Review of Telemicrobiology

Daniel D. Rhoads, MD; Blaine A. Mathison, BS; Henry S. Bishop; Alexandre J. da Silva, PhD; Liron Pantanowitz, MD

• *Context.*—**Microbiology laboratories are continually pursuing means to improve quality, rapidity, and efficiency of specimen analysis in the face of limited resources. One means by which to achieve these improvements is through the remote analysis of digital images. Telemicrobiology enables the remote interpretation of images of microbiology specimens. To date, the practice of clinical telemicrobiology has not been thoroughly reviewed.**

***Objective.*—To identify the various methods that can be employed for telemicrobiology, including emerging technologies that may provide value to the clinical laboratory.**

***Data Sources.*—Peer-reviewed literature, conference proceedings, meeting presentations, and expert opinions pertaining to telemicrobiology have been evaluated.**

***Conclusions.*—A number of modalities have been employed for telemicroscopy, including static capture techniques, whole slide imaging, video telemicroscopy, mobile devices, and hybrid systems. Telemicrobiology has been successfully implemented for several applications, including routine primary diagnosis, expert teleconsultation, and proficiency testing. Emerging areas of telemicrobiology include digital plate reading of bacterial cultures, mobile health applications, and computer-augmented analysis of digital images. To date, static image capture techniques have been the most widely used modality for telemicrobiology, despite newer technologies being available that may produce better quality interpretations. Telemicrobiology adds value, quality, and efficiency to the clinical microbiology laboratory, and increased adoption of telemicrobiology is anticipated.**

(*Arch Pathol Lab Med.* 2016;140:362–370; doi: 10.5858/arpa.2015-0116-RA)

The role of informatics in the clinical microbiology laboratory is growing, which includes the practice of telemicrobiology.[1] Telemicrobiology is the use of telepathology technologies within the subdiscipline of clinical microbiology. Telepathology allows a human interpreter to have access to a digital image at a time or location that is separated from physical access to the specimen. The digital image can be a macroscopic picture (eg, gross pathology, culture plate) or a microscopic image (eg, histopathology, special stain of microorganisms). The telepathology system, in simplistic terms, requires a mechanism for image acquisition, a telecommunication link for image transmission and/or access, and a mechanism (display) to remotely review and interpret those images.

Telepathology has been successfully used for clinical purposes, such as remote interpretations (telediagnosis) and second opinions or consultations (teleconsultation). In practice, telepathology has been largely employed in anatomic pathology. As a result, there is a large body of literature devoted to the use of telepathology for intraoperative frozen sections, teleconsultation for second review of histology slides, and telecytology for rapid, on-site evaluation. More recently, the benefits of telehematology for remote interpretation of digital peripheral blood smears has been revealed. However, telepathology is also applicable to other areas of the clinical pathology laboratory in which images may be of value, such as microbiology (macroscopic images of culture plates and microscopic images of microorganisms) and chemistry (macroscopic images of gels and microscopic images of fluids, including urinalysis).

To date, only a few published articles have mentioned the use of telepathology in microbiology. Despite this paucity of literature, several successful telemicrobiology consultation services have been established, such as the US Centers for Disease Control and Prevention's (CDC's) Division of Parasitic Diseases and Malaria's service for diagnostic assistance (DPDx). The scope of this review is to evaluate different applications of telemicrobiology that relate directly to the clinical microbiology laboratory, including primary diagnosis, teleconsultation, and proficiency testing. Nonclinical applications of telemicrobiology for research and educational purposes are not discussed.

TECHNOLOGY FOR TELEMICROBIOLOGY

Telemicroscopy can employ a number of modalities.[2] The most common modes of telemicroscopy include using photomicrographs (still images), static or dynamic whole slide imaging (WSI), live or recorded video telemicroscopy, or a hybrid of these. Of these modes, sharing photomicrographs is the most technologically rudimentary mode, but because it

Accepted for publication July 14, 2015.
Published as an Early Online Release August 28, 2015.

From the Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania (Drs Rhoads and Pantanowitz); the Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia (Messrs Mathison and Bishop and Dr da Silva); and the Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Laurel, Maryland (Dr da Silva). Dr Rhoads is now with the Department of Laboratory Medicine, Cleveland Clinic, Cleveland, Ohio.

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Daniel D. Rhoads, MD, Department of Laboratory Medicine, Cleveland Clinic, 9500 Euclid Ave, LL1-123, Cleveland, OH 44195 (email: danrhoads@gmail.com).

Table 1. Comparison of Different Telemicroscopy Modalities					
	Static Image Capture of a Single Field	Human-to-Human Live Video	Remotely Operated (Robot-Aided) Live Video	Recorded Video	Whole Slide Imaging (Static or Dynamic)
File created[a]	Yes	No	No	Yes	Yes
File size	MBs	No file generated	No file generated	Variable	MBs to GBs
Bandwidth demand	Low	Moderate	High	High	Moderate to high
Image generation, time	Instantaneous	Instantaneous	Instantaneous	1–10 min	10–100 min
Image area, X–Y dimensions	Minimal	Maximal	Maximal	Variable	Maximal
Image depth, Z dimension	1 Z plane	Typically, multiple Z planes in areas of interest	Typically, multiple Z planes in areas of interest	Typically, multiple Z planes in areas of interest	1 plane (static) or multiple planes (dynamic)
On-site expertise required for screening	Yes	Yes	No	Yes	No
Capital equipment costs	Minimal	Minimal	Maximal	Moderate	Maximal

[a] Image can be analyzed after slide has been filed.

is inexpensive and easy to implement, this approach has been the most commonly used telemicrobiology modality. It requires only a microscope with an attached digital camera and a means to share a relatively small digital image file (eg, e-mail or remote server). More-sophisticated equipment and infrastructure may be required for WSI and live video telemicroscopy. However, these more-advanced microscopic imaging technologies can provide unique advantages, such as access to an entire glass slide or dynamic (ie, multiple Z plane) viewing (Table 1). Most WSI scanners are not yet capable of digitizing glass slides using ×100 oil-immersion magnification (Figure 1, A through F), which is a major drawback because ×100 magnification is often used in clinical microbiology microscopy.

