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COMMENTARY

Automatic Digital Plate Reading for Surveillance Cultures

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The automation of specimen processing and culture workup has rapidly emerged in clinical microbiology laboratories throughout the world and more recently in the United States. While many U.S. laboratories have implemented some form of automated specimen processing and some have begun performing digital plate reading, automated colony analysis is just beginning to be utilized clinically. In this issue of the *Journal of Clinical Microbiology*, M. L. Faron et al. (J Clin Microbiol 54:2470–2475, 2016, http://dx.doi.org/10.1128/JCM.01040-16) report the results of their evaluation of the performance of the WASPLab Chromogenic Detection Module (CDM) for categorizing chromogenic agar plates as negative or "nonnegative" for vancomycin-resistant enterococci (VRE). Their major finding was 100% sensitivity for detection of "nonnegative" specimens using CDM compared to manual methods for specimens plated on two different types of VRE chromogenic agar plates. Additionally, utilization of digital plate reading in conjunction with automated colony analysis was predicted to result in significant savings based on greatly reduced labor costs.

Automation in clinical microbiology began decades ago with automated identification and antimicrobial susceptibility testing (ID/AST) systems along with instruments continuously monitoring blood cultures. More recently, automated systems that include specimen processing and plate handling capabilities and digital plate readers have begun to be used routinely in some U.S. microbiology laboratories. Implementation of these technologies, which had historically been reserved for use with chemistry and hematology specimens, has been ushered in by a transition to liquid-based microbiology specimens, adoption of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for organism identification, perceived reductions in the skilled microbiology labor force, and laboratory consolidations in which more volume must be handled in limited spaces, among other drivers (1–3). Automation for specimen processing and handling may be limited to automatic plate inoculation or may include total laboratory automation solutions that allow automated incubation, digital plate reading, transport of inoculated plates, and, perhaps soon, direct interfacing with ID/AST systems (4).

Digital plate reading systems consist of incubators with linked imaging devices that obtain images at predefined intervals. Plates are typically moved from the incubator to the imager for a short period of time. The amount of time that plates spend outside the incubator is very small compared to that required for the traditional approach of removing plates from the incubator in batches for manual collation and reading, leading to more-rapid growth. Following image acquisition, images of plates are read by a skilled technologist using instrument-specific middleware that may present the images along with additional analysis in a variety of formats. Multiple plate images collected over time and from different medium types are automatically collated for individual cultures and may be viewed individually or in a "contact sheet" format, and multiple cultures from the same patient are often available to the technologist for reference in reading the digital images. Increasingly, the instrument middleware of digital plate reading systems offer some type of automated colony analysis, the most basic of which allows identification of plates with "no growth." This allows the technologist to group negative plates and to quickly confirm the no-growth call in a batch mode, improving efficiency. Additional advances in artificial intelligence, neural networks, and other digital interpretive techniques allow more-sophisticated automated colony analysis, including identification of organisms based on colony morphology and/or specific reactions on chromogenic or other differential agar (5, 6). Additional potential benefits of digital plate reading include the possibility of telemicrobiology (i.e., remote consultation with clinical staff and/or the laboratory director that includes transmission of plate images), decreased time of physical exposure to pathogens, and improved ergonomics (7, 8).

The expectations for automated specimen handling and digital plate reading in clinical microbiology are manifold, and yet well-controlled studies demonstrating the financial and clinical benefit of such systems have been sparse. The standardization and quality of specimen plating using automated systems appear to be superior to those achieved by manual inoculation (9). However, it remains unclear if improved reproducibility of sample streaking impacts turnaround time or the accuracy of specimen workup, with some studies demonstrating results comparable to those achieved using manual approaches (10) and others showing reductions in turnaround time (11, 12). Studies addressing clinical outcomes related to the observed workflow efficiencies associated with automation do not exist.

Faron et al. report the results of their evaluation of the WASPLab Chromogenic Detection Module (CDM) (Copan, Brescia, Italy) for vancomycin-resistant enterococcus (VRE) screening using two types of chromogenic agar (13). Specimens included in the study were collected and analyzed at 3 sites using each site's standard-of-care chromogenic agar, which included Colores VRE (Biomed Diagnostics, White City, OR) and Oxoid VRE (Oxoid,

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Basingstoke, United Kingdom). Depending on the site's standard-of-care procedure, digital images were obtained after 24 or 40 h of incubation and scored for growth by the CDM software as well as manually read by a technologist using digital images displayed on a high-definition monitor. Of 104,730 specimens included in the study, 87,793 (84%) were negative, and the CDM score (negative for VRE or positive for VRE) agreed with the technologist's call 90.1% of the time. Discordance between the CDM call and the technologist call was entirely a consequence of potential "false positives" categorized by the CDM as "nonnegative" (100% sensitivity). Since cultures were no longer available for additional workup, discordant analysis was performed for the 10,438 discordant specimens by manual rereview of the digital plate images. Following this review, most (8,234) of the discordant results were judged to be false-positive CDM calls resulting from the presence of pigment due to residual specimen matrix or growth of yeast. In addition, 1,616 were determined to be negative on review but with the presence of colors judged to represent borderline results (no package insert-defined color). Interestingly, 498 results were determined to represent cultures that were true positives but were missed by the manual read of the technologist. Accepting the limitation of the inability to perform further definitive workups on cultures that were discordant, it appears that the CDM method not only demonstrated 100% sensitivity compared to manual reads but also identified a significant number of cultures as positive that were missed by the technologist's first manual read.

