

## ACID FAST BACILLI – FACT SHEET

*Mycobacterium tuberculosis* is the causative agent of tuberculosis, a disease causing significant worldwide morbidity and mortality and a major public health problem.

Acid-fast staining serves as a rapid and inexpensive screen for AFB. The cell wall of mycobacteria contains a higher content of complex lipids including long chain fatty acids called mycolic acids. Mycolic acids make the cell wall extremely hydrophobic and enhance resistance to staining with basic aniline dyes so mycobacteria are not visible with the Gram stain.

Under certain conditions arylmethane dyes are able to form stable complexes with the mycolic acids within mycobacterial cell walls. In the presence of phenol and applied heat, carbol fuchsin dye can be used as performed during Ziehl–Neelsen staining, which also utilizes methylene blue as a counterstain. Since these cell wall dye complexes are resistant to destaining with mineral acids, mycobacteria are referred to as “acid-fast bacilli” or “AFB”.

Alternatively, mycobacteria can be stained by fluorescent dyes (auramine O alone or in combination with rhodamine B). These are non-specific fluorochromes that bind to mycolic acids and that are resistant to decolorization with acid-alcohol.

### Slide examination

Each slide made from a clinical specimen should be thoroughly examined for the presence of AFB. When a carbol fuchsin-stained smear is read, a minimum of 300 fields should be examined (magnification, X1000) before the smear is reported as negative. The fluorochrome stain is read at a lower power (X250) therefore, more material can be examined in a given period. At this lower magnification, a minimum of 30 fields of view should be examined.

It is a good practice that all positive smears be confirmed. This can be accomplished by either a second observer, re-staining of the slide using a Ziehl-Neelsen stain, or by initially preparing two smears, one for the fluorescent stain and the other for ZN staining in the event of a positive.

### Reporting Smear Results

Both CDC and WHO have proposed standardized methods of reporting the average number of AFB observed (Tables 1 and 2).

**Table 1.** CDC reporting recommendations

| No. AFB seen at 1000x | Report           |
|-----------------------|------------------|
| 0                     | Negative for AFB |
| 1-2 per 300 fields    | No. seen         |
| 1-9 per 100 fields    | 1+               |
| 1-9 per 10 fields     | 2+               |
| 1-9 per field         | 3+               |
| >9 per field          | 4+               |

**Table 2.** WHO reporting recommendations

| No. AFB seen at 1000x | Result           | Grading                  |
|-----------------------|------------------|--------------------------|
| 0 per 100 OIFs        | Negative for AFB | 0                        |
| 1-9 per 100 OIFs      | Scanty           | Record exact number seen |
| 1-9 per 10 OIFs       | Positive         | 1+                       |
| 1-10 per OIF          | Positive         | 2+                       |
| >10 per OIF           | Positive         | 3+                       |

### Suggested readings

1. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*. 2016;4:33-43.
2. Pfyffer G.E. *Mycobacterium*: General characteristics, laboratory detections, and staining procedures. In: Jorgensen et. al., ed. *Manual of Clinical Microbiology*. Vol 1. 10th ed. Washington, DC: ASM; 2015:536.
3. Clinical and Laboratory Standards Institute. Laboratory Detection and Identification of Mycobacteria; Approved Guideline. Wayne, PA.: Clinical and Laboratory Standards Institute; 2008:M48-A Wayne, PA.
4. Global Laboratory Initiative. Mycobacteriology Laboratory Manual First Edition, April 2014 Available at: [http://www.stoptb.org/wg/gli/assets/documents/gli\\_mycobacteriology\\_lab\\_manual\\_web.pdf](http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf)