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Review Article

A review of the current state of digital plate reading of cultures in clinical microbiology

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Abstract

Digital plate reading (DPR) is increasingly being adopted as a means to facilitate the analysis and improve the quality and efficiency within the clinical microbiology laboratory. This review discusses the role of DPR in the context of total laboratory automation and explores some of the platforms currently available or in development for digital image capturing of microbial growth on media. The review focuses on the advantages and challenges of DPR. Peer-reviewed studies describing the utility and quality of these novel DPR systems are largely lacking, and professional guidelines for DPR implementation and quality management are needed. Further development and more widespread adoption of DPR is anticipated.

Key words: Automation, digital pathology, microbiology, plate reading, telepathology

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INTRODUCTION

Traditionally, clinical microbiologists handle and read routine bacterial culture plates on the open laboratory bench. At the beginning of each shift, stacks of inverted plates are removed from incubators and set on the benchtop. Throughout the day, the technologist works through the stack of culture plates. This work includes collecting plates from different incubators, inspecting media for bacterial growth, examining colony morphology, isolating pure cultures, performing biochemical testing on isolates, preparing media for antimicrobial susceptibility testing (AST), interpreting AST, and discarding or archiving old culture plates. This work also includes reviewing notes from the previous days' interpretations of any growth and deciding the next step in analysis and/or reporting for these cultures. The plates are eventually returned to their incubator(s), and the same process recommences the following day. With the advent of digital imaging, digital technology has recently been applied in the clinical microbiology laboratory to perform digital plate reading (DPR) in some laboratory settings. The DPR approach is similar to the aforementioned manual reading process, but is highly modified.

With DPR, technologists still read plates, but they can now do so "virtually" without physically touching the culture plate. DPR today in the clinical laboratory consists of a digital camera juxtaposed or attached to an incubator which, through automation, moves the culture plate to the camera for the image to be captured. The incubator and DPR combination allow for continuous incubation of cultures and scheduled digital image capture of those plates. DPR is typically combined with a middleware system that resides between the image capture system and the laboratory information system. These middleware

systems have functionality that can present to the technologist images of the cultures plates not only from a single culture but other cultures where there is perhaps growth from other sources. These middleware solutions are available from several manufacturers. Using a computer workstation, the technologist can perform the work that has historically been relegated to the open bench; DPR and middleware solutions allow images to be captured, colonies to be circled, zones to be measured, annotations to be made, and next steps to be planned. With DPR, the time required for collecting and collating plates can be reduced, and software enables stored images to be referenced when working up a culture. The duration at which the cultures are at suboptimal incubation temperature (i.e., physically on the counter) is reduced, and the productivity of the technologist is improved. With DPR the ability to incorporate computer aided tools and image analysis to support decision making is of great potential.

To date, there is limited literature about this novel tool. This review aims to discuss the advantages and challenges associated with DPR and explores some of the existing platforms that incorporate this technology into an overall solution for total laboratory automation (TLA) within the clinical laboratory space.

DIGITAL PLATE READING SYSTEMS

Perhaps the earliest DPR system used in clinical microbiology was the BIOGRAM (Giles Scientific, New York, NY) system, which was only partially digital.[1] The BIOGRAM system employed electronic calipers, which a technologist could use to measure the inhibition zone size on a culture plate created from an antimicrobial disk. The measurement from the calipers was automatically transferred to a computer, which converted the measurement to a minimal inhibitory concentration value. An updated platform, BIOMIC Video (Giles Scientific, New York, NY, USA), was subsequently developed and demonstrated to be reliable and feasible for clinical use in AST using disk diffusion testing.[2] One study demonstrated that it was more cost effective than a more automated AST alternative.[3] The latest generation of this system, BIOMIC V3 (Giles Scientific; Santa Barbara, California), incorporates a color analysis software tool that is designed to facilitate the interpretation of microbial growth on chromogenic media (i.e. CHROMagar) (http://youtu.be/koU9h8ioOyY).[4] The BIOMIC V3 is also capable of counting colonies (http://youtu.be/CT-0rzgyk_w) and analyzing certain ancillary tests such as Etest assays (http://youtu.be/b2fXjs37VZI). The BIOMIC V3 requires manual loading and unloading of one culture plate at a time, and it is not designed or marketed for comprehensive routine analysis of primary culture plates. Other semi-automated AST DPR systems that are similar to the BIOMIC V3 have been developed.[5] For example, i2a (Montpellier, France) developed the Sirscan 2000 which has been reported to have similar accuracy and better precision than manual measurement of AST zone sizes.[6] Therefore, it appears that DPR may reduce inter-operator variability, at least when measuring AST zone sizes.[6]

Some early clinical microbiology imaging systems were custom-built and used for comprehensive telemicrobiology, which included off-site DPR. These initial systems used extremely manual techniques and custom system designs that would not be feasible for the daily workflow of a contemporary, high-volume clinical microbiology laboratory.[7-9]

As stated above, newer automated systems are comprised of an incubator that incorporates an automated imaging component that interfaces with DPR middleware for viewing and analyzing images. Currently, at least four companies are offering or developing "off the shelf" automated DPR technologies: BD Kiestra (Drachten, Netherlands), bioMeriéux (Marcy-l'Étoile, France), Copan (Murrieta, California), and i2a. BD Kiestra's systems are currently the most widely implemented DPR systems with dozens of installations in Europe.[10] Its systems include TLA and work cell automation platform lines, which use Kiestra's ReadA Browser software for DPR analysis. The bioMeriéux TLA solution incorporates Myla as a middleware solution. Copan's TLA system is the WASPLab, and it uses a web-based interface. The i2a system is the Maestro, and this is still in development.

Currently available systems have been registered with the United States Food and Drug Administration as Class I devices. Giles Scientific's BIOMIC is registered as a microbiology "automated zone reader,"[11] and BD Kiestra's ReadA Browser is registered as a microbiology "manual colony counter."[12] Copan's WASPLab image acquisition station, interface software, and computer hardware are registered as clinical chemistry "data processing modules for clinical use."[13] As additional functionality is added to these DPR systems, it remains to be seen how the regulatory landscape may evolve.

The intent of this paper is not to compare the specific features of each of these systems, but rather to review advantages and disadvantages of digital microbiology in general, using examples that may be common to all of these systems or unique to only one of them. Given that the technology incorporated into DPR systems and software is still emerging, it should be borne in mind that some of the systems' details may be subject to modifications and updates.

IMAGE ACQUISITION AND ANALYSIS

Currently, DPR enables the capture of and analysis of images from traditional bacterial culture plates. The systems can also be used for yeasts, but they are not

currently designed for use with mycobacteria or fungi. The incubators have at least three components: A holding area for inoculated plates, a robotic handling mechanism for the plates, and an image capture station [Figure 1]. These incubators allow for automated image capture at user-defined intervals and on demand while being incubated continuously. Acquired digital images are between 9 and 27 megapixels in size. About half a minute per plate is needed for image capturing because the plates have to be robotically moved to the image capture station, and then numerous lighting strategies are used to capture multiple images of each plate [Figure 2]. As is the case with manual plate reading, different lighting conditions highlight different aspects of the culture plate and its colonies. For example, backlighting is used to highlight hemolysis, and tangential lighting is used to enhance colony texture. Captured images are compiled into a composite image, which incorporates the advantages of each original primary image [Figure 3]. The use of composite images is useful for several reasons. A composite image helps to consolidate key visual data from each original image, so that the technologist viewing the composite image can quickly interpret the relevant visual information contained in multiple original images. Another advantage is that a composite image may appear more similar to what the plate might actually appear as if viewed manually. Multiple image capturing and compositing strategies can therefore be useful for optimizing DPR.

Although the time required to photograph an individual culture plate is relatively short, the time required to photograph every plate in an incubator can be substantial. Hence, the frequency of photographing culture plates may be limited by the number of specimens in an incubator. For example, a full incubator may require 8 h of constant imaging to complete a photograph cycle and capture images of each plate. It is important to consider that a delay between image capture by the system and image analysis by a technologist could lead to challenges.[14]

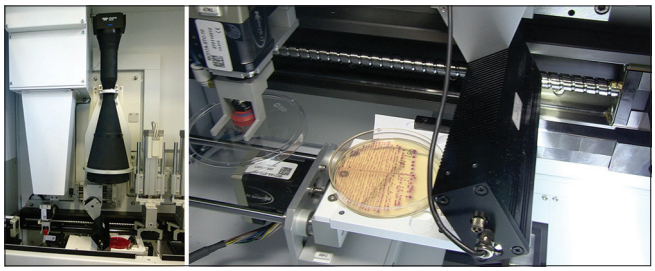

Figure 1: Copan's WaspLab image capture station (left) and a close up picture of the imaging stage (right) are pictured. The front end operation of digital plate reading is an automated system that retrieves a stored culture plate at user-defined intervals, moves the plate to the stage for imaging, removes the lid of the plate, illuminates the plate in multiple ways during the photographic process, and returns the plate to its storage location. This is all performed within a climate controlled incubator. Images are courtesy of Copan

One strategy that can be used is to prioritize imaging frequency by specimen type. For example, it may be appropriate to photograph specimens that are of the greatest clinical importance more frequently (e.g., spinal fluids every 4 h, compared to urine cultures that may only need to be photographed every 12 h).

Software and middleware tools are used with DPR to facilitate and expedite the analysis of the cultured specimens [Figures 4 and 5]. These tools can include a contact sheet of all the plates associated with a single specimen, side-by-side temporal comparison of a culture plate, side-by-side primary specimen gram stain and primary culture plate (in development), pop-up magnification of an area of interest, and automatic zone measurement. These software tools facilitate plate reading for technologists and potentially enhance their ability to interpret cultures beyond what is possible when performing manual plate reading.

ADVANTAGES

Digital plate reading has advantages (some potential and some realized) over traditional (manual) plate reading [Table 1]. Culture plates remain in incubation during routine DPR examination, so cultures have decreased time to the detection of growth.[15] The modified incubators used in DPR do not alter the appearance of colonies and culture plates, so the cultures appear the same as cultures that are incubated in traditional incubators.[16] DPR systems enable a reduction in time spent by skilled staff in transporting, sorting, and retrieving culture plates, which in turn allows

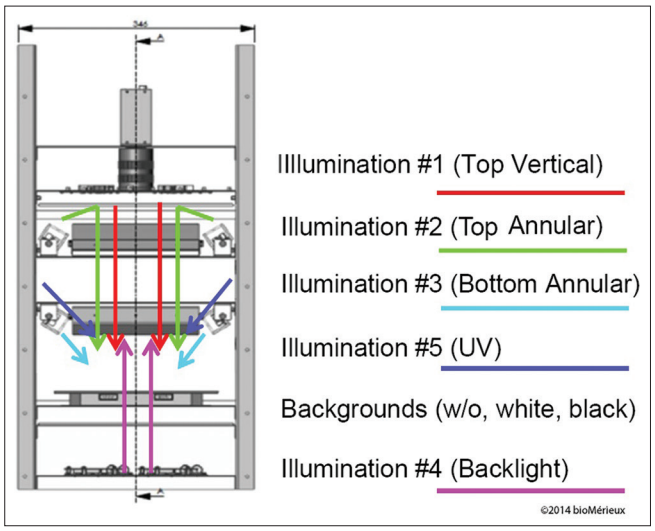

Figure 2: A diagram of the image capture station in a bioMeriéux incubator is depicted. Digital image capturing stations feature the capability to use different illumination wavelengths, lighting angles, lighting directions, light diffusion patterns, and backgrounds (without a background color, with a white background, or with a black background). Different image capture conditions have different advantages and disadvantages. For example, backlighting can help reveal hemolysis patterns but may make it difficult to discern details of colony texture. Figure is courtesy of bioMeriéux