The authors estimated that the technologist time required to process negative VRE screening cultures on chromogenic agar would be reduced from 9.6 min per specimen to approximately 2 min per specimen (mostly reflecting the time required to set up specimens for plating), resulting in a savings of ~$5 per negative specimen. Obviously, depending on screening volume and prevalence, individual institutions could realize very significant reductions in the operational costs associated with screening programs by implementing automation with digital plate reading and CDM. Of course, capital costs associated with instrument acquisition, installation, and training must first be offset.

Similar results were observed previously by using the same CDM tuned to interpret chromogenic agar designed to detect methicillin-resistant *Staphylococcus aureus* (MRSA) (6). In that study, 57,690 nasal swabs collected for MRSA screening purposes at 4 sites were plated to one of three chromogenic screening agars designed to detect MRSA (MRSASelect [Bio-Rad Laboratories, Redmond, WA], chromID MRSA [bioMérieux, Marcy l'Etoile, France], or CHROMagar MRSA [BD Diagnostics, Sparks, MD]). The WASPLab CDM was tuned to detect colonies exhibiting the characteristic color specified for MRSA colonies in the package insert of each chromogenic agar. Digital images were obtained at 0 h and again following 16 or 24 h of incubation and interpreted by manual examination of digital plate images and by the CDM method. Again, the sensitivity for detection of positive plates was 100% for all medium types tested and the specificity ranged from 90.7% to 92.4%. Discordance analysis was performed by a second manual review of the digital plate images associated with 5,507 CDM false-positive results and revealed an additional 153 plates that were judged to be true positives (missed by the first manual read), with the remainder exhibiting either residual matrix (1,189) or borderline colors (3,868).

Taken together, the studies demonstrated the excellent sensitivity as well as the versatility and robustness of the CDM, as it performed well for two different bacterial species with a total of 5 different types of chromogenic media. Obviously, the CDM cannot operate in a completely autonomous manner, since CDM-positive calls will continue to require technologist review, given the number of false positives observed; however, the fact that no positive screening specimens were missed suggests that negatives (which comprised 84% and 88.6% of specimens included in the two studies, respectively) could reliably be automatically read and reported by the system.

Those studies were significant in that they demonstrated the possibility of improving plate reading efficiency by implementing automatic colony analysis in the clinical microbiology laboratory to identify negative and nonnegative plates. Digital plate reading for chromogenic agar also allows technologists to read plates after the optimal incubation period, as images may be obtained even during shifts that are not staffed. This would likely improve the specificity of the chromogenic agar screening method, since extended incubation may lead to nonspecific breakthrough growth (14). In addition, the turnaround time for screening cultures (and thus, depending on infection control protocol, potentially the time to isolation) may be reduced, as plates do not need to be read in a batch but instead may be viewed immediately or very shortly after the prescribed incubation period.

Debate exists regarding the optimal methods that should be used for MRSA and VRE surveillance programs, with labor costs and turnaround time as the major limitations of culture-based methods that employ chromogenic agar (15–18). Conversely, molecular screening methods offer improved turnaround time at the expense of increased reagent costs and, in some studies, reduced sensitivity and specificity compared to phenotypic methods, the latter resulting from detection of resistance genes from species other than the target species and/or variability in the organization or sequence of genetic loci encoding resistance (e.g., *mecA* variant or dropout strains, nonfunctional or unexpressed *vanA* genes, and *vanB* genes present in gastrointestinal commensals other than enterococci) (17, 19–24). The ability to use technology to reduce labor costs (and, potentially, turnaround time) in the context of better performance characteristics (relative to molecular methods) may warrant reexamination of optimal approaches to implementation of MRSA and VRE screening programs for the purpose of infection control and epidemiologic surveillance in some centers. Studies evaluating the clinical impact of such approaches compared to molecular methods are also necessary.

In summary, options for automation of specimen handling and digital plate reading are now readily available for implementation in U.S. clinical microbiology laboratories, while technologies for automated colony analysis and further isolate workup are rapidly evolving. Studies demonstrating the performance of such systems relative to conventional approaches with regard to turnaround time, reproducibility, efficiency, and cost-effectiveness are beginning to appear, and the results look promising. The clinical impact of microbiology laboratory automation and the performance relative to alternative methods (such as molecular techniques) remain to be elucidated.

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