



PARAWORLD

PARAWORLD™ Training Resources

Frequently Asked Questions

FAQs: Photobleaching (Fading) in Fluorescent AFB Samples

▶ What is photobleaching?

Photobleaching is a physical phenomenon that occurs in all fluorescent technology that can limit the time during which a sample can be observed by fluorescence microscopy. In practice, this can result in an observable fading of the fluorescent sample over time. In some cases, the sample will ultimately no longer be visible.

▶ Why does photobleaching occur?

Photobleaching occurs because each fluorophore has a limited number of excitation/emission cycles that it can undergo. After progressing through this number of cycles, the fluorophore will no longer release visible photons of light.

▶ How does photobleaching affect the performance of fluorescent microscopy for detection of AFB?

After continuous exposure to the excitation light for an extended period of time, some fluorescent bacilli may become difficult or impossible to see.

▶ What can be done to control the effects of photobleaching?

Awareness and proper training of laboratory microscopists are the best ways to alleviate the impact of photobleaching and ensure good results. Additionally, two specific precautions can be of particular use:

First, limit the amount of time that each microscopic field is exposed to the excitation light. Do not remain on a single field for longer than it takes for the microscopist to identify and count any AFB that may be present. If confirmation of the result is necessary, turn the light source off while waiting for a second technician.

Second, limit the exposure of the sample to extraneous light in the laboratory. In general, the slides should be read immediately after staining, but if it is necessary to delay reading the slides, they should be stored in a cool, dark area. Exposure to both sunlight and artificial fluorescent light will contribute to fading of the samples over time.

▶ Can my reagents have an effect on photobleaching?

Yes. Always use well-prepared distilled water to rinse the slides during the staining process. Do not use tap water as the chlorine that can be found in some water sources will contribute to an increased rate of fading. Also, ensure that the pH of the distilled water is between 5.8 and 7.0 as the rate of fading is affected by the pH of the solutions during staining. Lastly, be sure to store staining reagents according to the manufacturer's suggested conditions and be observant of any physical changes (i.e. color, odor, etc.) of the reagents before use.

▶ Can photobleaching be reversed?

It is possible for fluorescence to recover from fading. If a sample is faded, turn off the excitation light for several minutes. When the light source is turned on again, the sample may be visible.

▶ Can I re-stain a sample that has experienced photobleaching?

Yes. It is possible to re-stain a sample that has lost fluorescence. However, repeated re-staining may affect sensitivity, so use other means of preserving fluorescence whenever possible.

▶ Are other tools available to overcome the effects of photobleaching?

Laboratories may consider the use of a microscope camera specifically designed for fluorescence microscopy, to capture fluorescent images for future evaluation. This will limit the need for extended exposure of the sample to the excitation light and allow for confirmation of results by photograph rather than direct observation. QBC Diagnostics offers a small, low cost microscope camera for use in such conditions. Please visit our website or consult product literature for additional information.

If you have additional questions, please contact QBC Diagnostics Technical Support at 866-265-1486 (+1-814-692-7661 outside the U.S.) or qbcsupport@qbcdiagnostics.com.



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