

Abstract

The QBC ParaLens Advance is a durable, easy-to-use option for providing LED fluorescence microscopy capabilities to any compound light microscope. Among its many applications, the ParaLens Advance can improve the sensitivity of malaria detection when viewing blood samples stained with fluorescent acridine orange dye. The QBC Malaria Test combines acridine orange with other patented breakthroughs to provide superior results to Giemsa thick films.¹ This application note demonstrates how the ParaLens Advance can work with the QBC Test to improve malaria detection for users.

Background

It is estimated that 350 to 500 million people each year are infected with malaria, and that over 1 million die annually from the disease.² Despite these numbers, malaria is treatable with early diagnosis and the proper use of anti-malarial medicines. To ensure proper diagnosis, the World Health Organization recommends both clinical and parasitological diagnosis of malaria before beginning a course of treatment.³ One of the most effective means of parasitological diagnosis involves staining a patient's blood sample with acridine orange, a fluorescent cationic dye.⁴ Acridine orange dye bonds with the nucleic acid in malarial parasites, causing them to appear green or red-orange against a dark red blood cell (RBC) background when excited by blue light in a fluorescence microscope.

The QBC Malaria Test was developed to take advantage of the benefits of fluorescence in parasite detection. The test consists of a precision capillary tube coated internally with acridine orange stain. It is centrifuged during preparation, allowing the components of blood, and parasites contained therein, to separate by density. This process concentrates parasites into distinct cell layers, simplifying and speeding detection. The test features a plastic float, which mechanically expands and thins the buffy coat and upper red blood cell layer to a few cell layers thick, further increasing sensitivity. While the benefits of the QBC Malaria Test are numerous, standard fluorescence microscopes used to review the test are expensive, bulky, and use dangerous mercury or xenon lamps as light sources. Because of these limitations, access to the QBC Malaria Test has been limited for many users who could benefit from the test's increased sensitivity and speed.

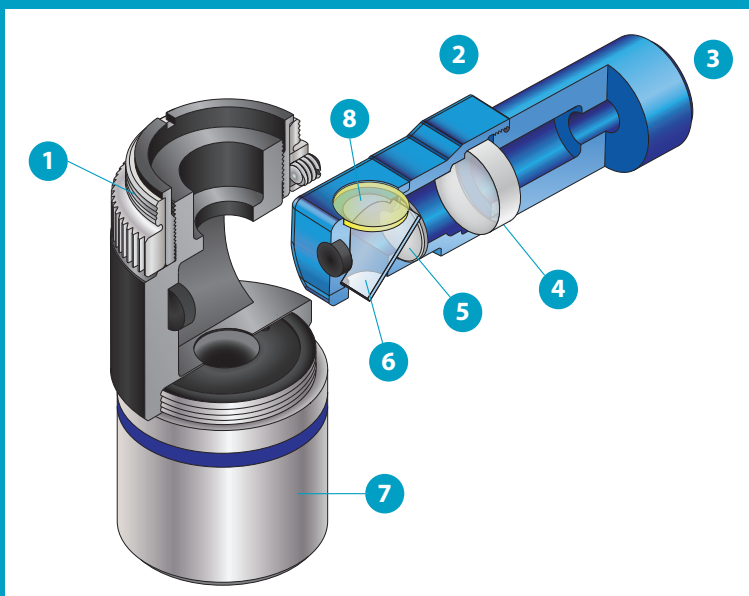
This application note will demonstrate how the ParaLens Advance, an LED fluorescence microscope attachment, provides an easy, affordable option for using the QBC Malaria Test to improve malaria detection. The ParaLens Advance can easily upgrade any compound light microscope for fluorescence microscopy through the use of a bright, durable and easy-to-power LED light source. By reducing the costs and hassles associated with traditional

fluorescence microscopes, the ParaLens Advance can place the benefits of the QBC Malaria Test in the hands of more clinicians on the front lines of the battle against malaria.