The benefits and limitations of WSI as a modality in surgical pathology have been discussed elsewhere.[3–6] With respect to static WSI use for telemicrobiology, important issues to be addressed are the capability of these devices to produce digital slides at high enough magnification and with sufficient depth of field (Z stacking) to be able to easily discern microorganisms. Traditionally, depth of field (focusing) is often limited to a single Z plane in static WSI. Also, resolving power is typically less than is used in routine clinical microbiology. Microbiologists often view slides using a ×100 oil-immersion objective lens, whereas WSI in anatomic pathology is typically performed using ×20 or ×40 magnification. Some authors have suggested that employing this lower power magnification for WSI may be inadequate to confidently rule out the presence of microorganisms, such as *Helicobacter pylori*, even with immunohistochemical staining.[7] However, it has been demonstrated by other investigators that when using more resource intensive dynamic WSI with a ×40 objective in which multiple Z-dimensions are captured, the digital slides produced have the equivalent diagnostic potential for identifying *Helicobacter pylori* in gastric biopsies (not stained with immunohistochemistry) to examining a glass slide using a traditional microscope.[8] Another study identified that appropriately photographed microscopic fields captured with a ×100 objective lens yielded telemicrobiology interpretations on par with the accuracy of glass slide interpretations, but the diagnostic accuracy of ×40 static WSI interpretations was inferior to glass slide and ×100 photomicrograph interpretations.[9]

CLINICAL MICROBIOLOGY APPLICATIONS

Telemicrobiology has been used for multiple applications in clinical testing. These applications include the routine use of telemicrobiology as part of daily operations, use of telemicrobiology for internal consultations within a health system, and the use of telemicrobiology for external consultations. More than a decade ago, the German Army Medical Service began developing and implementing telemicrobiology services, including telebacteriology, teleparasitology, and televirology for routine daily use in its field laboratory operations.[10,11] In the late 1990s, Veterans Integrated Service Network (VISN) 12, which is part of the Veterans Health Administration in the United States, began feasibility tests and implementation of a telepathology system to connect its medical centers.[12] This network was used for telemicrobiology consultative services within the VISN.[12–14] In 1998, the CDC's DPDx began practicing teleparasitology, which pathologists from all over the world now use as an external consultative service. Recently, a growing number of laboratories have been exposed to telemicrobiology with digital samples for proficiency testing. Another emerging and quickly growing application of telemicrobiology is digital plate reading, which is the use of digital images of bacterial culture plates for intralaboratory analysis. With the ubiquitous use of the Internet and mobile devices, we are beginning to witness additional mobile health applications of telemicrobiology.

Telemicrobiology for Routine Work

Scheid and colleagues[10] reported their clinical experience using telemicrobiology in German Army Medical Service field microbiology laboratories. Their initial work involved telebacteriology (eg, assessing bacterial plate cultures) and teleparasitology (eg, diagnosing malaria, leishmaniasis, and foodborne parasites, such as worm ova) in 2002 and 2003, followed by examination of cell cultures for televirology in 2005 and 2006.[11,14] Their system employed 2 microscopes, 1 for high-powered examination of cells and 1 for low-powered examination of colony morphology on nutrient media. They used a digital camera to capture 1360- by 1024-

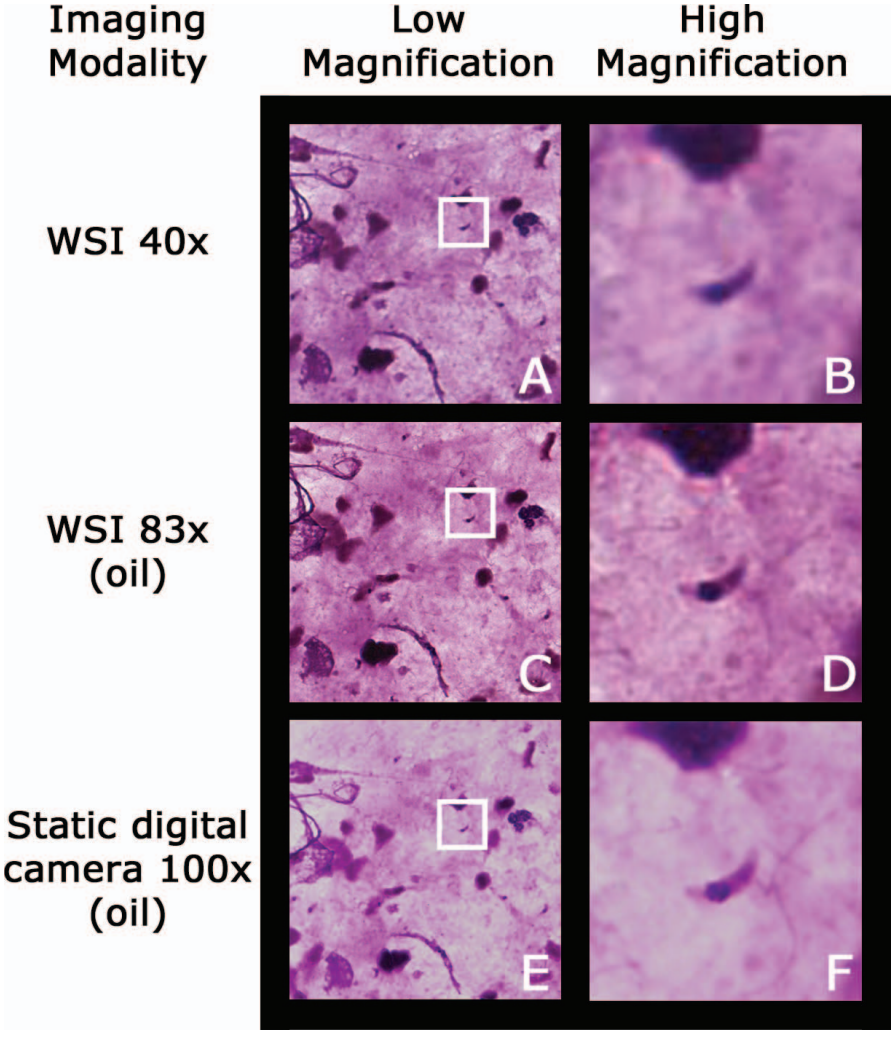

Figure 1. *A bronchoalveolar lavage cytospin with toxoplasmosis is shown (Romanowsky stain). Three imaging techniques were used to digitize the glass slide to create this comparative figure, including whole slide imaging (WSI) using the Aperio ScanScope XT (Vista, California) (A and B), an oil-immersion WSI using Aperio CS-O (C and D), and an oil-immersion static image captured using an Olympus U-TV0.5XC-3 camera (Center Valley, Pennsylvania) (E and F). At low magnification (A, C, and E), all of the images appear to be of similar quality. However, when viewing a* Toxoplasma gondii *tachyzoite at higher magnification (B, D, and F), there is a marked difference. In the ×40 WSI image (B), the tachyzoite's nucleus is difficult to resolve when maximally zooming in on the image. In the ×83 WSI image (D), the nucleus is clearly visible, but nuclear details are still not entirely evident. In the ×100 static capture image (F), the nucleus is clearly visible, and some nuclear detail is evident (original magnifications ×40 [A and B], ×83 [C and D], and ×100 [E and F]). (The ×83 images were generously provided by Mohamed E. Salama, MD.)*

pixel images.[11,14] DISKUS software (Hilgers, Königswinter, Germany) was used to process, transmit, and archive their images; and it enabled a remote expert to measure objects in the photomicrograph. After transmission of one or more photomicrograph, a video conference between the expert and the laboratory photographer was commenced to discuss cases.

This group performed a variety of validation studies in which the photographer and the interpreter were both blinded to results.[10] Malarial smears, stool examination for parasites, Gram stains of bacterial suspension, Gram stains of colonies, Gram stains of specimens from various sources, and colonies growing on nutrient media were all analyzed in this blinded fashion. The photographers had limited microbiology experience and were described in the study as "medical assistants," whereas the interpreters were bacteriologists and parasitologists. This telemicrobiology system enabled the expertise of microbiologists to be used remotely in field laboratories where microbiology expertise was limited. Not surprisingly, the remote microbiologists provided more-accurate interpretations than the on-site assistant.

As is the case with most store-and-forward telepathology setups, the importance of the photographer in selecting appropriate fields of view for capturing representative images is of "decisive importance." The photographers "cannot transmit what they do not notice," and the inexperience of photographers was deemed a "limiting factor" by Scheid et al.[10] In some instances, such as parasitologic stool diagnosis, the sensitivity of testing was as low as 76% (68 of 90), which the authors attributed to the photographer submitting nondiagnostic images to the expert for interpretation. The authors stated that telemicrobiology "is not a replacement for adequately trained staff,"[10] and they emphasized that all laboratory persons using the telepathology system should be trained in a central location using identical equipment to that used in the field.[11,14] This training not only prepares assistants for field work but also ensures that the experts who train the assistants personally know the field staff.[11,14] Overall, Scheid and colleagues[10] concluded that their telemicrobiology system "provided significant benefits to the health of deployed German forces." The North Atlantic Treaty Organization is also encouraging the use of this type of telemicroscopy to extend microbiology services and to help decrease the number of deployed medical staff.[15]

Telemicrobiology for Internal Consultation

The Veterans Health Administration's VISN-12 contains 8 Veterans Administration Medical Centers that are connected via a telepathology network.[12] McLaughlin and colleagues[14] reported the feasibility of telemicrobiology among

Table 2. Information Required by DPDx[a] When Requesting Telediagnosis and the Importance of These Data	
Information Required for Sample Submission	**Importance of Information Required**
Multiple images of the suspected parasite	Facilitates ease and accuracy of telediagnosis
Pertinent patient history (including travel history) Suspected diagnosis	Helps direct differential diagnosis
Type of specimen Date of collection Stain used Lens magnification used	Objective information about the sample source, its preparation, and the captured image facilitate analysis
Two unique patient identifiers A standardized requisition form[b]	Linking a specimen to the correct patient, test ordered, and ordering physician is necessary in telemicrobiology, the same as in all clinical laboratory testing

[a] DPDx is the Centers for Disease Control and Prevention's Division of Parasitic Diseases and Malaria's service for diagnostic assistance.
[b] http://www.cdc.gov/laboratory/specimen-submission/pdf/form-50-34.pdf. Accessed May 19, 2015.

these Veterans Administration Medical Centers in 1998. In that study, the authors explored the use of static digital photomicrographs to interpret Gram stains of specimens, such as sputa, stools, and wounds. In their study, 3 pathologists examined 30 cases. Each case consisted of a pair of 1024- by 768-pixel images, one of which was captured using a ×40 objective lens, and one of which was captured using a ×100 objective lens. The pathologists also examined the actual glass slide from each case using a conventional light microscope. They reported that the accuracy of static telemicrobiology was on par with the interpretation of glass slides; 95% of samples from each mode produced no major discrepancies.[14] Interpretation of the digital images took half the time (2.1 minutes) compared with examining glass slides (4.3 minutes). However, it took about 15 minutes to digitally capture each case. So, although analysis time by the consulting pathologist was reduced when using telepathology, the total time required for each case was greater.

Several reasons may account for discordant interpretations when using telemicrobiology for the remote analysis of Gram stains, all of which occurred in the McLaughlin et al[14] study: (1) poor field selection by the photographer, which decreased the sensitivity of the interpretation; (2) poor image quality, which compromised the accuracy of interpretation; (3) too few fields photographed, which resulted in diagnostic uncertainty by the interpreter; and (4) inappropriate case identification by the interpreter, which resulted in mispairing of the case number and its interpretation.

The VISN-12 telepathology network includes both robotic and nonrobotic telemicroscopy elements, using a hybrid of static capture and live video-image acquisition.[13] The network is used by both pathologists and technologists. Images shared over the network include static micrographs of stained slides and macroscopic images of culture plates. Additionally, live dynamic video of wet-mounted slides can be viewed to examine the potential motility of microorganisms. Several points are important for operational success, which they[12] have learned since implementing this telemicrobiology system: (1) effective technologic change requires enthusiasm and technical expertise at all sites; (2) when problems occur, it is imperative to have technical support rapidly available and to communicate directly and frequently with the parties involved; and (3) remote analysis requires careful attention to specimen preparation and examination in the same manner as is required for all laboratory work.

Telemicrobiology for Expert Consultation

The CDC's DPDx offers worldwide telediagnosis of parasitic infections. The DPDx now receives more than 400 telediagnostic cases annually.[16,17] In total, DPDx has received more than 3300 requests for telediagnosis from more than 60 different countries since it began accepting telediagnostic requests in 1999.[16,17] Telemicroscopic consultations are advantageous because they can be completed more quickly and can cost 80% less[18] (approximately $95 for a traditional consultation versus approximately $20 for a teleconsultation)[18] than traditional diagnostic consultations. Traditional consultations require the physical shipment of a specimen, but digital consultation can reduce the total turnaround time of a consultation by eliminating the time the specimen spends in transit. Images received by DPDx for consultation include mostly digital photomicrographs of blood and stool specimens, but images of tissue sections, arthropods, and worms have also been analyzed.[19] The DPDx's telemicroscopic consultations are performed within a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, although CLIA currently does not have any requirements specific to telepathology. The DPDx service requires digital specimens be accompanied by much of the same information as any CLIA-certified clinical laboratory would require, but additional information is also needed because of the unique approach of consult telemicrobiology (Table 2). The CDC laboratory staff can also access telediagnostic requests via secure mobile devices.[17,19] The DPDx has more experience than any other entity in performing consultative teleparasitology, which has a number of advantages and disadvantages over traditional parasitology consultation (Table 3).

