F.A.S.T.™ Lab System

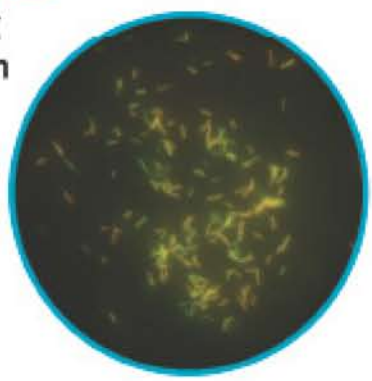
The complete Fluorescence Microscopy Solution from QBC

Complete User Guide

A person with active TB can infect 10 to 15 other people per year[1]
Nearly 1 million people die annually from malaria[2]

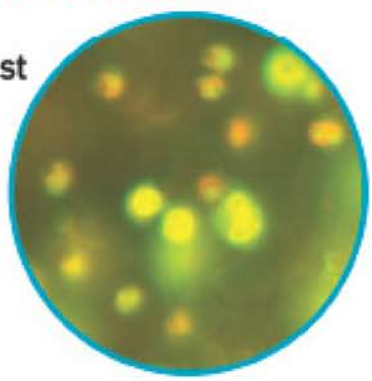
F.A.S.T.™ TB

- Reduce staining time to less than 3 minutes



F.A.S.T.™ Malaria

- Malaria Slide
- Rapid Malaria Test



Fluorescence and Staining Technologies (*F.A.S.T.*) is a revolutionary product line developed to help achieve the mission of the Stop TB Partnership, the Global Malaria Programme and the World Health Organization by providing complete solutions for fast, simple and accurate TB and malaria detection.

It's critical to have TB detection that is *F.A.S.T.*™

The proprietary *F.A.S.T.* 3-minute TB staining process dramatically reduces the conventional Auramine O smear preparation time from 20 minutes to 3 minutes while maintaining the high sensitivity standards of fluorescence microscopy. Whether your lab needs a complete TB microscopy system or simply TB microscopy supplies, we have a fast and complete solution.

F.A.S.T.™ and accurate detection of Malaria

In addition to the QBC Malaria Test, QBC Europe now offers a range of solutions for fast, accurate detection of malaria, including the *F.A.S.T.* Malaria Slide and *F.A.S.T.* Malaria Rapid Test.

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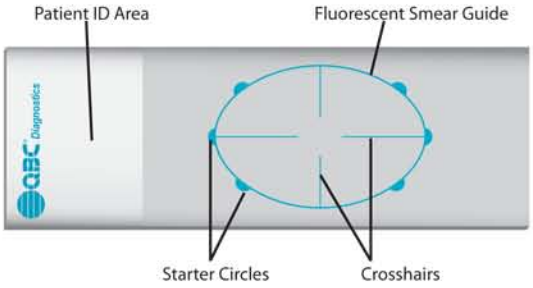
1 World Health Organization Website, 23 March 2009. Web 3 September 2009 (http://www.who.int/features/factfiles/tuberculosis/en/index.html)
2 World Health Organization Website, March 2009. Web 21 October 2009 (http://www.who.int/features/factfiles/malaria/malaria_facts/en/index1.html)

SureFocus™ Microscope Slide User Guide



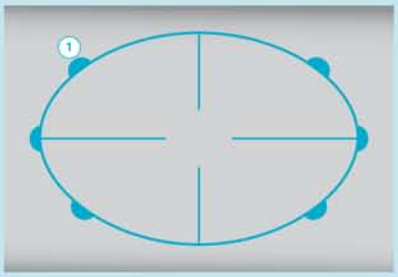
Patient ID Area: Etching area for proper labeling of specimen or patient ID

Fluorescent Smear Guide: A thin fluorescent oval that provides users with a standard area for smear preparation

Starter Circles: Large fluorescent markers that provide a reference point for focusing on slide

Crosshairs: Fluorescent lines that aid the user in quantifying fields as well as allowing proper focusing throughout the examination pathway, as explained below