The ParaLens Advance

The main body of the ParaLens Advance (as seen below in Figure 1) can be attached as an objective to any conventional light microscope with standard Royal Microscopy Society (RMS) threading (1). (Ring transition adapters are available for non-standard microscopes.) All filters required for malaria detection are contained within a detachable blue filter set arm (2), which is inserted into the main body and held in place by a pair of powerful magnets. The blue LED light source attaches to distal end of the filter set arm (3) and produces powerful blue light with a wavelength of approximately 410-511 nm. The LED is DC powered and can be run on any wall outlet using the included AC to DC Power Pack, or by additional power options, such as the ParaLens Advance Portability Pack accessories (including 12 volt battery clips, a solar powered battery pack, a USB cable, and more) or the QBC Mobile Power Station (a 12 volt

Figure 1



ParaLens Advance Cutaway Diagram

battery station that can power the ParaLens Advance and other electronic devices).

Inside the filter set arm, light from the LED light source passes first through a focusing lens (4) and then through an excitation filter (5) that allows only light in the 385-480 nm range into the ParaLens Advance main body. A dichroic beam splitter (6) redirects the light downward to the specimen. Powerful objective lenses (7) magnify the fluorescent light emitted by the specimen. The ParaLens Advance is available with a 60x objective configuration (p/n 424330) designed for use with the QBC Malaria Test.

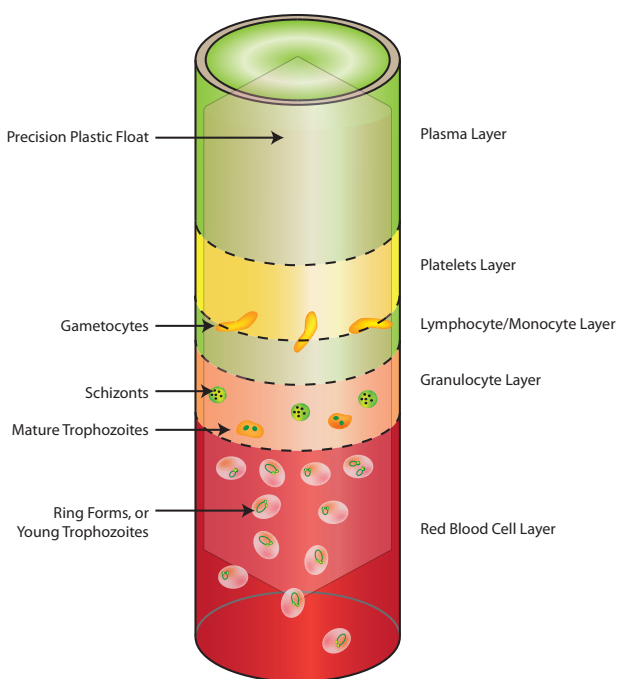
When the light returns to the beam splitter, light with a wavelength of ~510 nm or higher is allowed to pass through. Because the specimen has been treated with acridine orange, malarial parasites will appear green and/or red-orange and proceed through to the viewer. An emission filter (8) reduces background noise and optimizes the fluorescence signal transmitted to the observer.

Reviewing the QBC Malaria Test Set-Up And Focusing

To perform fluorescence microscopy using the ParaLens Advance with the QBC Malaria Test, first prepare and centrifuge the capillary or venous blood sample according to the QBC Malaria Test instructions. Then, insert the QBC tube into the center groove of the ParaViewer tube holder (as seen in Figure 2). The sealed end should extend over the depressed area of the holder (1), while the open end should pass under the retainer bar and rest against the retainer stop (2). It is important to rest the tube against the retainer stop, as it will prevent movement while the tube is manually turned during examination. The ParaViewer should then be placed on the microscope stage and clamped into place.

