Malaria Diagnostics Technology Landscape:

Enhanced Visual Parasite Detection

Project DIAMETER (Diagnostics for Malaria Elimination Toward Eradication)

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MAILING ADDRESS
PO Box 900922
Seattle, WA 98109
USA

ADDRESS
2201 Westlake Avenue
Suite 200
Seattle, WA 98121
USA

TEL: 206.285.3500
FAX: 206.285.6619

www.path.org

PATH

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Enhanced visual parasite detection

The need

As national malaria control programs contemplate their options for shifting tactics and tools to support malaria elimination,[1] it is useful to compare and contrast how decreased transmission shifts the diagnostic focus:

- While control phase priorities aim to reduce the morbidity and mortality from malaria, elimination prioritizes driving human infection to zero.[2] Thus, passive infection detection strategies that dominate a control program focus need to be augmented by active infection detection tactics in an elimination context.
- In an elimination context, parasite reservoirs are often characterized by lower density, sub-patent infections. Thus, tests capable of accurate detection at levels below that of traditional microscopy and rapid diagnostic tests (RDTs) are desired.
- Changes in malaria epidemiology that are associated with low transmission often result in clustered populations of both symptomatic and asymptomatic individuals that represent a transmission risk. To achieve the most efficient diagnostic and treatment coverage with active detection tactics targeting these groups, portable, point-of-care tests that provide rapid results without loss to follow-up are needed.
- Successful control programs targeting *Plasmodium falciparum* (*P. falciparum; Pf*) often result in a proportional increase in *Plasmodium vivax* (*P. vivax; Pv*), *Plasmodium malariae* (*P. malariae*; *Pm*), and *Plasmodium knowlesi* (*P. knowlesi; Pk*) and non-malaria febrile illness incidence – thus, differential diagnoses of individual malaria species from other febrile illness gain importance in the elimination context.
- The costs and risks associated with investigating and treating false positives in low prevalence, elimination settings increase the emphasis on diagnostic specificity.
- Decreased budgets and the cost of maintaining strong surveillance systems and national surveys emphasize the importance of cost-efficient diagnostic systems in elimination context.
- The risk of malaria strains developing resistance to drugs and detection emphasizes the importance of detect-and-treat programs with high levels of temporal efficiency. Thus, high coverage levels and high diagnostic sensitivities are paramount to expedient elimination.

Microscopy

Microscopic examination of Giemsa-stained blood slides has generally been considered the gold standard in malaria diagnosis.[2,3] Various studies that have sought to evaluate new malaria diagnostics have often compared their results to expert microscopy. Microscopy remains the only diagnostic method in which the parasite is visualized. It allows the identification of different parasite species, various parasite stages including gametocytes, and the quantification of parasite density.[4] The World Health Organization recommends the use of microscopy in the control phase and from the pre-elimination phase onwards because of these attributes, and the establishment of microscopy capabilities is one of the pre-conditions for certification of elimination.

Microscopy, however, relies heavily on the knowledge, level of skill, and judgment of the user,[5] which could affect its sensitivity and specificity.[6] Studies have shown considerable intra- and inter-observer inconsistencies in parasite density determination.[2,7] Additionally, errors in diagnosis are likely in elimination settings where it may be difficult to maintain microscopists' proficiency as a result of low prevalence of infection.[2,8] In elimination settings, however, it is critical to detect all infections, including sub-clinical infections which are often characterized by low parasite densities.[2,9]

Given these inherent challenges with microscopy, there have been recent efforts to improve the efficiency and objectiveness of reading blood slides by automating the process.[10] Other related technologies are based on cell-phone microscopy. Furthermore, other technologies have attempted to enhance the visualization of parasites through the use of fluorescent dyes.