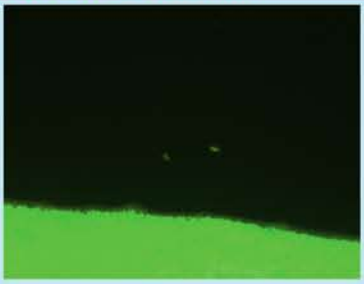
1. Upper Left Starter Circle

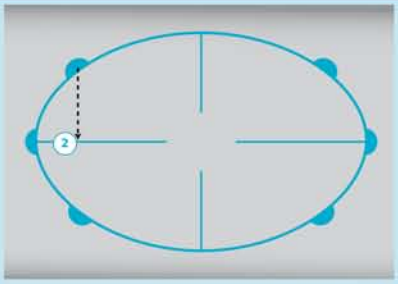


Focus on this upper left starter circle, and travel down toward the left crosshair. AFB may gather close to the fluorescing mark, as shown in the image to the left.

Note: All photographs in this section were taken at 600x magnification with limited digital modification.

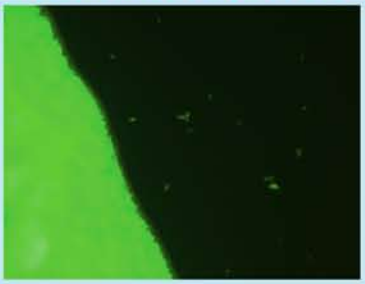
2. Left Crosshair

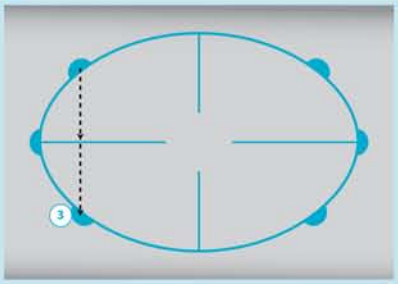




Once the crosshair has been reached, refocus (if necessary) and continue downward to the bottom-left starter circle. Two (2) fluorescing bacilli are seen in this picture.

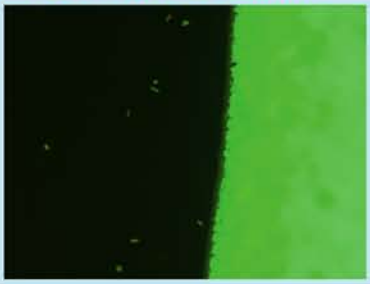
3. Lower Left Starter Circle

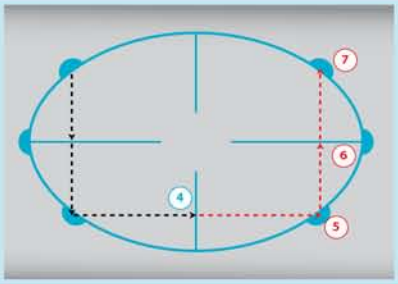




After reaching the lower left starter circle, refocus (if necessary), and continue moving right toward the bottom crosshair. Both single and clumped bacilli are shown in the image to the left.

4. Bottom Crosshair





Once the final crosshair has been reached, quantify the number (#) of bacilli seen, and use the standard recording system on the next page to report findings, based on magnification.

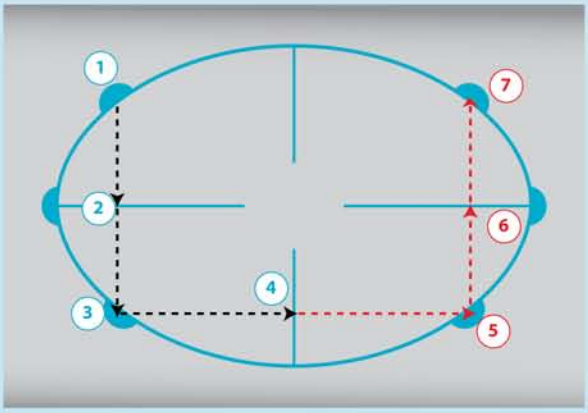
If further fields are needed for viewing (to confirm a scanty or an unclear result), observe the sample along the path designated by numbers 4-7. Use the same methods as previously described.