To bring the tube into focus, apply 2 to 3 drops of fluorescence optical immersion oil to the buffy coat region of the tube. To most easily focus the ParaLens Advance, view the descent of the lens from a side perspective. Attempt to focus the microscope on the buffy coat layer, which should appear slightly fluorescent when properly aligned with the ParaLens Advance. Gradually lower the ParaLens Advance until it touches the oil. There will be a distinct wicking effect when they touch. Now look through the eyepieces, and continue to focus downward. When part of the sample is in focus, gently adjust the Y-axis until most of the surface area is in focus. Once a sufficient percentage of the sample is focused, you should refrain from adjusting the Y-axis again. Instead, manually rotate the tube using the tube closure to see areas at the upper and lower borders of the sample. Turning the tube can cause some of the immersion oil to spread down the sides of the tube, so be sure to add more oil as needed.

Figure 3

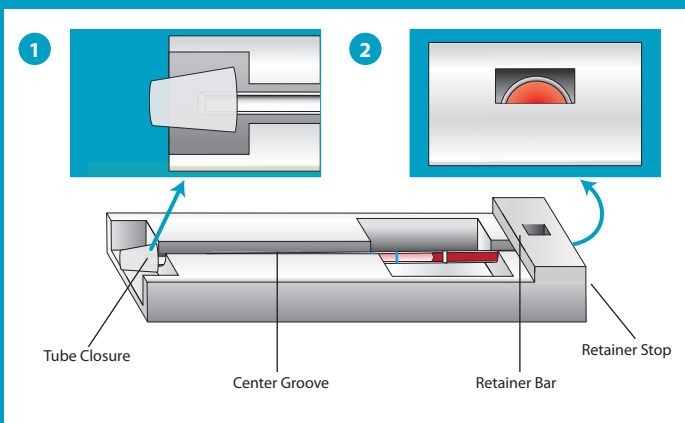


Cutaway Diagram of the QBC Malaria Test with Falciparum Malaria Parasites

Performing a Malaria Test

To begin searching for malaria parasites, slide the field-of-view along the X-axis to the interface of the Granulocyte and RBC layers (please refer to Figure 3 for a visual guide). Manually rotate the QBC tube using the tube closure to examine the entire circumference of the tube. From here, examine the tube toward the closure. Once you have reached

Figure 2



The QBC Malaria Test in the ParaViewer

the end of the tube, return to and scan the buffy coat, where some of the more mature malaria life forms can be found. An experienced technician should be able to perform a QBC Malaria Test in approximately 3 minutes.

Malaria parasites will often appear as young trophozoites, taking a signet ring shape. In the QBC Malaria Test, the “stone” of the ring will be green, while the “band” will be green to red-orange, and may or may not appear hollow. These ring forms are most easily found in the red blood cells near the granulocyte layer. Other signs possibly indicating the presence of malaria include yellow, sickle-shaped gametocytes (usually seen in *plasmodium falciparum* malaria); dark brown malaria pigment in mature trophozoites; schizonts; and gametocytes and distinct merozoites in mature schizonts.

Diagnosing Specific Forms of Malaria

Plasmodium falciparum – Falciparum malaria (seen in Figure 4) is the most deadly and most common cause of malaria. Falciparum malaria can infect all stages of RBCs and, therefore, typically will be distributed equally throughout the packed RBC layer. Some other common features that can help to distinguish *P. falciparum* parasites from other parasites include:

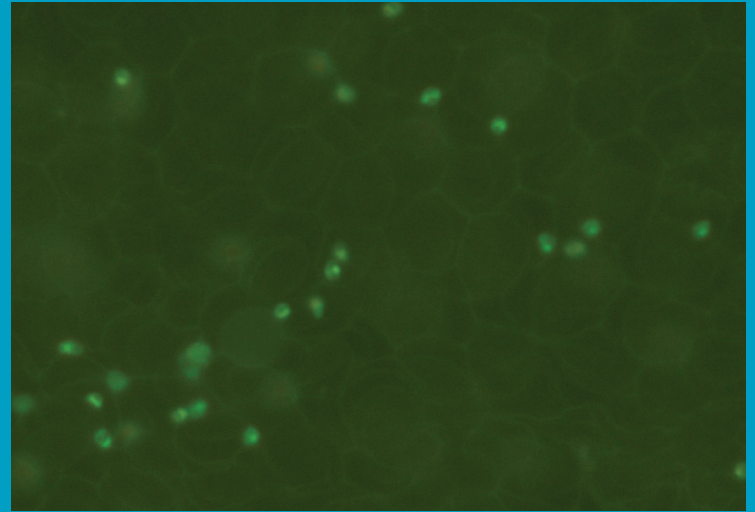
- Greater ratios of immature trophozoites to mature trophozoites and schizonts
- Double infections, i.e. two trophozoites in one RBC
- Headphone shaped trophozoites
- Yellow-green crescent, or sausage-shaped, gametocytes
- Equal distribution of ring forms throughout the RBC layer

Plasmodium vivax – Vivax malaria (seen in Figure 5) attack reticulocytes, which represent a small portion of blood cells (normally < 0.7%). These parasites will usually be found predominantly in the top of the packed red blood cell layer. Some common features that distinguish vivax parasites from other parasites include:

- Smaller ratio of immature trophozoites to mature trophozoites and schizonts
- Single, ring shaped infections
- Round gametocytes
- Schizont merozoite count of 7 to 12

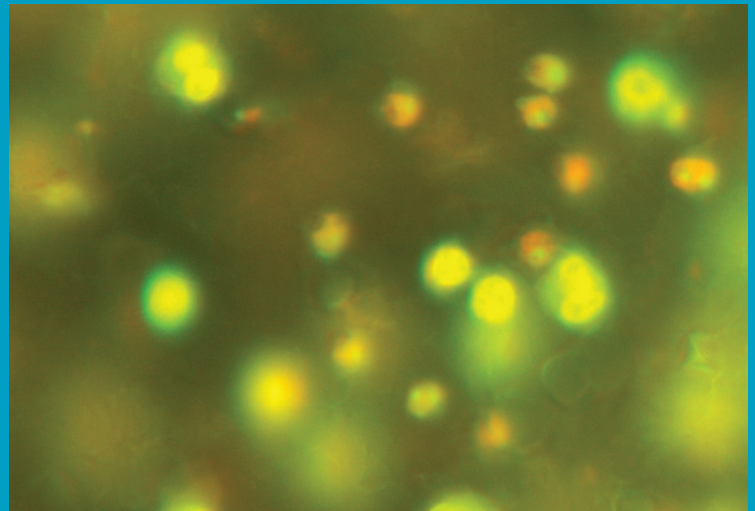
The ParaLens Advance can also be used to diagnose other, less common forms of malaria, such as *P. Ovale* and *P. Malariae*. Both forms exhibit characteristics similar to Vivax Malaria in the QBC tube. If *Ovale* or *Malariae* are known to be endemic to the area, additional testing may be indicated for samples that exhibit Vivax characteristics.

Figure 4



Falciparum Rings in the RBC Layer

Figure 5



Vivax trophozoites in white blood cell layer

Other Suggestions

We recommend that any technician unaccustomed to the ParaLens Advance or the QBC Malaria Test should first view several blood samples that are negative for malaria. This will allow the viewer to better understand what healthy blood looks like under the ParaLens Advance and in the QBC tube as well as to help identify common staining artifacts.

Fresh blood samples should always be used, if possible. Store unanalyzed blood specimens at 2 to 8°C (35 to 46°F).

Conclusion

An experienced microscopist can effectively use the ParaLens Advance Microscope Attachment and QBC Malaria Test

to provide accurate diagnosis in the treatment of malaria. The ParaLens Advance technology, with its unique LED light source, can turn any light microscope into a powerful fluorescence microscope at a minimum of cost or burden. Thus, the ParaLens Advance and QBC Malaria Test can serve as important tools in the worldwide battle against malaria.

References

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