Automated slide preparation

The use of automated slide makers and stainers is increasingly becoming more popular in automated hematology labs, particularly in developed countries, but there is a dearth of information on their performance characteristics.[11] Automated slide making/staining machines could be of benefit in standardizing the preparation of blood slides. They may increase the consistency and quality of blood slides.[12]

Automated image capture and reading

The concept of using computer-based algorithms to analyze images captured from microscopic examination of stained thick or thin blood smears—used to identify and quantify malaria parasites—has been tested by a number of research groups.[13,14,15,16,17] Additionally, leveraging the widespread availability of cell phones even in low-resource settings, their use as diagnostic devices has been explored

by various groups.[18,19,20] By enabling users to perform on-board image analysis and/or wirelessly transmit those images off-site for further analysis by experts or for record keeping, these technologies may enable greater consistency in parasite detection.

Fluorescent microscopy

Fluorescent microscopy is based on the principle that certain fluorescent dyes have an affinity for the nucleic acid in the parasite nucleus and will attach to the nuclei. When excited by UV light at an appropriate wavelength, the nucleus will fluoresce strongly, thus enhancing the detection of malaria parasites. This markedly reduces the length of time required for the detection of malaria parasites. Fluorescent microscopy is a viable and rapid alternative to traditional microscopy.

The aim of this report is to assess opportunities within this diagnostic technology category of enhanced visual parasite detection and identify specific investment opportunities to advance infection detection technologies to advance malaria-elimination goals.

Landscape of technology solutions

The technologies have been broadly categorized as automated slide preparation, automated image capture and analysis, cell phone microscopy, and fluorescent microscopy.

A. Automated slide preparation

i. The UniCel® DxH™ Slide Maker Stainer (SMS) Coulter® Cellular Analysis System by Beckman Coulter

The DxH SMS requires minimal operator intervention.[21] By combining and automating the slide maker and stainer into one module, the DxH SMS offers a small footprint in the lab with very little maintenance. The equipment has been validated for multiple vendor tube types and can make and stain slides from different blood sample tubes. The system automatically aspirates about 25µl of blood for slide preparation from vacutainers containing venous blood. This implies that a larger volume of blood would have to be drawn from patients. The DxH SMS also provides the option of making slides, making and staining slides, and staining manually prepared slides. The staining protocol can be adjusted according to each lab's staining preferences. It has a "load n' go" feature which allows the user to load up to 180 slides. The DxH SMS uses the proprietary Hemasphere technology to capture the relevant measure of blood, thus providing a more reliable and consistent smear regardless of the blood consistency. It has a tracking system that allows users to track individual patient slides, and also includes default protocols as well as an open system where users can select a stain suitable for their laboratory. The DxH SMS, however, requires

other consumables, including specific microscope slides, which must be procured separately, and its overall cost may limit widespread use in low-resource settings.

ii. The Sysmex SP-1000i™ automated hematology slide preparation unit by Sysmex

The technology essentially operates on the same principles as the DxH SMS described earlier to provide rapid, automated preparation of peripheral blood smears to help hematology labs meet and standardize smear review turnaround times.[22] It is capable of processing up to 120 slides per hour, and a larger model, the 2SP-1000i slide maker stainer, processes 240 slides in an hour.

B. Automated image capture and analysis

i. The World Health Technology (WHT) autoanalyzer by Hydas World Health

The WHT autoanalyzer comprises a scanner and a computer. Its software technology reads digital images of standard Giemsa- or Fields-stained thick and/or thin blood slides. Algorithms are able to distinguish malaria parasites from other blood constituents and artifacts. Digital microscopes or imaging scanners may be used to acquire the images that are stored and subsequently serve as the input for the algorithm to locate, identify, and count the parasites. The software is enhanced to be more proficient as the database of images increases. This technology has been evaluated against the WHO55 test (a deck of 55 WHO-validated slides) and 140 slides obtained from a malaria indicator survey in Equatorial Guinea.[10] The WHT analyzer received an overall score of Level 4, which is the lowest score attainable among expert microscopists with reference to the WHO55 test. The WHT analyzer was able to detect parasites, identify species, and quantify parasites in 75%, 45%, and 7% of the slides respectively. Having gone beyond proof-of-concept to establish the feasibility of using this technology for malaria diagnosis, Hydas World Health is working to develop the technology further. The developers estimate they can achieve a lower limit of detection (LOD) of 25p/µl or better (personal communication Sept 17, 2013).

ii. TBDx™ by Applied Visual Sciences Inc.

