

Abstract

The QBC ParaLens Advance is a durable, easy-to-use option for providing LED fluorescence microscopy capabilities to any light microscope. Among its many applications, the ParaLens Advance can be used to improve sensitivity in the detection of tuberculosis Acid Fast Bacilli (AFB) when reviewing direct smear sputum samples treated with F.A.S.T. AFB Stain, which can stain direct smear samples for review in just three minutes. This application note demonstrates how to use the ParaLens Advance with F.A.S.T. AFB Kits to simplify and improve AFB screening for more users around the world.

Background

It is estimated that 1/3 of the world's population is infected with tuberculosis (TB), and that almost two million people die each year from the disease.¹ The World Health Organization (WHO) and the Stop TB Partnership have the stated goal of reducing the prevalence of TB by 50% from 1990 levels by the year 2015, and reducing the disease to one per million population by 2050.²

The key to this goal is the advocacy of Directly Observed Therapy Short Course (DOTS), a multistage approach to developing TB awareness and support. One of the five elements of DOTS strategy involves improving the detection of *Mycobacterium tuberculosis*, the acid-fast bacterium (AFB) that causes TB. Traditionally, detection of AFB in suspected pulmonary TB cases has been performed using light microscopy on sputum samples treated with Ziehl-Neelsen stain. While this type of stain can be effective, fluorescence microscopy review, using auramine stain, is more sensitive and faster to perform than traditional methods.³ Unfortunately, the fluorescence microscopes needed for these tests are expensive and bulky and use dangerous and often fragile mercury or xenon lamps as light sources. Additionally, despite its improved sensitivity and decreased examination time, conventional auramine stain requires approximately the same amount of preparation time (20 minutes) as Ziehl-Neelsen stain to prepare direct smear samples, representing a missed opportunity for decreased workloads.

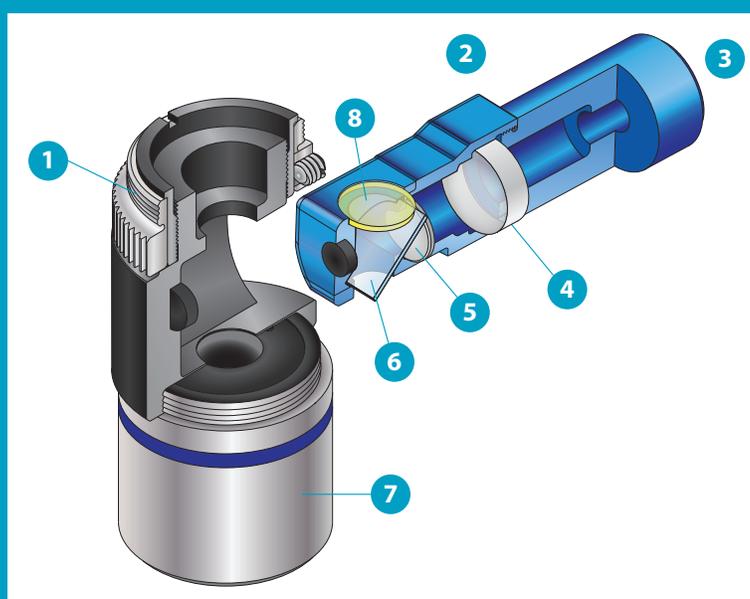
This application note will demonstrate how the QBC ParaLens Advance™ LED fluorescence microscope attachment and QBC Fluorescence and Staining Technologies (F.A.S.T.)™ AFB Kits can work together to overcome these obstacles and help TB programs better meet WHO and Stop TB Partnership goals. The ParaLens 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. The WHO has recognized the benefits of LED fluorescence microscopy in AFB detection, recommending that labs replace light microscopy and standard fluorescence microscopy with the increased sensitivity, durability and portability

of LED fluorescence.⁴ The ParaLens Advance works hand-in-hand with F.A.S.T. AFB Kits, which simplify AFB sample processing and staining with breakthroughs such as a 3-minute auramine stain and the patent-pending SureFocus microscope slide, which keeps samples in focus throughout review. Together, the ParaLens Advance and F.A.S.T. AFB Kits can increase the speed and efficiency of direct smear review, helping to reduce the prevalence of TB worldwide.

The ParaLens Advance

The main body of the ParaLens Advance (as seen in Figure 1) can be attached as an objective to any conventional light microscope with standard Royal Microscopy Society threading (1). (Ring transition adapters are available for non-standard microscopes.) All filters required for TB 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 the 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

Figure 1



ParaLens Advance Cutaway Diagram

can be run 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 battery station that can be used to 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. For AFB detection, the WHO has recommended that specimens be analyzed first under lower magnification, and that suspected AFB should be confirmed under a higher magnification.⁵ To facilitate this, the ParaLens Advance is available in a configuration (p/n 424330) that includes two main body assemblies, with 20x and 40x objectives.

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 Auramine dye, AFB will appear yellow-green or yellow-orange and proceed through to the viewer. An emission filter (8) reduces background noise and optimizes the fluorescence signal transmitted to the observer.