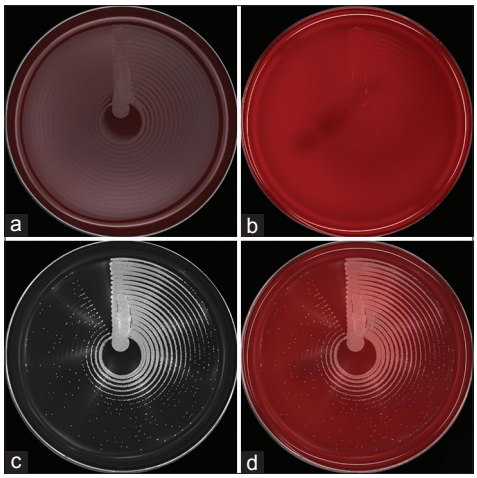

Figure 3: Three captured images and one composite image of *Eikenella corrodens* are shown. These were generated using bioMeriéux's digital plate reading system. The blood agar plate was inoculated with bioMeriéux's Previ Isola instrument and then incubated. Primary images were collected using top annular illumination, (a) bottom annular illumination, (b) and in high contrast black and white. (c) The Myla software then created a composite image, (d) which incorporates features of each primary image. Full resolution images are available at http://goo.gl/kbbeuw or by using this QR code

for increased time spent actually analyzing cultures, and these changes enable technologists to be more efficient.[17-20] The use of a modern DPR system can enhance technologist efficiency and decrease time to organism detection, which leads to decreased turnaround times.[20]

When using DPR, plates are physically handled less often. Time spent performing repetitive mundane tasks like labeling culture plates is reduced. Barcodes are applied to plates by the automated frontend of the TLA system. This automated labeling reduces the need to manually apply printed stickers or to manually write on culture plates, which can increase the efficiency of the process and decrease errors made in the laboratory when reading plates. These barcodes enable the laboratory to identify and track culture plates while in the DPR incubator, while being manipulated on the bench, and when returning the plates to the incubator. Because culture plates need to be handled less often, workstations can be configured to optimize ergonomics and minimize risk of repetitive motion injuries.[21,22] Also, the decreased physical exposure to pathogens provides a potential decrease in the risk of laboratory acquired infections for laboratory workers.[23]

Software tools associated with DPR provide unique advantages. These analyses include identifying no growth or enumerating colonies in cultures, measuring zone sizes on AST plates [Figure 5], and identifying a colony color, which can be used to identify organisms