The TBDx is a microscopy system that automatically loads slides, digitally captures images, and uses computerized algorithms to count acid-fast bacilli.[23] The system comprises a microscope, a camera, a slide rack, and a computer. The slide rack can be preloaded with a maximum of 200 slides. The system can potentially be adapted for use in smaller laboratories by using the camera and processing power available in mobile phone technology to capture and analyze images without the need for more expensive computers. The developers indicate that this fully automated hardware-software laboratory slide management and computer-vision detection platform has capabilities for future application to many other disease-detection challenges, such as malaria.

C. Cell-phone-based microscopy

The CellScope by CellScope

The CellScope was developed in a lab at the University of California following the demonstration that a mobile phone-mounted light microscope had potential for clinical use by imaging *P.falciparum*-infected red blood cells in bright field.[18] It is capable of performing image analyses. A patent application was filed by the University of California, Berkeley in 2009. Although the team is currently evaluating the CellScope for tuberculosis (TB) diagnosis, cytopathology, and ophthalmological screening in Thailand, India, and Cameroon, there is limited information on the use of the technology for malaria diagnosis beyond the proof-of-principle phase.[24]

Other research groups working on proof-of-concept cell-phone-based technologies have developed a compact, lens-free digital microscope that can be attached to the camera unit of a cell phone[25] and a category of lens-free microscopes that permits three-dimensional imaging of samples by relying on computation to partially undo the effects of diffraction that occur between the object and the detector planes.[26]

D. Fluorescent microscopy

i. The CyScope® by Partec

The Partec CyScope® (Partec GmbH, Münster, Germany) is a microscope that employs two light sources: in addition to the option of normal light microscopy, fluorescent light detection through incident UV light may also be used.[27] The microscope uses prepared and ready-to-use glass slides labeled with dry/lyophilized fluorescent dye that detects intraerythrocytic *Plasmodium* DNA, thus obviating the need for reagents, and special storage conditions. It integrates a powerful high-efficiency LED light source, enabling an extremely long lifetime of several thousand hours. It has been designed for mobility and has the ability to operate for several hours without a regular power supply. The CyScope uses built-in rechargeable batteries or AC line voltage (100–240 volts). The battery operation works up to six hours. The device has a built-in camera interface and an optional CMOS color camera upgrade may be ordered separately for further image analysis on a Windows™-based PC. It has been designed for use in malaria and tuberculosis diagnosis in resource-poor settings. The Cyscope® has been commercialized and is available through Partec subsidiaries and distributors in about 60 countries worldwide. The CyScope Plus Malaria—which is a binocular fluorescence microscope comprising UV LED (365 nm) excitation, white LED for transmitted light, 20X, 40X, and 100X (oil) objectives, and 25 malaria test slides—costs USD 5,240, while the CyScope Malaria, which is a monocular fluorescence microscope with the same components, costs USD 3,360. A pack of 200 ready-to-use slides costs approximately USD 229. Compared to traditional light microscopy, which uses about 10µl of blood, the Cyscope uses only a small

volume of blood (5µl) and is less labor-intensive and faster to use, therefore having a better turn-around time. It also requires less training and expertise. Furthermore, as the reagents are already dried onto the slides, there is no need for reagent preparation and staining of smears, thus minimizing variation and errors. Because it can operate on batteries, it is ideal for fieldwork and areas with no electricity. On the other hand, the Cyscope is not suited for species differentiation.

