clinical Laboratory Improvement Act (CLIA) requirements for laboratory-developed test (LDT) method verification. These consortiums could share specimens, such as rare blood culture isolates, and provide feedback to achieve the ultimate goal of rapid assessment of new technology. Multisite evalua-

tion networks, sharing a common ground with an organization such as the American Society for Microbiology (ASM), would allow users to quickly and easily see deidentified results of technology assessment across the United States, increasing the statistical power of any evaluation in terms of accuracy, precision, turnaround time, etc. While ensuring accurate data is the critical first step to technology implementation, it is clearly not the only critical aspect; the cost-benefit model must be fully justified. Many organizations were verbally reporting requirements by administrators for a capital return on investment in 1 to 2 years. This is an almost impossible task for clinical laboratories to achieve without some form of group collaboration and assessment of recovered costs and overall hospital savings. The laboratory budget cannot be considered on its own for some of the new technology platforms; benefits must be documented at the level of the health care organization itself. These assessments require organizational collaboration with finance, pharmacy, and laboratories. Building clinical microbiology networks would enable faster translation of new technology as well as much faster assessment of the cost benefit of new technology. Virtual meeting venues, software to links specimen biorepositories, encrypted data, and access to biostatisticians were among the key infrastructure elements identified as capabilities that the clinical laboratory would need to create before such a plan could succeed. These networks could be used as venues for clinical trial participation when an assay enters the FDA pathway or for assessment of evidence-based laboratory intervention programs for new technology. Standardized methods evaluation protocols, in compliance with the CLIA requirements, and statistically based assessment of new technology will be imperative for laboratories to create bench-to-bedside pipelines for rapid evaluations and, if warranted, adoption of new technology. These consortiums could also work through ASM to help ensure appropriate reimbursements for testing of multiplex or broad-range technology. Finally, member laboratories could share business model strategies successfully employed for the purchase of high-cost technologies in which "fill-in-the-blank" financial formats are provided.

(ii) Formalized consortiums between the clinical microbiology professionals, the infectious disease pharmacy professionals, and the medical community to plan the cost benefit, clinical utility, and antimicrobial stewardship experiments could occur in conjunction with initial laboratory use. Such collaboration at the national and local level should enable more accurate and timely assessment of the impact of new technologies on patient care and provide financial evaluation of the technology impact. Recent literature suggests that these collaborations may be critical to the success of new technologies to drive antibiotic de-escalation and thus decrease mortality, decrease costs, and support antimicrobial stewardship (27).

(iii) Culture methods are not obsolete, nor will they be in the future. Even with the advent of increased molecular-based and protein-based microbial identification testing, there is an enormous need to continue culture-based testing to assess susceptibility for all antimicrobials and to identify microbes with mutations that may escape detection by new technology.

(iv) As more-rapid microbiology test methods become standards of care and as evidence-based studies prove the benefit of rapid testing and interpretation of microbiology results, laboratories will need to maintain or expand their clinical practices to operate around the clock every day of the year, expanding coverage for microbiology testing on all shifts. The evidence is clear in its support of rapid and targeted antibiotic administration, a practice that is driven by the speed in which the laboratory can provide an actionable result. Notification of positive blood culture bottles and associated pathogen identification appear to be vital components to drive the de-escalation process; therefore, laboratories must expand collaborations with pharmacists and physicians and strive for rapid de-escalation of broad-spectrum antibiotics to optimize patient care and reduce mortality.

(v) Initiatives for publication of diagnostic method evaluation exist, and the working group urges adoption of initiatives, such as the STARD (standards for reporting of diagnostic accuracy) program (6). Authors could elect to meet the STARD criteria for their publication, which would increase its value and enable easier and more standardized comparison of publications for meta-analysis, evidence-based review, or general use.