From October 2005 through September 2009, about one-third (423 of 1192) of telediagnostic requests to DPDx were for the potential diagnosis of malaria.[18] Of those, about 70% (298 of 423) resulted in a confident diagnosis (positive identification of *Plasmodium* to the species level, positive identification of *Babesia* spp., or negative for parasites), but physical samples were requested for the remaining 30% (125 of 423) of telediagnostic requests to achieve a more-confident or specific diagnosis. In instances in which this additional sample was received by DPDx after it was requested, a confident microscopic diagnosis of the infecting *Plasmodium* species could be made in 46% (36 of 79) of the samples. In some instances, physical specimens were received by DPDx even after a confident telediagnosis was made, although the physical specimens were not requested; and follow-up testing of those physical specimens resulted

Table 3. Advantages and Disadvantages to Static-Image Teleparasitology as Performed by DPDx[a]

Advantages	Disadvantages
Rapid identification or screening (usually within 1–2 h) Clinically relevant examples: • Differential identification of organisms where treatment differs (eg, differentiating *Babesia* and *Plasmodium* species) • Recognize or rule-out acutely life-threatening pathogens (eg, free-living amoebae) • Identification of organisms where there may be infection-control needs (eg, nosocomial *Sarcoptes scabiei*) • Determination of when follow-up is warranted for further molecular analysis (eg, discriminating *Entamoeba histolytica* versus *Entamoeba dispar*, resolving *Cyclospora* to species level for outbreak investigations, confirming the presence of amastigotes in suspect cases of leishmaniasis, or characterizing extraintestinal microsporidiosis) • Investigation of potential infection via transfusion or transplant (eg, *Babesia* sp in a blood recipient) Less expensive than traditional specimen submission (ie, physically mailing slides and related materials) No risk of physical damage to the specimen (eg, slide breakage) or permanent specimen loss (eg, fluid leakage) Avoids shipment of and potential exposure to potentially infectious pathogens Diagnoses are reported in accordance with CLIA guidelines Facilitates information sharing and training including: • DPDx image library • DPDx monthly case studies and other teaching resources • Publications	Submission of images may be biased based on the presumptive diagnosis of the submitter (eg, submitting only images of features that support the presumptive diagnosis and not giving a clear overall picture of the case) Static images do not provide multiple Z planes, which can impair the quality of the analysis in some cases (eg, quantifying *Entamoeba* sp nuclei, visualizing both the nucleus and kinetoplast in *Leishmania* spp or *Trypanosoma cruzi* amastigotes) The quality of the consultant's interpretation can be limited by the poor quality of the submitter's images. Common examples that render images unsatisfactory for analysis include: • Poor focus • Poor magnification choice for the suspected organism • Poor image exposure (eg, overexposure) • Poor cropping • Too few images submitted to give a clear overall picture of the case The quality of the consultant's interpretation can be limited by the submitter's omission of important data regarding the submission (eg, size, magnification, specimen type, stain used, relevant travel history) Some organisms are inherently difficult to image well, for example: • Amastigotes of *Leishmania* sp or *Trypanosoma cruzi* in tissue sections • Microsporidia • Coccidian protozoa • Adult (gross) helminths

Abbreviation: CLIA, Clinical Laboratory Improvement Amendments of 1988.

[a] DPDx is the Centers for Disease Control and Prevention's Division of Parasitic Diseases and Malaria's service for diagnostic assistance.

in a diagnosis that was discordant with the original telemicroscopic diagnosis 7% (5 of 71) of the time. Two of these 5 cases were originally diagnosed as malaria, but follow-up analysis reclassified the diagnoses as babesiosis.[18] Two cases were *Plasmodium* infections that were misidentified at the species level, and one case produced a false-negative result by telemicrobiology because photomicrographs submitted to DPDx did not contain images of parasites. These findings demonstrate that telemicroscopic images sent to DPDx for malarial parasite identification usually yield a rapid and accurate diagnosis; but in this scenario, telemicroscopy is not as sensitive or specific for achieving *Plasmodium* species identification as traditional microscopy.[18] However, the rapidity associated with a DPDx consultation is essential to provide appropriate treatment in cases that might be fatal if not properly managed. For example, the morphologic similarities between *Babesia* spp. and *Plasmodium falciparum* is a common pitfall for sentinel laboratories, which can lead to misdiagnosis and subsequent clinical mismanagement. Rapid and accurate differentiation between babesiosis and *P falciparum* malaria has been achieved through DPDx telediagnosis.[18]

Proficiency Testing

Currently, the College of American Pathologists offers proficiency testing surveys that use telemicroscopy. Some surveys (eg, parasitology) use single-field photomicrographs. Other surveys (eg, virtual Gram stain and vaginitis screen) use static WSI (www.cap.org/web/home/lab/proficiency-testing/digital-scope-technology, accessed May 19, 2015) for proficiency samples. There are several advantages to using telemicroscopy for proficiency test samples. Telemicrobiology ensures that all proficiency testing sites have access to the same quality of sample. Only a small amount of physical and potentially infectious sample is required, which can then be imaged and shared electronically with an unlimited number of laboratories. This approach decreases the potential for exposure to potentially infectious substances that may be present in proficiency samples. Telemicrobiology provides cost savings because of decreased postage, consumables, and preparation time needed. It also relieves the laboratories of needing to keep track of a physical specimen. Disadvantages of using telemicroscopy for proficiency testing include using a method of analysis (visual digital image analysis) that is typically different from that which is used in routine laboratory testing. Moreover, digital image analysis requires a different workflow than what is used for physical specimens. For example, telemicroscopy bypasses testing the quality of the laboratory's preparation technique and microscope quality. The focus quality of the image is determined before it arrives to the laboratory, which may limit interpretation, especially for images with only a single Z plane. Nevertheless, the cost saving and specimen standardization advantages of using electronic samples for proficiency testing are 2 factors that will help ensure the continued and growing use of telemicrobiology in proficiency and competency testing.

Digital Plate Reading

In addition to telemicroscopy, telemicrobiology may also involve the remote analysis of digital images of culture media,

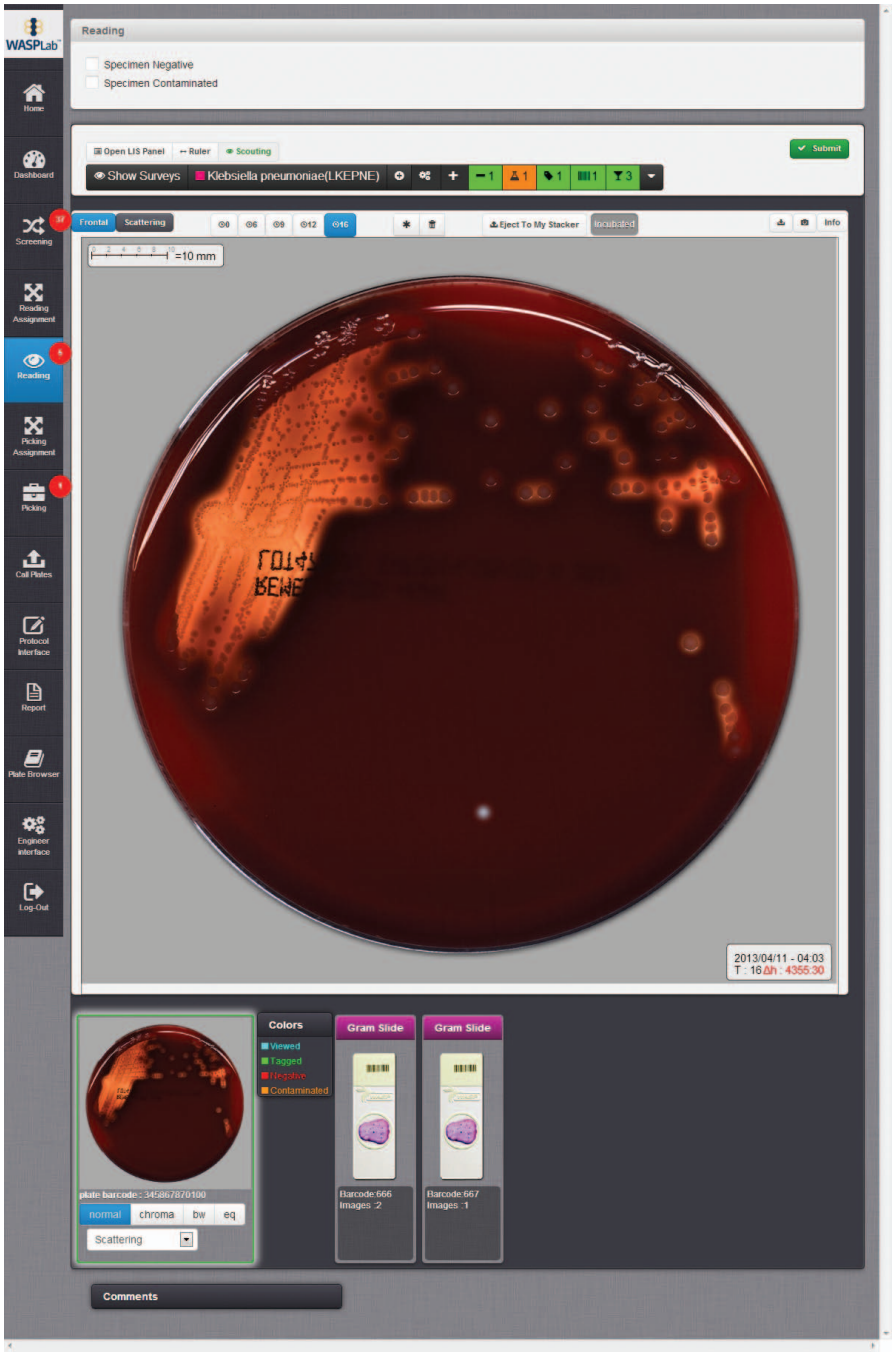

Figure 2. *Copan's WaspLAB software (Murrieta, California) interface for digital plate reading. The software displays a digitally photographed culture plate and provides tools to annotate and analyze the image. Thumbnail images of the Gram-stained specimen are visible at the bottom of the browser window, and these slides are meant to be viewed using the same software interface. Image is courtesy of Copan Diagnostics, Inc.*

which is referred to as *digital plate reading* (DPR). The emerging practice of DPR has been previously reviewed.[20] As described above, Scheid et al[10] and McLaughlin et al[14] have used custom-built systems for low-throughput DPR. However, commercial high-throughput DPR systems are currently being employed in some clinical microbiology laboratories, which aim to improve efficiency and quality. A DPR system enables a technologist to analyze bacterial culture plates remotely.[21] The DPR systems require a front-end component that can simultaneously incubate culture plates and employ automation to move the plates to an image-capture station at defined time points. In this system, removal of the culture plates from the incubator is minimized, which helps to avoid delays, repetitive manual tasks, and exposure to suboptimal incubation temperatures. The images are analyzed and interpreted by microbiologists, who view them on a computer display using proprietary software interfaces provided by the manufacturer (Figure 2). Work is being done to integrate digital images of Gram stains into this DPR analysis pipeline.[22] An initial report has demonstrated that DPR can help improve the productivity of microbiology technologists.[23] Although dozens of clinical laboratories, predominantly in Europe, have implemented DPR systems; peer-reviewed studies using these systems are needed. Copan (Murrieta, California), BD Kiestra (Drachten, Netherlands), and bioMérieux (Marcy l'Etoile, France) are companies currently marketing digital plate-reading systems for routine use.

Mobile Telemicrobiology

Many cellular telephones are now equipped with high-resolution digital cameras and cellular Internet connectivity. This combination of features has fostered the rapid emergence of mobile telemicroscopy, including mobile telemicrobiology.[24–26] Mobile telemicrobiology offers hope of affordable, accurate, and rapid teleconsultation for developing communities worldwide.[27] The most commonly investigated uses of mobile telemicrobiology are in the areas of tuberculosis detection and parasite identification.

One of two general strategies is typically used in mobile telemicrobiology. One approach is to use a portable microscope and an unmodified cell phone camera. In 2011, Tuijn and colleagues[26] used this approach. They described the utility of using mobile telephones containing cameras to capture and transmit microscopic images and short videos of pathogens, such as *Plasmodium* spp, *Giardia*, and *Mycobacterium tuberculosis*.[26] Their investigation concluded that cell phone cameras can capture and transmit images capable of verifying the presence of *Plasmodium* spp in blood smears and identifying the parasites to species, verifying bacterial vaginosis, verifying *Giardia* spp by viewing a video that captured its motility pattern, and verifying positive *M tuberculosis* smears. Tapley and colleagues[28] developed a prototype system that acts similarly to a cellular phone's camera and display screen to capture and display a fluorescent microscopy live feed. They demonstrated that minimally qualified individuals could effectively use the system to interpret sputa for the presence of mycobacteria, and the authors suggest this method could be enhanced by transmitting some of the images to remote experts. In 2009, Zimic and coauthors[29] described the use of cellular telephones to transmit images of mycobacteria grown in a drug-susceptibility test system. These images were first captured with a digital camera, and then, the data were transferred to a cellular telephone and transmitted via the phone to off-site experts for analysis. Presumably, if this study was performed today, the images could be capture directly onto a cell phone using the phone's camera. Zimic et al[29] reported concordance of interpretation between off-site analysis of digital images and on-site direct microscopic analysis of the cultures in 74 of 75 instances, and the authors concluded that the use of cellular phones for transmitting images would facilitate the diagnosis of multidrug-resistant tuberculosis in settings in which adequately trained staff is a limiting resource.

A second approach to mobile telemicrobiology is to modify an existing cell phone camera's lens to transform the phone's camera into a microscope. In 2013 and 2014, Bogoch and colleagues[30,31] used this type of approach. They described the use of a simple 3-mm ball lens (Edmund Optics, Barrington, New Jersey) taped to the iPhone 4S (Apple, Cupertino, California) camera lens that could be used directly on microscope slides containing stool preparations to detect worm eggs.[30,31] In their studies, they analyzed the samples' images directly on the phone, but this method could easily be adapted to enable an expert to remotely view the cell phone camera's live video feed. Unfortunately, the modified cell-phone camera provided very poor specificity (36%) when compared with using standard microscopy.[31] Switz and colleagues[32] demonstrated proof of concept that a reversed lens in a mobile phone's camera can potentially perform better than a 6-mm ball lens, and they demonstrated its ability to resolve an *Ascaris lumbricoides* egg with the help of image postprocessing. These studies did not attempt telemicrobiology, but remote analysis is a logical next step in these studies.