ii. The QBC® Malaria Test by QBC Diagnostics

The QBC® Malaria Test is a qualitative fluorescent microscopy-based malaria diagnostic test. It uses the fluorochrome acridine orange, which labels the DNA of *Plasmodia* and leucocytes.[28] Although acridine orange is a very intense fluorescent stain, it is non-specific and stains nucleic acids from all cell types. Centrifugal force is applied to the blood sample within a QBC capillary tube coated with acridine orange, potassium oxalate, sodium heparin, and K_2EDTA, resulting in density gradient layering of blood components. Although easy-to-use, fast, and easy-to-read, it requires specialized equipment such as a centrifuge, and a special attachment called the QBC ParaLens Advance, which provides fluorescence capabilities to any light microscope. A QBC Malaria system including a microscope with 4X, 10X, 40X, and 100X objective, the ParaLens Advance microscope attachment with a 60X objective, a QBC centrifuge, and a box of 2,000 QBC capillary tubes costs USD 8,420; a system without the microscope costs USD 7,320. A box of 2,000 QBC capillary tubes costs USD 4,210. The QBC Malaria Test has received clearance from the US Food and Drug Administration and has CE certification. The process of centrifuging the blood sample simplifies detection for users, as the parasites concentrate into specific, easy-to-locate layers in the tube. Also, the test concentrates a relatively large volume of blood (50-65µl), thus providing benefits in cases of low parasite density. The preparation and review of the test takes about eight minutes, which is far less than the time it takes to prepare and read a slide for traditional microscopy.

Table 1: Comparison of technologies

Technology	Light microscope	CyScope®	QBC®	WHT automated scanning	TBDx™ (currently for TB diagnosis only)
Stage of product development	Commercialization	Commercialization	Commercialization	Development	Validation
Quality of evidence[1]	Has been in use for over 100 years	3	2	1	2
Sensitivity (%)	Dependent on microscopists' expertise	Ranges from 62–100	Ranges from 55.9–99.0	89[a] 100[b] 92[c]	75.8
Specificity (%)	Dependent on microscopists' expertise	Ranges from 16.6–98.3	Ranges from 77.0–95.0	70[a] 94[b] 90[c]	43.5
Limit of detection	5–10 parasites/µl by experts ≥100 parasites/µl by average microscopist	400 parasites/µl	<10 parasites/µl may not detect low-level parasitemia	Estimated at 140 parasites/µl	NA
Parasite quantification?	Yes	Yes	No	Yes	Yes
POC compatible?	Yes	Yes	Yes	Yes	Yes
Blood quantity per test	10 µl	5 µl	50–65 µl	Same as microscopy (10 µl)	Same as microscopy (10 µl)
Throughput	1 slide	1 slide	Centrifuge in batches of 20 but tubes read individually	8 or 160 depending on scanner used	Up to 200
Time to results	30–60 minutes per slide	5 minutes per slide	Up to 8 minutes per tube (centrifugation plus reading)	5 minutes for scanning each slide using a 40x objective and results in less than 1 minute	2 minutes per slide: 1 minute for camera to autofocus and 1 minute to acquire 100 FoV (6–7 hrs to process a full slide loader of 200 slides)
Species differentiation	Yes	Cannot differentiate between species accurately. May have limited utility in areas with more than 1 *Plasmodium* spp.	Cannot differentiate between species accurately. May have limited utility in areas with more than one *Plasmodium* spp.	Yes, was possible in 60% of cases during evaluation.	Possible
Target analyte	*Plasmodium* spp	Plasmodia DNA	Plasmodia DNA	*Plasmodium* spp	*Plasmodium* spp
Need for electrical power?	Yes, but can use solar energy	Yes, but can operate on rechargeable battery	Yes	Yes	Yes
Capital cost (USD)	100–7,000	3,360–5,240	8,420	NA	NA
Cost per test (USD)	0.12–0.40	0.50	2.47	NA	NA
Storage of tests	Slides can be stored for an extended period.	Slides cannot be stored for an extended period but images of slides may be stored in a computer if the optional camera upgrade is obtained.	After centrifugation, tubes can be stored at temperatures up to 37°C without refrigeration, or refrigerated at 4°C for up to 2 weeks prior to examination.	Images of slides can be stored in the computer. Stained slides can also be stored.	Images of slides can be stored in the computer. Stained slides can also be stored.

[a] Compared to the WHO55 (35 positive, 20 negative); [b] Compared to EGMIS (13 positive, 106 negative); [c] Pooled slides (48 positive, 126 negative)

Promising technology

The automated slide-reading technologies hold a lot of promise. Both technology platforms need very minimal human intervention. The WHT automated scanning technology has gone through the proof-of-concept phase and is being improved upon by its developers. Although the TBDx has been principally developed and used for TB diagnosis, the technology holds a lot of potential for malaria diagnosis. Its ability to handle up to 200 slides makes it ideal for high-volume settings, and it could be used as a front-end screening application to other diagnostic modalities.

Gap analysis

The technologies based on fluorescence microscopy (QBC and CyScope) are currently available commercially. Their adoption has been poor, however, and it could be surmised that their limited availability and use is because they require specialized equipment and are relatively more expensive compared to light microscopy or even RDTs. This conclusion, however, needs to be explored further. Although a major limitation of fluorescence microscopy is the non-specific staining of debris and non-parasitic cells, which lower specificity, and species-specific diagnosis is not reliable, this technology may be considered as a screening tool for passive case detection. Samples found to be positive using fluorescent microscopy may be evaluated further for speciation and quantification. The CyScope has a comparative advantage in that it is portable and can be battery operated, whereas the QBC requires significant instrumentation.

While the technologies based on automated image capture and analysis are promising, none has reached an advanced stage of development, and further research and development is required to improve and validate the feasibility and utility of the devices and their in-built software. The developers of WHT automated scanning technology are working to improve upon the LOD by improving their algorithms and examining a larger portion of the slides, adding on other capabilities such that basic hematology profiles can be obtained, and working on producing a portable device that could be battery operated (personal communication on October 03, 2013).

The TBDx on the other hand, has been validated for TB diagnosis, and the developers indicate the Aurum Institute in South Africa has placed orders for the TBDx system with funding from the President's Emergency Plan for AIDS Relief (PEPFAR). Although the developers state that the technology can be adapted for malaria diagnosis, there is no further information on how this has progressed. The company is planning to enhance the performance of the TBDx for TB diagnosis by exploring color-based staining and revised detection algorithms and is developing strategic partnerships with academic institutions such as the Clinical Microbiology Lab at the Stanford University Medical Center. Plans are also underway to evaluate the tool in Uganda and Nigeria in collaboration with the Joint Research Center in Uganda and the Liverpool School of Tropical Medicine,

respectively. The evaluation in Nigeria will be carried out by piggy-backing it on an European & Developing Countries Clinical Trials Partnership (EDCTP)-funded project. The device will also be evaluated for commercialization in China later in 2013 in collaboration with a Chinese Government TB hospital in Tsingtao[29]. The extent of financial support involved is unclear.

The use of cell phones for the acquisition, analysis, and transmission of assay data is currently an area of active research and development. While this technology could be immensely useful at the lower levels of health care (e.g., among community health workers), the unreliable nature of phone network signals in areas where community health workers typically work and quality assurance challenges surrounding the slide preparation process may limit utility at such levels. The CellScope mobile microscopy group obtained support from the Bill & Melinda Gates Foundation, Blum Center for Developing Economies, UC Berkeley Center for Information Technology Research in the Interest of Society (CITRIS), Microsoft Research, Vodafone Americas Foundation, Stop TB Partnership, Siemens AG, Research to Prevent Blindness, Francis I. Proctor Foundation, and UC Berkeley Big Ideas Fund. The extent of support received from these institutions is, however, not known.

Generally, the probability of detecting malaria infection is a function of the density of parasites and the volume of blood examined, but some characteristics of the parasite—mainly sequestration (*Pf*), dormancy (*Pv*), and submicroscopic carriage—may limit the utility of technologies based on visual detection of parasites.[30,31] Additionally, the relatively lower volume of blood examined during microscopy potentially raises the LOD as compared to other techniques such as polymerase chain reaction.[31]

Nevertheless, in an elimination setting we envision that individuals and technologies that support vertical malaria control programs (e.g., malaria-specific microscopists and microscopes) will need to become more flexible as malaria becomes less of a burden to public health. It is likely that there will be decreasing funding to support vertical programs for low-prevalence, low-impact diseases. Thus, training and technologies such as general microscopy and microscopes validated for multiple pathogens will be favored to support general surveillance activities in horizontally integrated health systems.