SureFocus™ Microscope Slide User Guide

Calculating Fields Viewed

By moving along the dotted lines from point 1 to point 4 (as seen to the right) the reader will view an appropriate number of fields to quantify AFB based on the WHO standards for AFB Smear Grading (as seen below). Use the following chart to determine the approximate number of fields read:

Total Magnification	Approximate # of Fields Viewed (from points 1-4)
200x	26
400x	52
600x	78



Reporting Results

Follow this recommended grading scale to quantify AFB (based on the WHO guidelines for AFB smear grading[1]):

Ziehl-Neelsen Stain	Fluorescence Microscopy: # of AFB and Fields Observed Using SureFocus Slide (approximate, using points 1-4)			Report
1000x (~100 fields)	200x (~26 fields)	400x (~52 fields)	600x (~78 fields)	
0	0	0	0	No AFB Seen
1-9 AFB total	1-29 AFB total	1-19 AFB total	1-12 AFB total	Report Exact Count
10-99 AFB total	1-10 AFB per field	20-199 AFB total	13-239 AFB total	+
1-10 per field	10-100 AFB per field	5-50 AFB per field	3-30 AFB per field	++
>10 per field	>100 AFB per field	>50 AFB per field	>30 AFB per field	+++

1. Fluorescent Stain Preparation. WHO laboratory services in tuberculosis control. Part II: Microscopy. WHO/TB/98.258. Geneva, Switzerland: WHO, 1998: 31-34.)

Slide Reading Tips

1. Use positive and negative control slides as a reference and quality control for each batch of slides stained. These controls also aid the viewer in reviewing the morphological characteristics of fluorescing organisms.
2. Bacilli size is roughly 1-10 µm long, and may appear as curved or bent rods. Some non-tuberculous species of mycobacteria may appear more coccoid or have longer rods.
3. Bacilli may appear alone or in small groups. Large clumps of organisms are rarely seen from direct specimen smears.
4. Fluorescing bacilli should appear as yellow-green rods against a darker background.
5. Non-mycobacterial artifacts are sometimes present in specimens and may fluoresce. Examples include hair, fiber and food particles. To avoid confusion, it is important to learn what a fluorescing bacillus looks like.

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F.A.S.T.™ AFB User Guide: Slide Preparation for Acid-Fast Bacilli (AFB) Detection

***F.A.S.T.* Products used in slide preparation:**

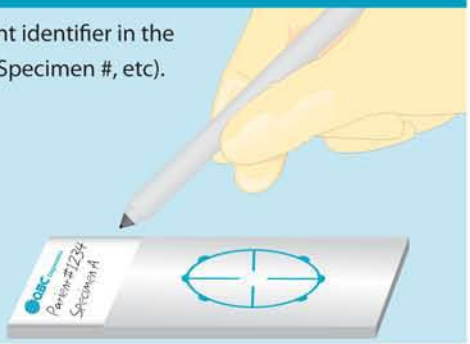
- One (1) *F.A.S.T.* Wooden Applicator Stick
- One (1) *F.A.S.T.* SureFocus™ Microscope Slide (or *F.A.S.T.* Microscope Slide)

1. Inspect Sample

Visually inspect sputum cup for leakage. Record patient and sample information (patient ID, sample amount, etc.) on laboratory record sheet.

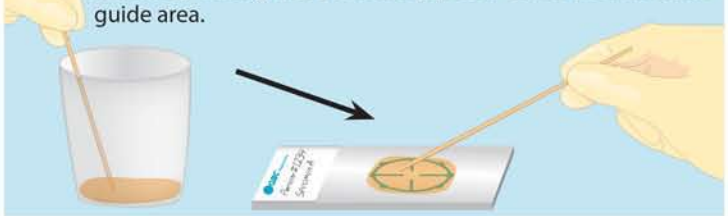
2. Label Slide

Label slide with patient identifier in the patient ID area. (ID #, Specimen #, etc).



3. Apply Smear*

Remove a small portion of the specimen and smear it evenly over the center of the slide. Avoid saliva and debris, and choose material that is thick, bloody, or discolored. If the specimen is extremely watery, use a transfer pipette. Cover the blue stain guide area.