These types of mobile telemicroscopy systems have the potential to extend the ability to perform effective microbiology microscopy in resource-limited or remote settings that lack a highly qualified microbiologist. In such circumstances, difficult cases could be simply and rapidly sent for remote consultation via telemicrobiology. It remains to be seen whether video (live or recorded) or static capture methods will be most effective when using cellular phones for capturing and transmitting microscopic images for telemicrobiology.

FUTURE DIRECTIONS

Using readily available commercial products and open-source software to build telemicroscopy networks may help overcome clinical microbiology infrastructure challenges that are present in resource-limited areas of the world. This may provide a mechanism to increase the capacity of disease monitoring through collaboration in these underserved areas, specifically in the realm of malaria diagnoses.[33] Some investigators have demonstrated that e-mailing static images of blood smears are sufficient to diagnose malaria and other parasites that have distinct diagnostic morphologic features.[34] Indeed, static telepathology for diagnosis and parasite identification is performed routinely as part of the services provided by CDC's reference-diagnosis DPDx. Such services will undoubtedly continue to have an important role in telemicrobiology in the future because many laboratories have limited on-site expertise in diagnosing rarely encountered parasitic infections.

Additionally, the frequency of routinely using interlaboratory telemicroscopy networks in resource-rich locations may also increase. As health systems work to centralize and consolidate microbiology laboratories, maintaining the skills and competency to perform rapid microbiology testing (eg, blood culture Gram stains, spinal fluid microscopy, sterile tissue touch preparations, among others) at satellite locations can be a challenge,[35] and the use of automated slide-staining equipment,[36] combined with novel telemicroscopy software solutions,[37,38] may fill a growing need in the consolidating microbiology laboratory.

Guidelines for telepathology use and validation have recently been published,[4,39] and the College of American Pathologists has recently updated its Laboratory General Checklist to include items that specifically address telepathology and WSI. However, specific telemicrobiology guidelines have not been published. In the United States, the US Food and Drug Administration has not yet approved a WSI system to be used for primary diagnosis.[39] In the future, telepathology regulatory requirements will hopefully become clearer, and this clarity will foster implementation of telemicrobiology.

As telemicroscopy equipment becomes more prevalent, less expensive, and as mobile telemicroscopy platforms become more feasible, live video-telemicrobiology consultation may become more routine. Real-time telediagnosis is a promising area of application that could enhance the diagnostic capabilities of telemicrobiology consultation, such as the DPDx system.[16] This approach would allow the submitting laboratory to provide a live feed to the consultant, and the consultant would be able to guide the microscope operator and discuss the case in real-time.

Services such as DPDx could be used to support additional global and environmental health needs if properly implemented. For example, remote parasitology services could be used to expedite the identification of causative agents (eg, Cyclosporiasis or trichinellosis) from clinical, environmental, or food samples during outbreak investigations.[40,41] Some areas of the world face constant health threats but lack the expertise required to provide optimal diagnostic care. Developing an infrastructure that would enable those areas to gain access to services, such as DPDx, could greatly improve the areas' health care, but establishing and maintaining front-end quality (eg, proficiency training) and functional systems (eg, maintained devices and connectivity) have to be considered and supported if this type of investment to strengthen global disease detection is pursued.

Others are working to modify cellular telephones to be used as portable telemicroscopy instruments capable of capturing, analyzing, and transmitting diagnostic images from sputa or blood smears for telemicrobiology consultation[27,42] or for reference by the treating clinician.[43] Widespread adoption of cell phone cameras may foster an increase in telemicrobiology consultation. Crowdsourcing and gamification of microscopic identification of infectious agents, such as *Plasmodium* spp, have been investigated.[44–46] It is difficult to imagine this approach to telemicroscopy gaining traction in the current era of patient-privacy vigilance and analyst-credentialing requirements, but it is a novel contemporary approach to disease screening and diagnosis.

Telemicrobiology is a strategy that can be used to expedite interpretation or improve interpretation of microbiology images. However, another approach that can be used to accomplish these goals is the use of computer-augmented image analysis (CAII) software. Moreover, CAII has been successfully applied to routine cytopathology analysis to facilitate automated Papanicolaou test screening. In the clinical laboratory, CAII is routinely used in some hematology[47] and urinalysis[48,49] testing, but CAII is not yet routinely used in clinical microbiology. In microbiology, CAII studies have been performed, which interrogate the software's quality in screening slides for mycobacteria,[50] interpreting Gram stains, counting bacterial colonies, and detecting parasites. The most extensive body of work is in the development of automated image-analysis tools to detect and characterize malarial parasites in blood smears.[51–55] There is interest in implementing CAII for use in routine clinical microbiology,[56] and some have partnered cell-phone camera image collection with CAII.[57] Novel portable microscopes may one day be used to detect parasites directly from samples by using CAII, which would decrease the need for off-site expert analysis using telemicrobiology.[58,59] Consumers' and software developers' growing interest in affordable, modular, application-based software designed for mobile platforms may facilitate the adoption of CAII on mobile devices and will likely augment human telemicrobiology consultation in the future (Figure 3).[57]

CONCLUSIONS

Telemicrobiology systems can be effectively used to increase the speed or quality or both of microbiology visual data interpretation. Only a few complete telemicrobiology systems have been described, which were built for routine use.[10,14] These complete systems were custom-built, primarily used static imaging, and proved to be effective in accomplishing their goal of remote expert microbiology analysis. Laboratories have used remote expert analysis for routine daily operations, for consultation within a health system, and for external expert consultation. Those who have used telemicrobiology for clinical testing have recognized that the screening quality of the person who acquires the visual data can limit the sensitivity of the analysis. The use of an alternate telemicroscopy method, such as WSI, may improve the sensitivity and accuracy of routine telemicrobiology because WSI enables the off-site expert to access the entire slide and identify the most diagnostically relevant area of the slide instead of relying on the on-site photographer.

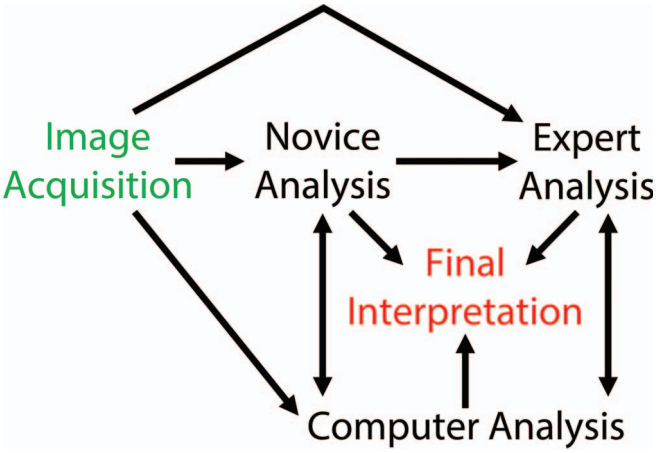

Figure 3. *The workflow of a telemicrobiology system begins with image acquisition (green), followed by a number of possible analysis pathways (black), and then a final interpretation (red). Typically, image acquisition and novice analysis are performed at the same physical location and do not incorporate the use of telemicrobiology. Telemicrobiology is used when consulting an off-site expert. Currently, computer analysis is not routinely used, but in the future, computer analysis is likely to be a valuable resource to enhance the quality and speed of digital image analysis that can be performed by on-site novices and off-site experts.*

Other telemicrobiology systems have been implemented for discrete applications within the laboratory, such as proficiency testing or DPR. The use of telemicrobiology in proficiency testing and plate reading is expected to continue to grow. Proficiency testing has been able to standardize assessments through implementing telemicrobiology. Studies have been performed to interrogate the utility of cellular phones and their cameras for capturing and transmitting microbiology visual data, and the greatest incentive for implementing these platforms is in resource-limited settings. Going forward, computer analysis of digital images will likely augment human analysis in the telemicrobiology workflow.