Investment opportunity

Based on the technology landscape and gap analysis, technologies that provide more consistent parasite detection with minimal human intervention have the potential to make an impact on malaria diagnostics. An investment that may be considered is work with Hydas World Health (developers of the WHT automated scanning technology), and the developers of the TBDx, to support continued development and technology optimization. The following activities may be carried out:

- Support Hydas World Technology to improve upon the performance characteristics of the WHT autoanalyzer and explore integration of automatic slide preparation techniques.

- Support the developers of the TBDx to develop algorithms for the diagnosis of malaria.
- Support both developers to adapt their technologies to be used for other infections.
- Evaluate the products in multi-country settings to assess performance, cost-effectiveness, and acceptability.
- Identify an appropriate regulatory pathway and a commercialization partner for each technology.
- Partner the developer of the most promising technology with an established and reputable manufacturer.

Appendix A: The TBDx™ System

Website: http://www.appliedvs.com

The TBDx system includes:

- Prior 200 Slide Loader, with four slide cassettes containing 50 slides each;
- Olympus Microscope;
- Olympus Camera;
- Prior Automated Slide Stage;
- Joystick for manual stage movement;
- Computer running TBDx integration, detection, and reporting software.

Steps involved:

Step 1 – Prepare, stain, and dry slides as is normally done.
Step 2 – Preload slide rack with 1–200 slides.
Step 3 – TBDx system automatically inventories and selects each slide, inserts it into the stage of the microscope, focuses the microscope, digitizes 100 fields of view at 40X magnification, and downloads these data to a computer which then uses proprietary algorithms to detect and count acid fast bacilli (AFBs) on the digitized field of view. Slide processing currently takes approximately two minutes (one minute for the camera to autofocus the slide and another minute to acquire 100 digital fields of view).

Country	Comparator	Population	N	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	QOE score
South Africa[32]	Sputum culture	Gold miners suspected of having TB	981	75.8 (70.3–80.8)	43.5 (39.9–47.3)	2

Support: The company is planning to enhance the performance of the TBDx by exploring color-based staining and revised detection algorithms, and is developing strategic partnerships with academic institutions such as the Clinical Microbiology Lab at the Stanford University Medical Center. Plans are also underway to evaluate the tool in Uganda and Nigeria in collaboration with the Joint Research Center in Uganda and the Liverpool School of Tropical Medicine, respectively. The evaluation in Nigeria will be carried out with funding from the EDCTP. The device will also be evaluated for commercialization in China later in 2013 in collaboration with a Chinese Government TB hospital in Tsingtao.

Appendix B: The CellScope

Website: http://cellscope.berkeley.edu/

The steps involved in the use of the CellScope have not been well described. However, the image captured by the camera may be analyzed with an in-built image system (Image J), or it may be transmitted off-site for analysis.

Country	Comparator	Population	N	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	QOE score
NA	NA	Samples obtained from University of California	NA	NA	NA	0

Support: The CellScope mobile microscopy group obtains support from the Bill & Melinda Gates Foundation, Blum Center for Developing Economies, University of California (UC) Berkeley Center for Information Technology Research in the Interest of Society (CITRIS), Microsoft Research, Vodafone Americas Foundation, Stop TB Partnership, Siemens AG, Research to Prevent Blindness, Francis I. Proctor Foundation, and UC Berkeley Big Ideas Fund.

Appendix C: The Partec CyScope®

Website: http://www.partec.com

Steps involved:

1. Take a drop of blood (5µl) from a finger prick.
2. Place the drop of blood on the Partec Malaria Test slide (already contains dried-in reagents).
3. Cover the slide with a cover glass and directly analyze with the Partec CyScope.