4. Air Dry

Allow slide to air dry approximately 5 to 10 minutes prior to heat fixing.

Save the sputum cup until after smear examination, then discard the cup into a biohazard container.

5. Heat Fix Slide

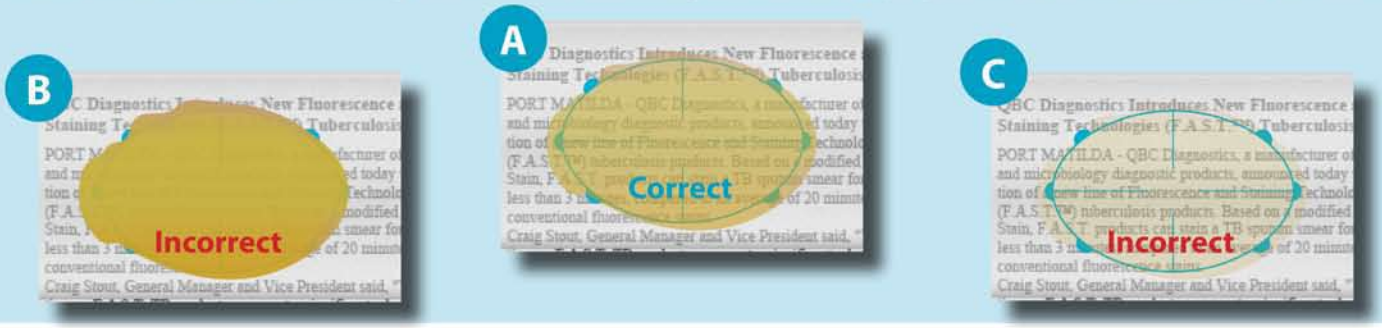
Heat fix the slide using a bunsen burner or electric heating block.

If using a bunsen burner, pass the slide through the flame 3 to 4 times. Do not allow the sample to char. For heating blocks, set to 65-75 °C and allow slides to heat fix for 2 hours.

OR

Examples of Slide Thickness

A prepared smear should be thick enough to read newsprint through (A). Avoid applying smears that are too thick (B) or too thin (C).



***Note: When working with patient specimens, Personal Protective Equipment (PPE) should be utilised per standard laboratory protocols. An example may be found at www.cdc.gov/od/ohs/tb/tbdoc2.htm.**

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F.A.S.T.™ AFB User Guide: Staining Procedures for Acid-Fast Bacilli (AFB) Detection

***F.A.S.T.* Products used in specimen staining:**

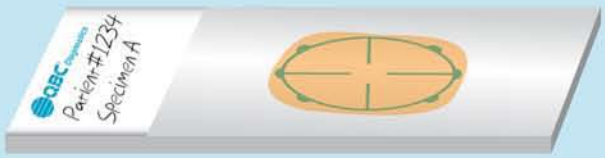
One (1) Bottle *F.A.S.T.* Auramine O AFB Stain

One (1) Bottle *F.A.S.T.* Decolorizer/Quencher

1. Process Sample

Process and prepare sample fully, as seen in the *F.A.S.T.* AFB User Guide: Slide Preparation.

Allow slide to completely cool before staining.



2. Apply Stain

Use 8-10 drops of *F.A.S.T.* Auramine O Stain, or until smear is covered. Let stand for approximately 1 minute.

3. Rinse

Gently rinse slide with sterile water. Be careful to avoid washing specimen off of slide. Carefully shake slide to remove excess water.

Note: Tap water may contain chlorine, which can interfere with fluorescence. Avoid using if possible.

4. Apply Decolorizer

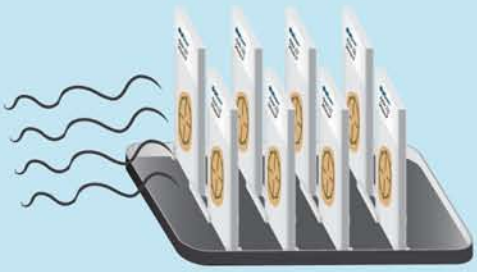
Use 8-10 drops of *F.A.S.T.* Decolorizer/Quencher, or until smear is covered. Let stand for approximately 1 minute.

