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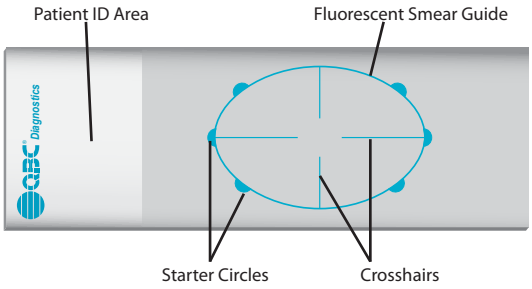
# PARAWORLD™ Training Resources

# SureFocus™ Microscope Slide User Guide



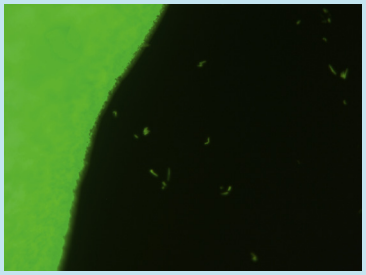

**Patient ID Area:** Etching area for proper labeling of specimen or patient ID

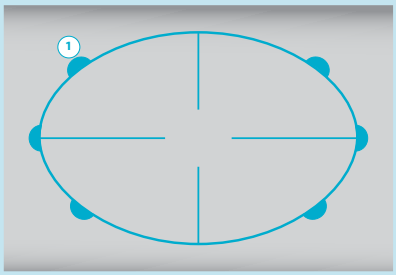
**Fluorescent Smear Guide:** A thin fluorescent oval that provides users with a standard area for smear preparation

**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

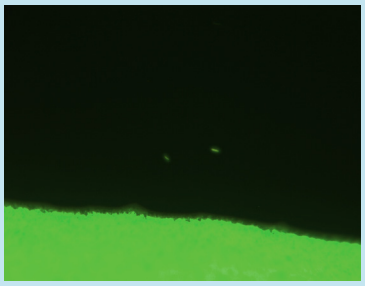


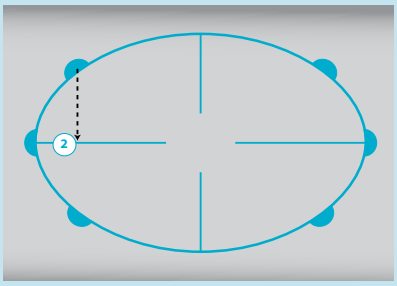



Focus on this upper left starter circle, and travel down toward the left crosshair. AFB may gather close to the fluorescing mark, as shown in the image to the left.

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

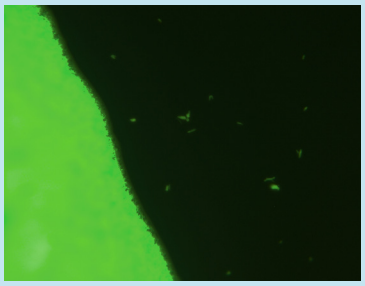
## 2. Left Crosshair

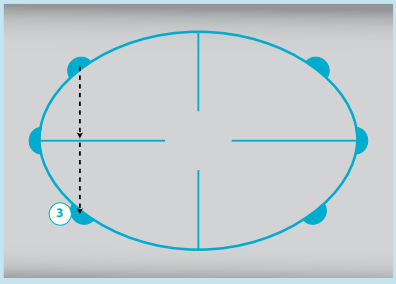





Once the crosshair has been reached, refocus (if necessary) and continue downward to the bottom-left starter circle. Two (2) fluorescing bacilli are seen in this picture.

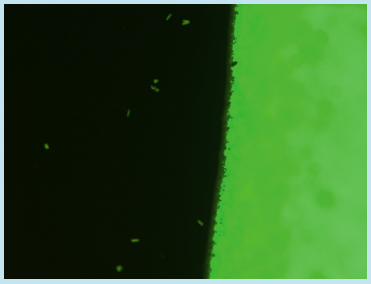
## 3. Lower Left Starter Circle

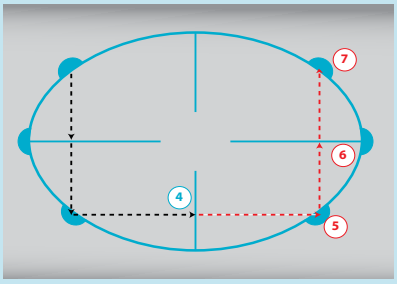





After reaching the lower left starter circle, refocus (if necessary), and continue moving right toward the bottom crosshair. Both single and clumped bacilli are shown in the image to the left.

## 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.

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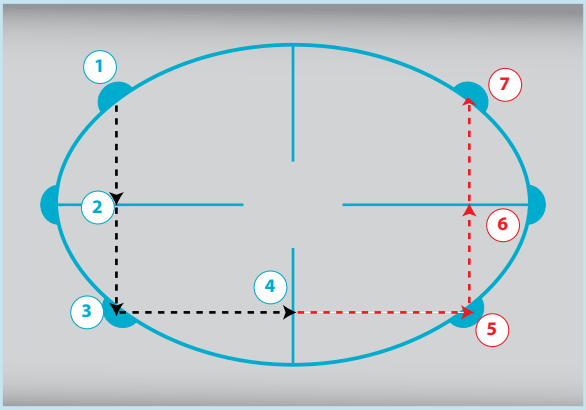
## Calculating Fields Viewed

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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