We thank Amanda Schmidt from Copan (Murrieta, California) for supplying Figure 2 and Mohamed E. Salama, MD, for his contribution to Figure 1, C and D.

References

1. Rhoads DD, Sintchenko V, Rauch CA, Pantanowitz L. Clinical microbiology informatics. *Clin Microbiol Rev*. 2014;27(4):1025–1047.
2. Kaplan KJ, Weinstein RS, Pantanowitz L. Telepathology. In: Pantanowitz L, Tuthill JM, Balis UGJ, eds. *Pathology Informatics Theory & Practice*. Chicago, IL: American Society for Clinical Pathology; 2012.
3. Cornish TC, Swapp RE, Kaplan KJ. Whole-slide imaging: routine pathologic diagnosis. *Adv Anat Pathol*. 2012;19(3):152–159.

4. Pantanowitz L, Sinard JH, Henricks WH, et al; College of American Pathologists Pathology and Laboratory Quality Center. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2013;137(12):1710–1722.

5. Williams S, Henricks WH, Becich MJ, Toscano M, Carter AB. Telepathology for patient care: what am I getting myself into? *Adv Anat Pathol*. 2010; 17(2):130–149.

6. Ghaznavi F, Evans A, Madabhushi A, Feldman M. Digital imaging in pathology: whole-slide imaging and beyond. *Annu Rev Pathol*. 2013;8:331–359.

7. Campbell WS, Lele SM, West WW, Lazenby AJ, Smith LM, Hinrichs SH. Concordance between whole-slide imaging and light microscopy for routine surgical pathology. *Hum Pathol*. 2012;43(10):1739–1744.

8. Kalinski T, Zwonitzer R, Sel S, et al. Virtual 3D microscopy using multiplane whole slide images in diagnostic pathology. *Am J Clin Pathol*. 2008; 130(2):259–264.

9. Rhoads DD. Applied telemicroscopy for microbiology: comparing the accuracy of whole slide images to static photomicrographs. Paper presented at: Pathology Informatics Summit 2015; May 7, 2015; Pittsburgh, PA.

10. Scheid P, Lam DM, Thommes A, Zoller L. Telemicrobiology: a novel telemedicine capability for mission support in the field of infectious medicine. *Telemed J E Health*. 2007;13(2):108–117.

11. Scheid PL. Use of telemedicine within the diagnosis of parasites and viruses. *Wien Klin Wochenschr*. 2012;124(suppl 3):10–13.

12. Dunn BE, Choi H, Almagro UA, Recla DL, Davis CW. Telepathology networking in VISN-12 of the Veterans Health Administration. *Telemed J E Health*. 2000;6(3):349–354.

13. Dunn BE, Choi H, Almagro UA, Recla DL. Combined robotic and nonrobotic telepathology as an integral service component of a geographically dispersed laboratory network. *Hum Pathol*. 2001;32(12):1300–1303.

14. McLaughlin WJ, Schifman RB, Ryan KJ, et al. Telemicrobiology: feasibility study. *Telemed J*. 1998;4(1):11–17.

15. Lam DM, Poropatich RK. Telemedicine deployments within NATO military forces: a data analysis of current and projected capabilities. *Telemed J E Health*. 2008;14(9):946–951.

16. Mathison B. Telediagnosis in the identification of parasites of public health concern—the DPDx perspective. Paper presented at: Pathology Informatics Summit 2015; May 7, 2015; Pittsburgh, PA.

17. Mathison BA. What can the CDC do for you?: telediagnosis and molecular technologies for diagnosing parasitic diseases. Paper presented at: The 114th ASM General Meeting; May 18, 2014; Atlanta, GA.

18. Mathison BA, Bishop H, Johnston S, Xayavong M, Arguin P, da Silva AJ. Trends in malaria diagnosis: combining the use of telediagnosis, microscopy and PCR in the identification of *Plasmodium* spp. Poster presented at: The 59th Annual Meeting of the American Society of Tropical Medicine and Hygiene; November 3–7, 2010; Atlanta, GA.

19. Mathison BA, Bishop H, Eberhard ML, Johnston SP, Long EK, da Silva AJ. Usefulness of telediagnosis in the identification of tissue parasites: an evaluation based on two years (from 2006–2008) of telediagnosis submissions to the CDC DPDx project. Poster presented at: The 57th Annual Meeting of the American Society of Tropical Medicine and Hygiene; December 7–11, 2008; New Orleans, LA.

20. Rhoads DD, Novak SM, Pantanowitz L. A review of the current state of digital plate reading of cultures in clinical microbiology. *J Pathol Inform*. 2015;6: 23.

21. Matthews S, Deutekom J. The future of diagnostic bacteriology. *Clin Microbiol Infect*. 2011;17(5):651–654.

22. Thomson R. Total lab automation in today's clinical microbiology lab: a buyer's perspective. Advance Health Network Webinar Series; December 9, 2014. http://laboratory-manager.advanceweb.com/webinar/webinar.aspx?rid=916. Accessed July 8, 2015.

23. Bentley N, Farrington M, Doughton R, Pearce D. Automating the bacteriology laboratory. Poster presented at: The 21st Annual Meeting of European Congress of Clinical Microbiology and Infectious Diseases; May 7–10, 2011; Milan, Italy. Poster 1792.

24. Skandarajah A, Reber CD, Switz NA, Fletcher DA. Quantitative imaging with a mobile phone microscope. *PLoS One*. 2014;9(5):e96906.

25. Frean J. Microscopic images transmitted by mobile cameraphone. *Trans R Soc Trop Med Hyg*. 2007;101(10):1053.

26. Tuijn CJ, Hoefman BJ, van Beijma H, Oskam L, Chevrollier N. Data and image transfer using mobile phones to strengthen microscopy-based diagnostic services in low and middle income country laboratories. *PLoS One*. 2011;6(12): e28348.

27. Breslauer DN, Maamari RN, Switz NA, Lam WA, Fletcher DA. Mobile phone based clinical microscopy for global health applications. *PLoS One*. 2009; 4(7):e6320.