(vi) In order for the clinical laboratory to assess the effectiveness of new technology and outcomes related to laboratory interventions, laboratorians must begin to think and train differently, with more emphasis on evidence-based interventions. There is a critical need to undertake initiatives to manage the large amounts of bioinformatics data that will soon be created by multiplex and broad-range technology platforms. Likewise, there is a critical need to undertake initiatives to manage the large amounts of medical and financial informatics required to assess the utility and cost benefit of new technology. Clinical laboratory science (CLS) programs should consider adding curricula to introduce medical informatics, bioinformatics, health care management, and financial management, at least at the level of master's degree programs. The American Board of Medical Microbiology Committee on Postgraduate Education Programs (CPEP) should offer the same type of curricula at the doctoral level. Clinical microbiology organizations are urged to offer certificate programs or continuing medical education (CME) to meet the future workplace needs in medical informatics and bioinformatics. In addition, we urge laboratory scientists at all levels of the profession to undertake management course work or degrees such as a master's in health administration. Additionally, increased training in biostatistics is warranted in support of quality improvement initiatives as well as translational and clinical research efforts. Clinical microbiology organizations are urged to offer certificate programs or CME to meet the workplace educational needs for management, financial planning, biostatistics, and bioinformatics.

Recommendations for mass spectrometry instrument vendors and the FDA. (i) We encourage regulatory agencies to recognize the urgent need for MALDI-TOF and similar rapid detection technologies for identification of pathogens directly from blood culture bottles, as well as to identify antimicrobial resistance and genotyping capabilities. If a fast-track option were to be considered for evaluation of 510(k) or premarket authorization (PMA) submissions, clinical microbiologists in the United States encourage the FDA to consider use as stakeholders with them in approaching this option. Access to new technology in Europe and Canada places clinical microbiolo-

gists in the United States at a professional disadvantage, and the working group strongly encourages faster availability for the U.S. market. Vendors should recognize the need for PCR/ESI-MS platforms that are smaller and less costly in terms of capital investment and in yearly maintenance costs to enable a wider user group within clinical laboratories. Further, improvements toward a more random access option for the technology, or a stat override of the 96-well format, were identified as desirable.

(ii) From a reimbursement perspective, the working group agreed that research-use-only (RUO) or laboratory-developed test (LDT) status will not be acceptable for microbial identification technologies in the long term. The submission of methods for expedited FDA review and approval is critical to the clinical microbiology community. Increased dialogue with the developers and the clinical microbiology community is warranted, as well as increased dialogue between FDA and the clinical microbiology professionals, who can advise agency representatives about the needs and potential benefits of new technology. New categories may be warranted, similar to the emergency use authorization (EUA), not for public health emergencies but, rather, for use in clinical laboratories to address (*a*) urgent workplace needs for automation and work flow reduction and (*b*) assays and technology with the potential for a high impact and a likelihood to improve medical care.

(iii) For evaluation of molecular- or proteomics-based assays, the working group recommended that phenotypic methods should not be used alone as the reference standard for comparison. Rather, sequencing methods such as 16S analysis for discrepancy testing should be included to ensure the highest accuracy.

WILL TECHNOLOGY BE AVAILABLE TO ALL OR ONLY TO LARGE REFERENCE LABORATORIES?

General recommendations. (i) By virtue of cost, complexity, and throughput, many of the newly developed technologies will only be affordable for large reference or university-based diagnostic laboratories. The working group strongly urges the development of platforms that could have utility for hospitals, regardless of their size. (ii) Based on preliminary reports of accuracy and assuming that the technology can be adapted to all hospitals, there is a strong possibility that molecular- and protein-based testing will replace traditional biochemical testing methods and interface with traditional susceptibility test systems to become the identification methods of choice. (iii) There is an important need to develop middleware that would enable users from multiple health care organizations to interface with new technology such as mass spectrometry.

Session discussants: Cara Bastulli, Mathew J. Binnicker, Deborah Boldt-Houle, Paul Bourbeau, Lynn Boyer, Paul Campognone, Larry Hambleton, Stephen G. Jenkins, Sue Kehl, Markus Kostrzewa, Michael Loeffelholz, Linda M. Mann, Elizabeth M. Marlowe, Anne Robinson-Dunn, Paul Schreckenberger, Ribhi Shawar, and Mary Ann Silvius.

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