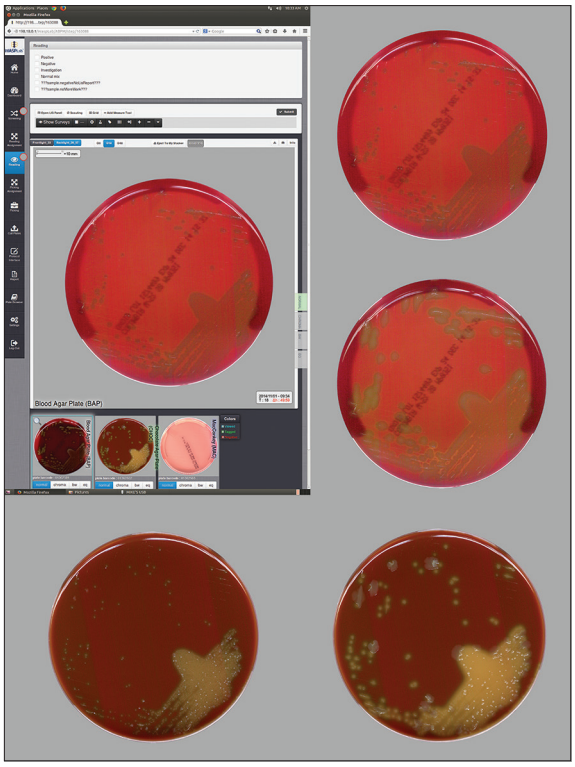

Figure 4: The software interface (top-left) and images of culture plates from Copan's WASPLab digital plate reading system are pictured. A culture of *Streptococcus pneumoniae* after 18 h of incubation on sheep blood agar is shown in this screenshot of the software. Other plates set up from the specimen, which include chocolate and MacConkey, are visible at the bottom of the screen. Although no growth is present on the MacConkey plate, the growth on the blood agar is visible at 18 h (top-right) and 48 h (mid-right), and the growth on the chocolate agar is visible at 18 h (bottom-left) and 48 h (bottom-right). Full resolution images are available at http://goo.gl/kbbeuw or by using this QR code

growing on chromogenic media [Figure 6].[24] Software can interpret simple culture results, such as identifying "no growth" plates, so these results can be released quickly.[25] Additionally, DPR software allows skilled technologists to annotate plates and delegate additional work-up to support staff or an automated colony picking instrument.[18,20] These software tools help to maximize the amount of time workers spend performing tasks at the top of their skill levels. All of these software features help increase the efficiency of DPR.

The use of preserved digital images is an advantage of DPR because it enables integrative interpretive analysis, rapid consultation, archiving important teaching cases, and sharing of visual information.[18] Archived images of culture plates at user-defined time points facilitates the analysis of specimens.[26] The development of software tools to enable simultaneous viewing of digital plates and digital gram stains will allow for a more

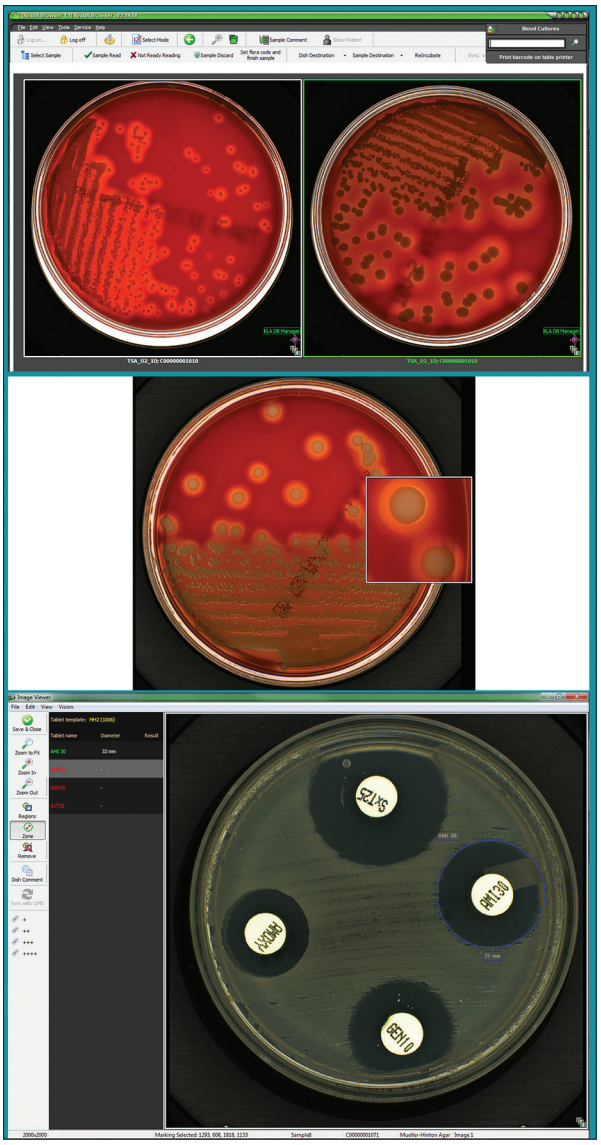

Figure 5: Examples of BD Kiestra's digital plate reading software tools are demonstrated. A plate of interest can be examined at multiple time points (top pane), which enables the microbiologist to more objectively analyze the change in a culture's appearance over time. An area of interest can be viewed at a greater magnification without the need to manually adjust the lighting, nor use a physical magnifying lens (middle pane). Zones of inhibition can be measured in silico without ever removing the plate from the incubator (bottom pane). Images are courtesy of BD Kiestra