5. Rinse

Rinse slide again with sterile water.

6. Dry Slide

Allow slide to air dry. If necessary, gently blot excess water with a lint free tissue.



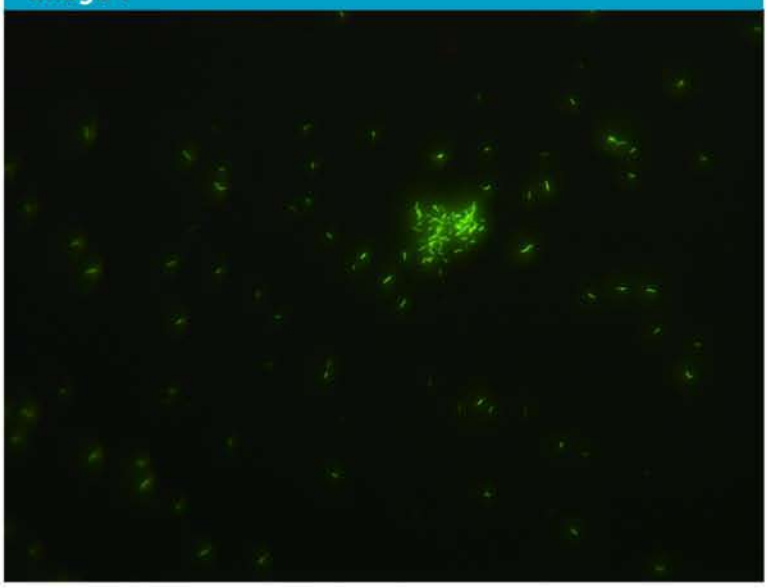
7. Examine

Examine the slide using the ParaLens Microscope Attachment, or other fluorescent microscope.

See reverse side for examples of AFB stained with *F.A.S.T.* reagents.