28. Tapley A, Switz N, Reber C, et al. Mobile digital fluorescence microscopy for diagnosis of tuberculosis. *J Clin Microbiol*. 2013;51(6):1774–1778.

29. Zimic M, Coronel J, Gilman RH, Luna CG, Curioso WH, Moore DA. Can the power of mobile phones be used to improve tuberculosis diagnosis in developing countries? *Trans R Soc Trop Med Hyg*. 2009;103(6):638–640.

30. Bogoch, II, Andrews JR, Speich B, et al. Mobile phone microscopy for the diagnosis of soil-transmitted helminth infections: a proof-of-concept study. *Am J Trop Med Hyg*. 2013;88(4):626–629.

31. Bogoch, II, Coulibaly JT, Andrews JR, et al. Evaluation of portable microscopic devices for the diagnosis of Schistosoma and soil-transmitted helminth infection. *Parasitology*. 2014;141(14):1811–1818.

32. Switz NA, D'Ambrosio MV, Fletcher DA. Low-cost mobile phone microscopy with a reversed mobile phone camera lens. *PLoS One*. 2014;9(5): e95330.

33. Suhanic W, Crandall I, Pennefather P. An informatics model for guiding assembly of telemicrobiology workstations for malaria collaborative diagnostics using commodity products and open-source software. *Malar J*. 2009;8:164.

34. Murray CK, Mody RM, Dooley DP, et al. The remote diagnosis of malaria using telemedicine or e-mailed images. *Mil Med*. 2006;171(12):1167–1171.

35. Sautter RL, Thomson RB Jr. Consolidated clinical microbiology laboratories. *J Clin Microbiol*. 2015;53(5):1467–1472.

36. Baron EJ, Mix S, Moradi W. Clinical utility of an automated instrument for gram staining single slides. *J Clin Microbiol*. 2010;48(6):2014–2015.

37. Goswami R, Pi D, Pal J, Cheng K, Hudoba De Badyn M. Performance evaluation of a dynamic telepathology system (Panoptiq) in the morphologic assessment of peripheral blood film abnormalities. *Int J Lab Hematol*. 2015;37(3): 365–371.

38. Rhoads DD, Ahmed I, Pantanowitz L. Feasibility of using the Panoptiq imaging system for telemicrobiology. Poster presented at: Pathology Informatics Summit 2015; May 6, 2015; Pittsburgh, PA.

39. Pantanowitz L, Dickinson K, Evans AJ, et al. American Telemedicine Association clinical guidelines for telepathology. *J Pathol Inform*. 2014;5:39.

40. Hall RL, Lindsay A, Hammond C, et al. Outbreak of human trichinellosis in Northern California caused by *Trichinella murrelli*. *Am J Trop Med Hyg*. 2012; 87(2):297–302.

41. Abanyie F, Harvey RR, Harris JR, et al; Multistate Cyclosporiasis Outbreak Investigation Team. 2013 multistate outbreaks of *Cyclospora cayetanensis* infections associated with fresh produce: focus on the Texas investigations. *Epidemiol Infect*. 2015:1–8.

42. Prasad K, Winter J, Bhat UM, Acharya RV, Prabhu GK. Image analysis approach for development of a decision support system for detection of malaria parasites in thin blood smear images. *J Digit Imaging*. 2012;25(4):542–549.

43. Tice AD. Gram stains and smartphones. *Clin Infect Dis*. 2011;52(2):278–279.

44. Mavandadi S, Feng S, Yu F, Dimitrov S, Yu R, Ozcan A. BioGames: a platform for crowd-sourced biomedical image analysis and telediagnosis. *Games Health J*. 2012;1(5):373–376.

45. Luengo-Oroz MA, Arranz A, Frean J. Crowdsourcing malaria parasite quantification: an online game for analyzing images of infected thick blood smears. *J Med Internet Res*. 2012;14(6):e167.

46. Mavandadi S, Dimitrov S, Feng S, et al. Distributed medical image analysis and diagnosis through crowd-sourced games: a malaria case study. *PLoS One*. 2012;7(5):e37245.

47. VanVranken SJ, Patterson ES, Rudmann SV, Waller KV. A survey study of benefits and limitations of using CellaVision DM96 for peripheral blood differentials. *Clin Lab Sci*. 2014;27(1):32–39.

48. Linko S, Kouri TT, Toivonen E, Ranta PH, Chapoulaud E, Lalla M. Analytical performance of the Iris iQ200 automated urine microscopy analyzer. *Clin Chim Acta*. 2006;372(1–2):54–64.

49. Shayanfar N, Tobler U, von Eckardstein A, Bestmann L. Automated urinalysis: first experiences and a comparison between the Iris iQ200 urine microscopy system, the Sysmex UF-100 flow cytometer and manual microscopic particle counting. *Clin Chem Lab Med*. 2007;45(9):1251–1256.

50. Lewis JJ, Chihota VN, van der Meulen M, et al. "Proof-of-concept" evaluation of an automated sputum smear microscopy system for tuberculosis diagnosis. *PLoS One*. 2012;7(11):e50173.

51. Di Ruberto C, Dempster A, Khan S, Jarra B. Analysis of infected blood cell images using morphological operators. *Image Vis Comput*. 2002;20(2):133–146.

52. Frean JA. Reliable enumeration of malaria parasites in thick blood films using digital image analysis. *Malar J*. 2009;8:218.

53. Ross NE, Pritchard CJ, Rubin DM, Duse AG. Automated image processing method for the diagnosis and classification of malaria on thin blood smears. *Med Biol Eng Comput*. 2006;44(5):427–436.

54. Tek FB, Dempster AG, Kale I. Computer vision for microscopy diagnosis of malaria. *Malar J*. 2009;8:153.

55. Racsa LD, Gander RM, Southern PM, McElvania TeKippe E, Doern C, Luu HS. Detection of intracellular parasites by use of the CellaVision DM96 analyzer during routine screening of peripheral blood smears. *J Clin Microbiol*. 2015;53(1): 167–171.

56. Falbo R, Sala MR, Signorelli S, Venturi N, Signorini S, Brambilla P. Bacteriuria screening by automated whole-field-image-based microscopy reduces the number of necessary urine cultures. *J Clin Microbiol*. 2012;50(4):1427–1429.

57. Chang J, Arbeláez P, Switz N, et al. Automated tuberculosis diagnosis using fluorescence images from a mobile microscope. *Med Image Comput Comput Assist Interv*. 2012;15(pt 3):345–352.

58. Mudanyali O, Oztoprak C, Tseng D, Erlinger A, Ozcan A. Detection of waterborne parasites using field-portable and cost-effective lensfree microscopy. *Lab Chip*. 2010;10(18):2419–2423.

59. Linder E, Grote A, Varjo S, et al. On-chip imaging of *Schistosoma haematobium* eggs in urine for diagnosis by computer vision. *PLoS Negl Trop Dis*. 2013;7(12):e2547.