PARAWORLD™ Training Resources

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

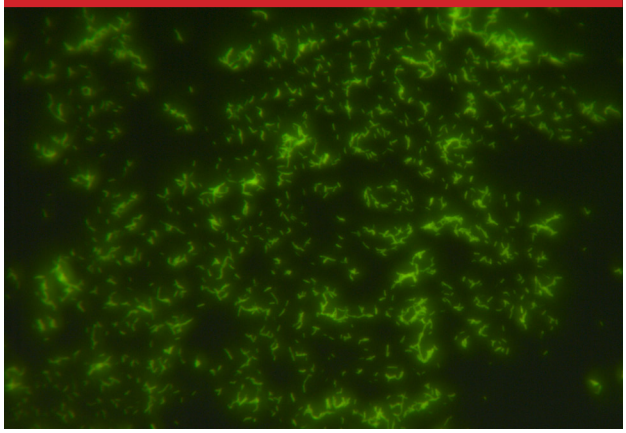
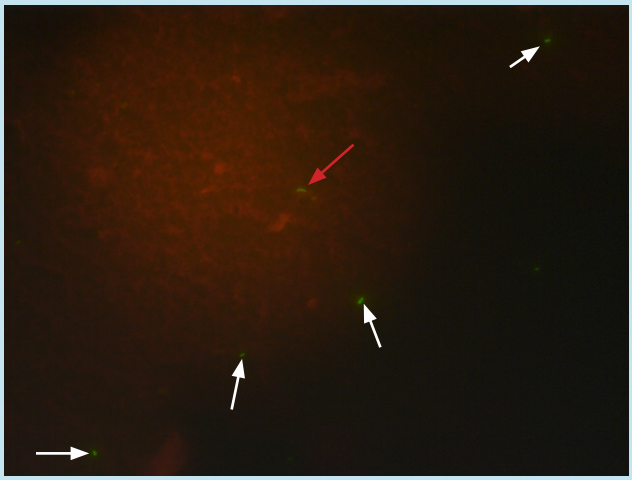
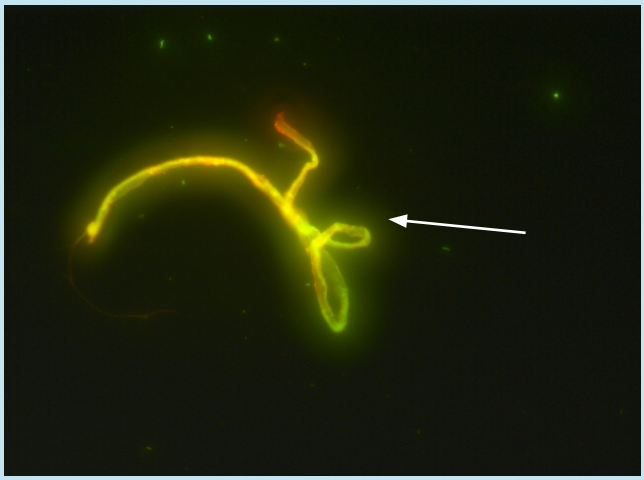


Image 1



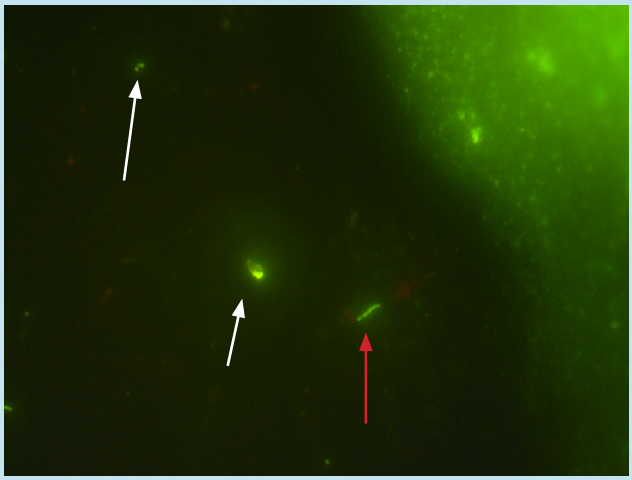
In this image, there is only one AFB, situated against the orange background. (400x)

Image 2



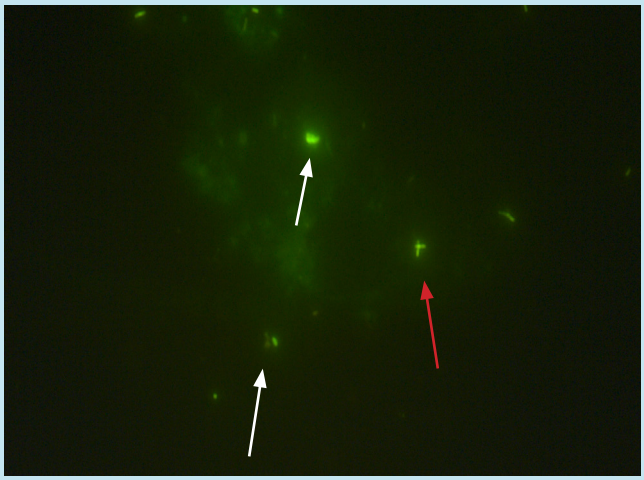
A large piece of fiber, with size and shape uncharacteristic of AFB. (400x)

Image 3



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

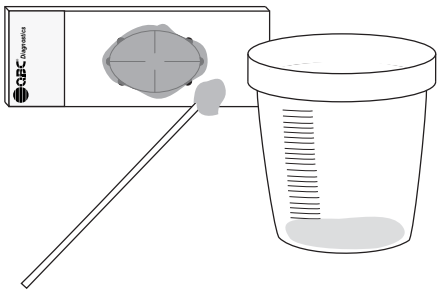
Image 4



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

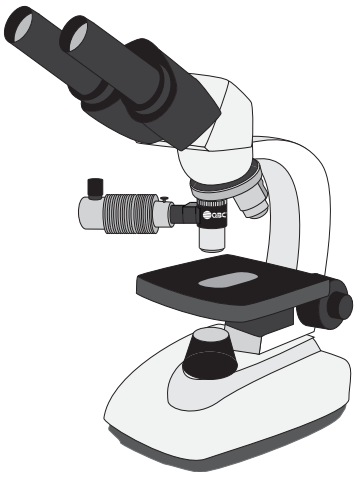


1. **Sputum Cup is leaking**
   Clean the outside with disinfectant, and transfer remaining specimen to a new container.
2. **Specimen material is too thick**
   Add a small amount of sterile water to dilute your specimen.
3. **Prepared smears are too thick/thin**
   Smears should be thin enough to read 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 Advance™



1. **Fluorescence Microscope is not working**
   Check the power supply as well as your fuses to make sure they are all functional.
2. **ParaLens Advance 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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