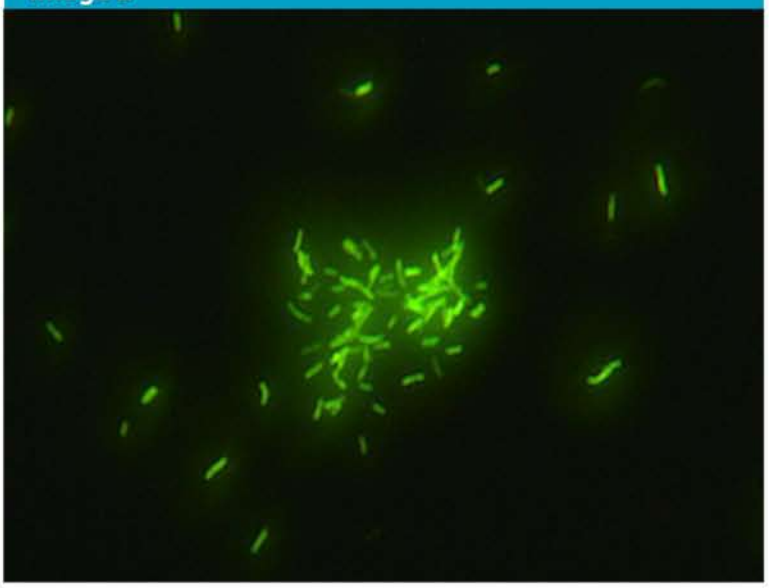
F.A.S.T. AFB Guide: Slide Review

Image 1



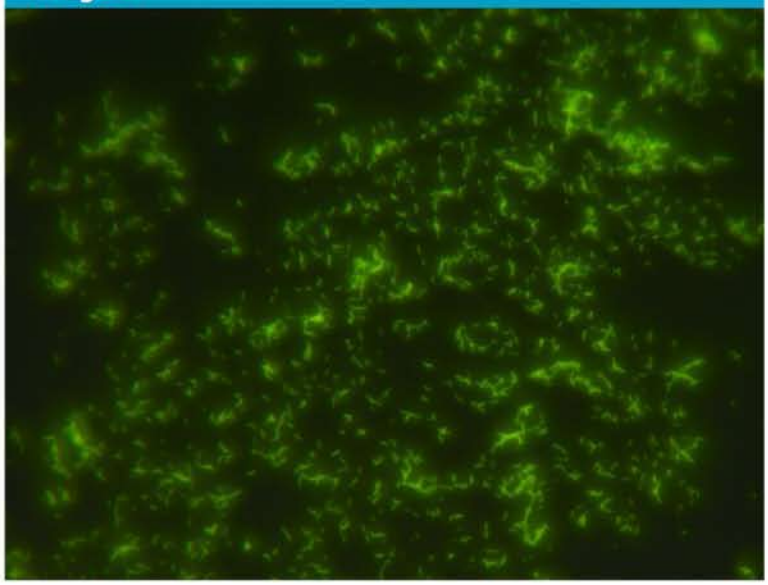
Mycobacterium tuberculosis, viewed at 400x magnification. A dark background helps to clearly observe the fluorescence of the AFB.

Image 2



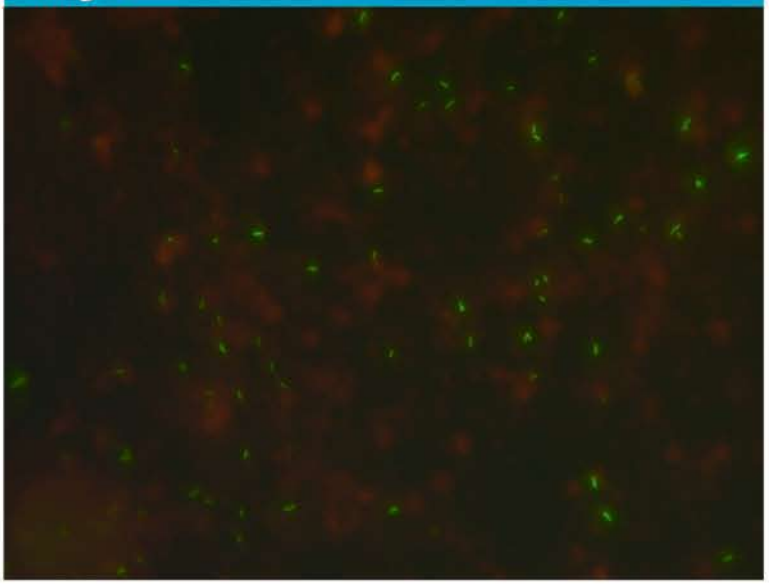
The clump of bacteria in the center is magnified at greater detail where single bacilli can be seen even clearer. (Original photo taken at 400x, Digitally modified)

Image 3



Mycobacterial bacilli sometimes fluoresce at varying degrees, as seen in this picture. Note that there are single bacilli as well as clumped bacilli here. (400x)

Image 4



AFB appearing as a brilliant green against an orange/black background (possibly due to residual stain). While the AFB are clear, it is best to wash the slides completely so that the background remains a contrasting black colour. (400x)

Note: For instructions on detecting AFB using SureFocus™ Microscope Slides, please consult the SureFocus Microscope Slide User Guide.

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F.A.S.T.™ AFB User Guide: Troubleshooting in Acid-Fast Bacilli (AFB) Detection

Artifacts (such as hair and fibers) as well as other organisms may fluoresce, so it is important to ask the following questions when looking at fluorescent slides:

- **Is the size correct?** *Is the size of the possible AFB consistent with the correct magnification size?*
- **Is the shape correct?** *Sometimes AFB can be short or long, but it should ALWAYS exhibit a bacillus (rod-like) or coccobacillus structure.*

Below is a series of four images that illustrate varying degrees of fluorescent objects, both artifacts (highlighted by white arrows) and AFB (highlighted with red arrows). When examining the items in these images, compare them in terms of size and structure to the AFB control image seen to the right. (400x)