F.A.S.T. AFB Kits

QBC F.A.S.T. AFB Kits feature QBC Diagnostics' revolutionary F.A.S.T. Auramine O stain and counterstain. The configuration of this stain permits sputum staining and counterstaining in just 3 minutes. F.A.S.T. AFB Kits also include the unique, patent-pending SureFocus™ microscope slide. SureFocus slides are printed with a fluorescent marker that is visible under the same wavelengths that excite Auramine O stains. This marker can assist users in finding the proper focal plane, and provides visible guide points to standardize review to meet WHO requirements.

F.A.S.T. AFB Kits are available in several different configurations suitable for meeting any lab's unique TB burdens. The F.A.S.T. AFB Smear Kit (p/n 427409) was developed for labs performing direct smear microscopy, and includes supplies for 400 tests (see Figure 2): one 120 mL bottle of QBC F.A.S.T. Auramine O Stain (1), one 120 mL bottle of QBC F.A.S.T. Decolorizer/Quencher (2), 432 SureFocus slides (3), five quality control slides (4), 400 wooden applicator sticks

Figure 2



F.A.S.T. AFB Kit Components

(5), and four bags of 100 sputum cups (120 mL) with lids (6). The F.A.S.T. AFB E-Z Smear Kit (p/n 427410), is a version of the kit designed for easy portability, with 10 individual packs containing materials for 5 tests, including one 3 mL bottle of F.A.S.T. Stain, one 3 mL bottle of F.A.S.T. Decolorizer/Quencher, five SureFocus slides, five wooden applicator sticks, and five sputum cups.

The QBC F.A.S.T. AFB Smear Kit with Digestion Solution (p/n 427408) is designed for labs performing culture or PCR. For more information on these procedures, please consult the ParaLens Advance application note: "Reviewing Slides Prepared With F.A.S.T. AFB Kits with Digestion Solution".

Reviewing Direct Smear Samples with the ParaLens Advance and F.A.S.T. AFB Kits

Sputum Sample Collection

If a patient has presented with any of the clinical symptoms of pulmonary tuberculosis, the patient should be asked to give two to three sputum samples over the next few days. The sputum cups contained in the F.A.S.T. AFB Kits are designed for this purpose. The patient should be instructed

to provide only sputum (consisting of mucus and phlegm), and not saliva. Sputum smear slides can be prepared from these samples to detect the presence of AFB.

(Note: For further guidelines and standards in the care of patients presenting with TB symptoms, please consult the International Standards of Tuberculosis Care (ISTC) at http://www.tbcta.org//Uploaded_files/Zelf/InternationalStandardsforTuberculosisCare1181209995.pdf and the Practical Approach to Lung Health (PAL) strategy documents at http://whqlibdoc.who.int/hq/2005/WHO_HTM_TB_2005.351.pdf and http://whqlibdoc.who.int/hq/2008/WHO_HTM_TB_2008.410_eng.pdf.)

Preparing a Direct Smear Sample

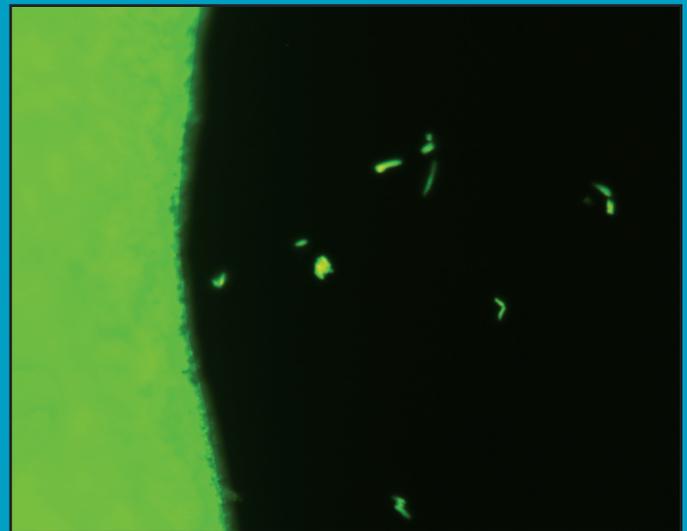
To prepare the slides for staining, use the applicator stick to take a small amount (~ 100 µl) of the sample and apply it to a SureFocus slide. Heat fix the slide to kill any active mycobacteria and permanently adhere the smear to the slide. The preferred method of heat fixing is a slide warmer set at 65 degrees Celsius for two hours, but heat fixing can be done with an open flame. If a flame is used, do not allow the sample to become charred.

Once the slides have been heat fixed, they are ready for staining. Cover the smear with *F.A.S.T.* Auramine O Stain and let stand for 1 minute. Rinse gently with deionized or tap water and drain. Cover the smear with *F.A.S.T.* Decolorizer/Quencher, and let stand for 1 minute. Again, rinse gently with water and drain. Once the slide is dry, it can be reviewed using the ParaLens Advance.