Country	Comparator	Population	N	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	QOE score
Sudan[33]	Microscopy	Febrile patients >18yrs	293	98.2 (90.6–100.0)	98.3 (95.7–99.5)	2
Uganda[34]	Microscopy	Healthy children in a cross-sectional study	980	92.1 (89.6–94.1)	28.6 (22.8–34.9)	3
Uganda	Microscopy	Healthy adults in a cross-sectional study	552	86.7 (79.3–92.2)	38.8 (33.6–44.1)	3
Ghana[35]	RT-PCR	Febrile patients <5yrs	489	62.0 (56.3–67.8)	96.0 (92.3–98.3)	2
Ghana[36]	Microscopy	Febrile patients <5yrs	263	100.0 (96.6–100.0)	97.4 (93.6–99.3)	2
Nigeria[37]	Microscopy	Febrile children 6m–12yrs	209	91.3	16.6	2

Appendix D: The QBC® Malaria Test

Website: http://www.qbcdiagnostics.com

Steps involved:

Step 1 – Fill tube: Fill the tube with capillary or venous blood to a level between the two blue lines.
Step 2 – Roll tube: Keep the tube horizontal and roll between your fingers at least three times to mix the blood with the white anticoagulant coating.
Step 3 – Tilt tube: Allow the blood to flow to the opposite end of the tube into the orange reagent coating. Return to horizontal and roll five times to mix blood with the coating.
Step 4 – Seal tube: Remove a closure from the test tray well and press the opposite end of the tube into the closure. Twist and firmly push on the closure until it is sealed and properly aligned.
Step 5 - Insert float: Using clean plastic forceps or a clean gloved hand, select a float from the test tray well and insert it into the unsealed end of the tube. Tap the float into the tube with the forceps.
Step 6 – Centrifuge: Place tube in the centrifuge as per instructions and centrifuge for five minutes.
Step 7 – Insert the tube into the paraviewer.
Step 8 – Clamp onto the microscope stage.
Step 9 – Apply immersion oil and slowly elevate stage until the ParaLens touches the oil.
Step 10 – Review under microscope.

Country	Comparator	Population	N	Sensitivity (%)	Specificity (%)	QOE score
Sechuan, China[38]	Microscopy	Healthy volunteers	364	87.2	95.0	3
Sechuan, China	Microscopy	Suspected malaria patients	90	99.0	92.9	3
Nigeria[39]	Microscopy	Suspected malaria patients	353	55.9	88.8	3
India[40]	Microscopy	Suspected malaria patients	90	84.0	77.0	3
India[41]	Microscopy	Suspected malaria patients	572	80.7	94.5	3
India[42]	Microscopy	Suspected malaria patients	406	87.5	99.0	3

Appendix E: Summary of Enhanced Visual Parasite Detection Technology Attributes

	Definitions		
	Bad	Neutral	Good
Detection despite sequestered *P. faciparum*	No	NA	Yes
Detection despite sequestered *P. vivax*	No	NA	Yes
Years to commercialization	10,9,8,7	6,5,4	3,2,1
Quality of evidence score	0,1,2	3,4	>4

CyScope	QBC Malaria test	WHT autoanalyzer	*TBDx
No	No	No	No
No	No	No	No
1-3	1-3	7-10	7-10
3	2	1	2

	Scoring Definitions Compared to Existing Microscopy		
Product Characteristics	Inferior	Neutral	Superior
Sensitivity	lower	same	higher
Specificity	lower	same	higher
Limit of detection	higher	same	lower
Ease of use	more difficult	same	less difficult
Time to results	slower	same	faster
Infrastructure requirements	higher	same	lower
Cost	higher	same	lower
Process variability	higher	same	lower
Results interpretation variability	higher	same	lower
Throughput	lower	same	higher
Portability	less portable	same	more portable

CyScope	QBC Malaria test	WHT autoanalyzer	*TBDx
lower	lower	lower	N/A
lower	lower	lower	N/A
higher	same	higher	N/A
less difficult	less difficult	less difficult	less difficult
faster	faster	faster	faster
lower	same	same	same
higher	higher	higher	higher
higher	lower	lower	lower
higher	higher	lower	lower
same	same	higher	higher
more portable	less portable	less portable	less portable

*this technology has only been evaluated for TB diagnosis but could potentially be adapted for malaria

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