Acid Fast Bacilli Control

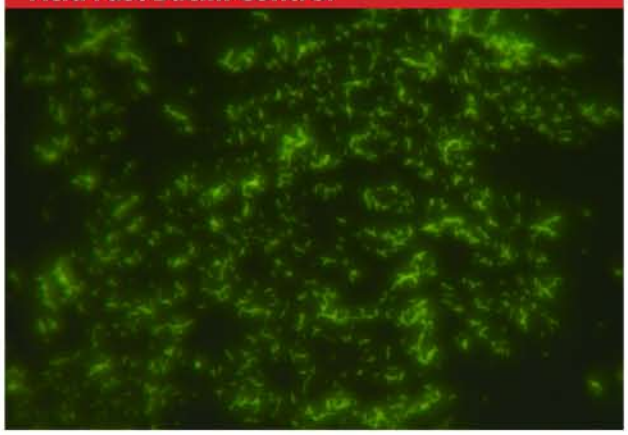
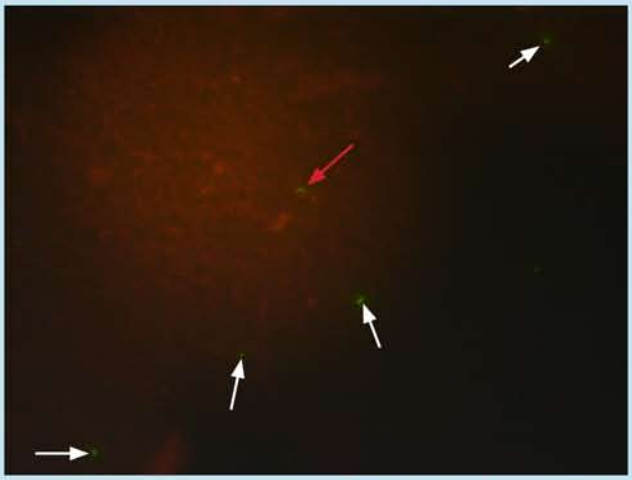
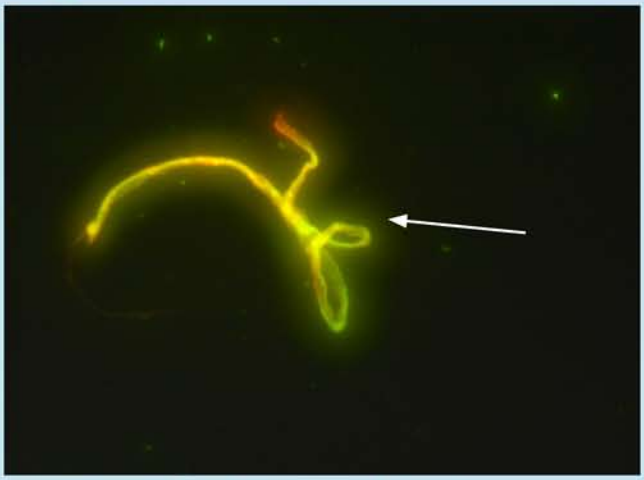


Image 1



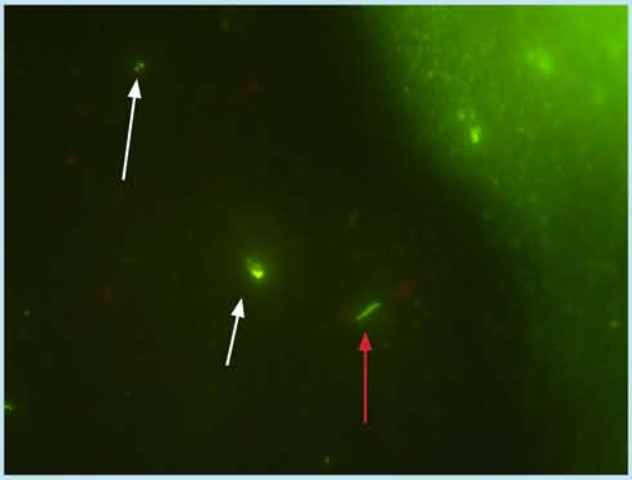
In this image, there is only one AFB, situated against the orange background. (400x)