Set-Up and Focusing the ParaLens Advance

To perform epifluorescence microscopy using the ParaLens Advance, first remove two objectives from the nosepiece of any compound light microscope. Screw the ParaLens

Figure 4



AFB, seen with the SureFocus Marker

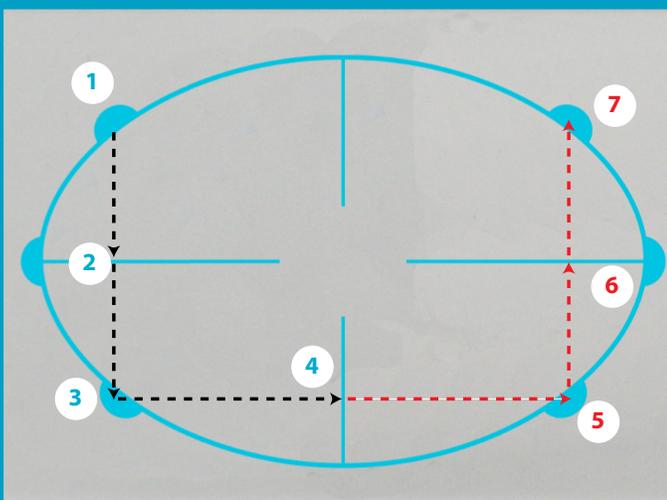
Advance 20x and 40x objective main body assemblies into the open slots. Insert the blue filter set arm into the 20x assembly. You should feel a distinct pull as the magnetic connection is established.

Place the blue LED light source on the distal end of the filter set arm, and manually tighten the small thumb screw. Select the power option of your choice. If using the included power pack, slide the proper international adapter for your location onto the adapter and press downward to click into place. Insert the plug end of power pack into an outlet, and the cord end into the power input of the LED light source. (Note: For instructions on the use of other power options, see the ParaLens Advance Operator's Manual.) Turn the black intensity control knob to turn on the LED light source.

Place the prepared SureFocus slide on the microscope stage and clamp it into place. To most easily focus the ParaLens Advance, center the 20x objective over the upper left starter circle of the SureFocus fluorescent marker, as demonstrated in Figure 3 (1). This can be accomplished in two ways: by turning on the microscope's light source and finding the starter circle using a low power objective, or by aligning and viewing the descent of the lens from a side perspective. Lower the lens until it is about 0.5 cm from the slide. Now look through the eyepieces, and continue to focus downward. Figure 4 shows the SureFocus slide under 600x magnification. (Note: The large green object on the left side is part of the slide marker, while the small green objects on the right are AFB.)

Once the starter circle is in focus, begin viewing the sample at one corner of the smear and work systematically through the smear. A suggested review path is to move from the upper left starter circle along the Y-axis to the left crosshair (2). Use the crosshair to refocus. Continue down the Y-axis

Figure 3



SureFocus Suggested Review Path

to the lower left starter circle (3). Again refocus. Now move right toward the bottom crosshair (4).

When using the 20x objective, you should be able to follow this path in approximately 26 fields. If you discover what you believe to be AFB, switch to the 40x ParaLens Advance objective to confirm. If further fields are needed for viewing (to confirm a scanty or an unclear result), observe the sample along the path designated by numbers 4-7. Use the same methods as previously described.

For more information on working with the SureFocus microscope slides, please consult the library of materials on the SureFocus slide at www.qbcdiagnostics.com.

Performing a TB Screen

Under the ParaLens Advance, TB mycobacteria should appear as yellow-green or yellow-orange, rod-shaped objects (as seen in Figure 4). Under a fluorescence microscope, they should stand out in stark contrast to the dark background. Occasionally, artifacts such as crystals, hair, or cells may appear in the sample.

The World Health Organization has defined a “smear positive” case as “the presence of at least one acid fast bacilli (AFB+) in at least one sputum sample in countries with a well functioning external quality assurance (EQA) system.”⁶ The following recommended grading scale, based on the current WHO standard, can be used to quantify AFB⁷:

Report	200x (~ 26 Fields)	400x (~ 52 Fields)
No AFB Seen	0	0
Exact Count	1-29 AFB total	1-19 AFB total
+	1-10 AFB per field	20-199 AFB total
++	10-100 AFB per field	5-50 AFB per field
+++	>100 AFB per field	>50 AFB per field

Quality Control

Adequate quality control is critical for the evaluation of laboratory staining and review procedures. To facilitate proper quality control, F.A.S.T. AFB Kits contain 5 quality control slides that contain one non-viable smear of Mycobacterium tuberculosis (contained in a circle marked “+”) and one non-viable smear of Escherichia coli (contained in a circle marked “-”). These smears should be stained and examined as with any patient specimen. QC should

be performed regularly and results should be recorded, in accordance with governing regulations.

Change Over

A slide stained for fluorescence microscopy can also be re-stained for light microscopy using the Ziehl-Neelsen method with no additional preparation. Simply perform the staining using standard methods.

Conclusion

The ParaLens Advance microscope attachment is capable of providing the benefits of an expensive fluorescence microscope at a fraction of the cost. It can be used with an existing light microscope, thereby saving precious lab resources. Combined with the increased staining speed of the QBC F.A.S.T. AFB Kits, the system is an important new weapon in the worldwide fight against tuberculosis.

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