integrative analysis of microbiology testing.[27-29] Similarly, simultaneous analysis of multiple specimens from a variety of sources all obtained from a single patient (including archived images from previous specimens) facilitates a patient-centric strategy of analysis instead of the source-centric microbiological analysis that is commonly employed.[18,20] Difficult to interpret cultures that demonstrate unusual or discordant findings can be shared electronically with the laboratory director for rapid consultation, which can streamline and expedite analysis of difficult cases.[28] Interesting cases that are useful for

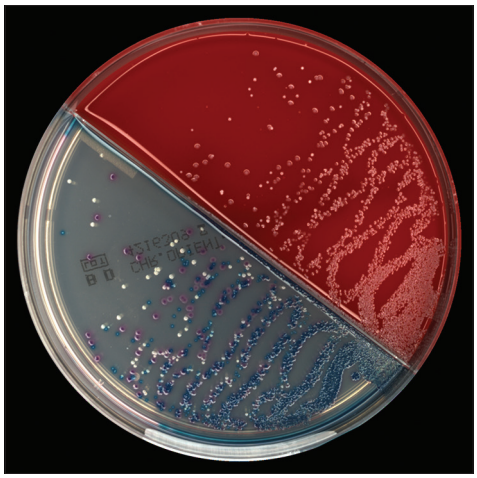

Figure 6: A bi-plate of BBL™ CHROMAgar™ Orientation medium and Columbia CNA agar is shown that was inoculated with the BD Kiestra InoqulA and imaged with BD Kiestra digital plate reading (DPR) system. Colonies suggestive of *Escherichia coli* (pink), *Enterococcus* (blue), and *Staphylococcus epidermidis* (white) are visible on the chromogenic agar, and only the gram positive organisms are evident on the CNA medium. DPR software can be used to quantify colony color and qualitatively interpret colony types by color. Image is courtesy of BD Kiestra. Full resolution images are available at http://goo.gl/kbbeuw or by using this QR code

teaching or training can be captured retrospectively.[26] Work is being done to integrate microbiology images into the hospital information system, so that clinicians can view images of finalized cultures and specimen gram stains.[28] Retrospective quality review of plate interpretations and work-up is also possible, which can be used as a means to monitor or measure the proficiency and competency of a technologist.[26]

In summary, the advantages of DPR can be attributed to one or more of the following: Image capturing is designed to occur within an incubator, so cultures can undergo routine analysis while maintaining continuous incubation; culture plates require less manual manipulation, which can save time and improve safety; software can be used to analyze and annotate digital images, which can increase objectivity and efficiency; storing images electronically enables increased flexibility in analysis and sharing of information.

CHALLENGES

The two most common and significant challenges to adopting and implementing DPR are likely the capital investment required to obtain the equipment and workflow changes required to implement DPR [Table 1]. As with the training and implementation associated with any new technology, one should anticipate a learning curve

Table 1: The current advantages and challenges of using digital plate reading for bacterial cultures in the clinical microbiology laboratory