Image 2



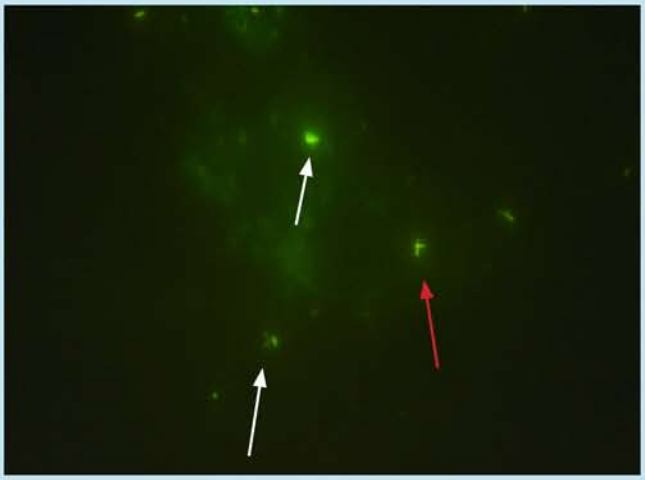
A large piece of fiber, with size and shape uncharacteristic of AFB. (400x)

Image 3



In Image 3 (600x), the long and slender AFB contrasts with the artifacts present.

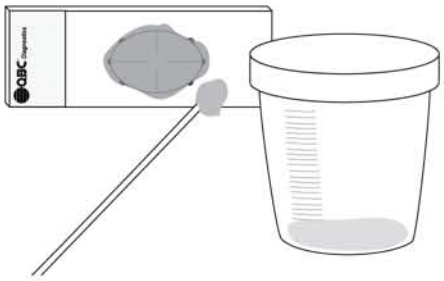
Image 4



The size and shape of the overlapping AFB are distinctive from the artifacts shown in this image. (400x)

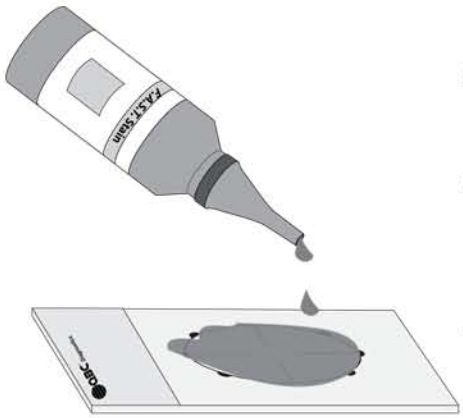
F.A.S.T.™ AFB User Guide: Troubleshooting

Smear Preparation



1. **Sputum Cup is leaking**
   Clean the outside with disinfectant, and transfer remaining specimen to a new container.
2. **Specimen material is too thick**
   Add a small amount of sterile water to dilute your specimen.
3. **Prepared smears are too thick/thin**
   Smears should be thin enough to read newspaper through them, but not too thick as to obscure viewing. Re-smear slide if necessary.

Staining and Reading Slides



1. **Smeared material washes off the slide when staining**
   Smears are too thick, or have not been heat-fixed properly. Make sure slides are stained carefully. Wash gently.
2. **Reagents are cloudy or turbid**
   Your stains may be contaminated. Check your chemicals and reagents, including your water source.
3. **Quality Controls are not working, or there is poor fluorescence**
   Stains may be expired or have been stored improperly, or the staining procedures were not performed properly. Repeat, and make sure to record for quality assurance.
4. **It is difficult to differentiate AFB from artifacts**
   Remember to rely on your control slides for the proper size and shape of AFB, and confer with fellow microscopists to determine slide positivity if there are uncertainties.

Fluorescence Microscope or ParaLens™

1. **Fluorescence Microscope is not working**
   Check the power supply as well as your fuses to make sure they are all functional.
2. **ParaLens is not working**
   Check that the power pack is plugged in properly and the LED light source is turned on. Make sure the microscope bright light is turned off. Rotate intensity control on light source.
3. **Fluorescent light is dim**
   Check that the power supply is constant. Adjust light source intensity. If using the ParaLens, ensure light source is pushed all of the way onto the side arm.
4. **Lenses are cloudy**
   Clean the lenses with lens paper both before and after use to prevent cloudiness and debris build-up. Avoid immersion oil or other liquids with 20x and 40x lenses.

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