Advantages	Challenges
Computer-aided analysis (e.g. identifying plates with no growth) facilitates objective and rapid resulting	Implementation requires significant changes in laboratory design and workflow
Increased rapidity of organism growth due to the decreased time that cultures are need to be outside of the incubator	Peer-reviewed studies describing patient-relevant improvements (e.g. decreased turn around time or decreased time to appropriate antimicrobial therapy) are needed
Decreased exposure to cultivated pathogens.	Many culture plates still need to be manipulated manually
Archived images of culture plates enable more thorough quality review and the development of real-world training sets	Increased data storage space is needed
Increased staff productivity	Peer-reviewed studies describing laboratory-relevant improvements (e.g. increased quality, increased efficiency, decreased costs, decreased staffing) are needed
Facilitates comparison of cultures from multiple sites and at multiple time points during incubation	Expert guidelines and regulations regarding the implementation and the expectations associated with the training, use, quality monitoring, maintenance, image storage, image discoverability, and downtime procedures are needed
Captured images can be incorporated into the medical record to enhance documentation and/or communication with clinicians	Informatics tools for sharing images need to be custom-built
Potential for enabling downstream automation (e.g. robotic colony picking and subsequent testing)	The rapidity of future development and implementation of system improvements are unknown
Cultures can be read at consistent, user-defined intervals (e.g. 24 h for urine cultures)	The frequency of image capturing can be limited due to physical constraints involving the time required to image an entire incubator of plates
	Significant capital needed for equipment purchase and maintenance
	Unrecognized challenges may exists because this is an emerging technology

among the laboratory staff. The capital investment for a DPR system is significant, but the return on investment may result in an eventual net cost-savings due to improved efficiency. However, only preliminary studies describing the increased efficiency associated with DPR and TLA are available, so extrapolation and best-guessing is currently required when estimating return on investment.

The willingness of technologists and technicians on the bench to change processes needs to be considered, and their expectations as well as concerns need to be heard.[30] Additionally, techs that do not feel confident or comfortable using computers may feel anxious and apprehensive when considering moving from a manual analysis of culture plates to DPR. Effective change management is thus paramount when attempting to successfully implement a DPR system. Medical leadership and administration need to agree on the goals of changing to a DPR system. Ideally, representatives from all stakeholder groups will be involved in all stages of planning, implementation, and process revision. Open communication of goals is important in order to foster a unified vision of change.

Digital plate reading is an emerging technology that is only now entering the early adoption stage in the United States, so the unknowns associated with DPR are largely unexplored. Additionally, emerging technologies can undergo a period of rapid evolution during which the cost of the technology can rapidly decline and during which the quality of the output can rapidly improve. It is unknown if early adoption of a DPR system will lead to its early obsolescence because of the rapid improvements that may occur in the near future, and it is unknown if current buyers are paying a premium price to be an early adopter of DPR. These concerns may stifle the readiness of financial decision makers to release large amounts of capital to immediately invest in DPR. The concerns associated with adopting this emerging technology can begin to be overcome by performing independent studies that objectively analyze and report quality and efficiency metrics associated with DPR (e.g., accuracy, precision, changes turnaround time, changes in productivity, changes in laboratory space needs, changes in staffing needs) and DPR's return on investment. Such studies will help others in clinical microbiology to make more informed decisions about the value associated with implementing DPR and TLA.

Some abstracts have been presented that identify other challenges unique to DPR. Fulchiron and colleagues identified a novel challenge associated with DPR, which is the loss of colony isolation.[14] This loss can occur because cultures continue to be incubated after their images have been captured, so colonies continue to grow after imaging. Therefore, if the images are not examined in a timely matter (i.e., <2 h after image capture), then colonies that

appeared to be isolated may have time to collide with others on the agar plate. This occurrence would require reanalysis of the sample, which could impair workflow and throughput. Additionally, DPR workstation design needs to be carefully considered and implemented because the potential exists for increased risk of repetitive motion injuries if design is suboptimal.[31] Nuances such as this, which are unique to DPR, need to be identified empirically, studied formally, and published in the peer-reviewed literature.

CASE REPORT

In 2010 and 2011; Farrington *et al*., described the process of TLA implementation and some of the changes they encountered after implementing DPR as part of TLA in a laboratory that processes approximately 500,000 samples annually.[19,20] In total, eight DPR workstations were installed. Their site preparation caused significant disruption within the laboratory for weeks leading up to the installation of the equipment. Each technologist underwent approximately 25 h of training before performing independent DPR, but full confidence with using the system was not achieved after the initial training.

During the first 6 months of DPR use, they surveyed 6 weeks of work and reported their findings. During those 6 weeks, 23,630 samples were processed from which 46,725 plates were inoculated (1.98 plates per sample) and 149,762 images were obtained (3.21 images per plate). Of those 46,725 plates; 9,552 (20.4%) were removed from the incubator for manual work-up or analysis.

Errors in the TLA system were most frequently identified immediately following system changes, such as following initial implementation of the system or following the addition of another incubator. Although these authors describe their system as being "generally consistently reliable," they did note a "significant breakdown" that lasted 5 h caused by a software error. The manufacturer's software engineers were able to resolve the error remotely. The authors report that these errors gave them the opportunity to improve "fall-back systems that will reduce or abolish the impact of any similar failures in the future."

Staffing requirements in their laboratory also changed. Before implementation of TLA, the laboratory was staffed by fourteen workers daily. Twelve of these positions were first shift, and two were second shift. After implementation, the number of staff required for daily operation decreased, but the number of second shift staff needed for optimal operation increased. After implementation, four staff were needed for first shift, and four staff were required for second shift. The full-time equivalents required to operate the laboratory decreased from 26.5 to 18.43 (30% reduction) after the implementation of DPR and TLA, even though the laboratory's sample volume increased.

DIGITAL PLATE READING IN THE FUTURE

Further advancements in DPR systems are anticipated as this technology matures. Through the use of DPR and TLA, automated colony picking and subculturing may become a reality in routine clinical microbiology.[21] Work is currently being done to improve the computer's ability to interpret DPR images.[32] Other developments are likely to include advanced colony analysis. The addition of automated colony analysis to an existing DPR system would most likely only require the validation of software and no additional capital equipment.[33,34] These advanced DPR informatics tools could include the use of neural networks[18] and artificial intelligence,[24] which would leverage a computer's ability to more accurately and reproducibly quantify image components that humans typically analyze qualitatively (e.g., color, size, speed of growth). Copan is supporting an effort (microbIA.org) that is working toward this type of advancement. The addition of such advanced computer-assisted analysis for DPR would facilitate specimen work-up and streamline analyses. The continued development and implementation of tools that could reduce the number of plates that need to be physically manipulated by individuals would help to increase the efficiencies associated with DPR. Although DPR will initially be performed locally within the laboratory, it has been proposed that it may even be feasible that DPR could be performed remotely by telepathology.[18] How DPR might fit into the routine analysis of more complex microbial culturing techniques, such as mold and mycobacteria cultures, is yet to be explored.

The development and adoption of DPR is so recent that no significant body of formal studies or peer-reviewed literature is currently available. The real-world challenges associated with DPR need to be better identified and reported to the clinical microbiology and clinical informatics communities. Successful (and unsuccessful) downtime strategies, downtime frequency, and downtime performance reports would be useful additions to the literature. Guidelines and recommendations are needed for DPR. Guidelines for validation and verification of quality measures as well as proficiency testing have not been established. Consensus recommendations as to the minimum specifications for image capture quality and quantity, as well as digital display quality have yet to be broached. Image archiving requires increased digital storage space, and guidelines as to the appropriate duration and integrity of culture image storage are needed. Moreover, practical recommendations as to whether or not these images should be part of the discoverable medical record are also needed.

CONCLUSION

Digital plate reading is a novel tool that should be added to the growing applications made possible by introducing digital imaging technology into the pathology laboratory. Laboratorians will begin to derive ensuing benefits from improved efficiency of laboratory workflow, expedited generation of results, and enhanced characterization of microbial isolates as the clinical microbiology laboratory becomes more automated, more digital, and more reliant on informatics tools. Initial studies and anecdotal evidence suggest that DPR can improve the clinical microbiology laboratory's efficiency while improving turnaround times. Improved turnaround times could in turn allow patients to more quickly receive optimal antimicrobial management. Unfortunately, rigorous studies of DPR have yet to be reported in the peer-reviewed literature, and guidance regarding implementation, management, and monitoring of DPR systems is currently lacking.

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