# Microbacteria Testing: AFB Smear and Culture

The purpose of microbiological lab testing is to **detect, isolate and identify** the mycobacterial species and determine the drug susceptibilities of the organisms to anti-TB drugs.

## Acid-Fast Bacilli (AFB) Staining and Microscopic Examination

AFB stained smears are the first bacteriological evidence of mycobacteria in a clinical specimen, giving a quick and easy preliminary confirmation of the diagnosis. Essentially, the specimen is put on a microscope slide, stained and examined under the microscope. Mycobacteria are rod shaped and referred to as bacilli. There are two types of stains used regularly: Auramine Rhodamine (fluorescence microscopy) and Ziehl-Neelsen (or Kinyoun). The NWT uses fluorescence microscopy as the primary method of staining and detection. If mycobacteria are present they will retain the dye. The number of acid-fast bacilli can then be visualized and counted.

An AFB smear result is reported within a **24 hour period** from the time the specimen is received at the lab.

AFB smears are ideal to identify mycobacteria but it is important to remember they do not differentiate between NTM and MTB. Therefore a positive AFB smear can be NTM or MTB and another test (culture and DNA probe) is necessary to tell the difference.

A negative smear does not exclude an active TB diagnosis. Patients with active TB disease can have a negative smear. AFB smears are considered the first step in diagnosing TB disease.

## Infectivity Smear Positive


Table 6.3: Infectivity Continuum

| Smear Positive TB (S+C+) | Culture Positive TB (S-C+) | Non-respiratory TB |
|---|---|---|
| AFB seen under microscope directly from a sputum sample | AFB not seen under direct microscopy but when placed on appropriate media, TB organisms grown on culture | Blood, serum or biopsy sample |
| Highly transmissible | Less transmissible, but still infectious | Rarely transmitted |
| Symptomatic | Usually symptomatic unless immunocompromised | Localized or systemic symptoms |

## AFB Smear Interpretation

Table 6.4: Number of Bacteria Seen on Microscopy and Laboratory Interpretation

| Number Of AFB Seen By Staining Methods | | |
|---|---|---|
| **Fuchsin stain (Ziehl-Neelsen) (1,000-fold magnification)** | **Fluorochrome (250-fold magnification)** | **Semi-quantitative grading system** |
| 0 in 300 fields | 0 in 30 fields | Negative |
| 1–2 per 300 fields | 1–2 per 30 fields | Inconclusive, repeat |
| 1–9 per 100 fields | 1–9 per 10 fields | 1+ (rare) |
| 1–9 per 10 fields | 1–9 per field | 2+ (few) |
| 1–9 per field | 10–90 per field | 3+ (moderate) |
| >9 per field | >90 per field | 4+ (numerous) |

The infectiousness of a TB patient is directly related to the number of droplet nuclei carrying *M. tuberculosis* (tubercle bacilli) that are expelled into the air, through ways such as coughing.

## Nucleic Acid Amplification Techniques (NAAT)

Nucleic acid amplification tests (NAAT) amplify segments of DNA and RNA from the mycobacteria (i.e. genetic elements in the mycobacteria), to reliably identify the microorganisms. Results are usually available within 24 hours of receiving a positive AFB smear. They are recommended only for airway secretion specimens that are smear positive. Both NTM and MTB will stain AFB smear positive. NAAT is used to differentiate the two.

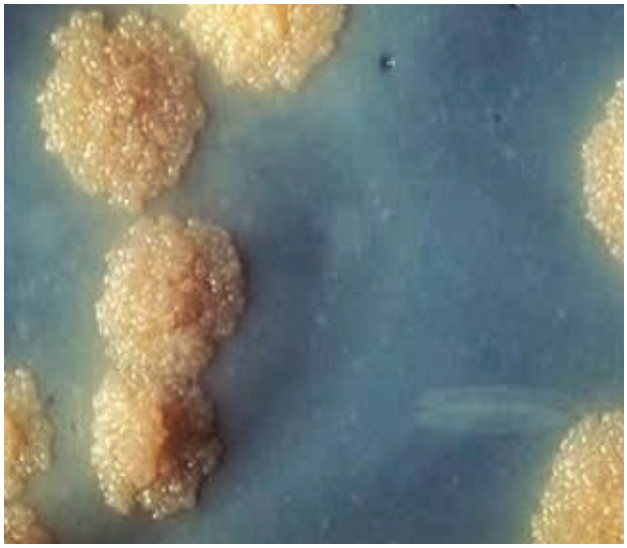
## Mycobacterial Culture

**Mycobacterial culture is the gold standard for diagnosing active TB disease**. It is also required to do drug susceptibility testing (DST) to direct therapy (see **Section 8, Treatment for Tuberculosis**). Cultures are performed on all specimens regardless of the AFB smear and NAAT results. Because mycobacteria are slow growing, it takes 6–8 weeks before obtaining a final result. Three sputum specimens are advised to increase the sensitivity of testing. Only 10–100 viable bacteria are necessary for a positive culture.

If the cultures are negative then the diagnosis of active TB disease must rely on the clinical presentation of the patient. It is also possible that mycobacteria are present in other body sites not examined or where specimens were not collected (see **non-respiratory TB,** this section).

Cultures demonstrating AFB have DNA testing to identify MTB. If MTB is confirmed, drug susceptibility testing is done and reported so treatment can be tailored and any drug resistance (if present) is identified.

Figure 6.6: Colonies of *Mycobacterium Tuberculosis* Grown in Culture



## Respiratory Specimen Collection

Due to the importance of lab testing in the diagnosis of active TB disease, collection and handling of specimens should be done carefully. When specimens are sent to the laboratory they must be collected in a leak proof container (laboratory approved). It must be accompanied with a requisition form and include the following information:

- Patient demographic information (name, date of birth, address, health information number)
- Submitting practitioner name
- Date and time of collection
- Specimen type and site
- Specimen collection method

Sputa are the preferred method of detecting the presence of MTB in the lung(s). Relevant information on the patient's status and initial diagnosis is advisable. Specimens should be collected before the start of anti-TB treatment:

- Sputum collection is best done in the morning upon rising and can be repeated at a minimum of every hour thereafter (particularly in the hospital setting).
- Three samples should be collected (if possible) to increase the sensitivity of testing.
- Ideally, in the community setting, at least three consecutive early morning samples of sputum should be examined before a diagnosis of tuberculosis is excluded. The ideal sample contains 5–10ml of solid or purulent material. (Equivalent: 1–2 teaspoon).
- Patient should NOT use antiseptic mouthwash or drink alcohol immediately prior to obtaining a sputum specimen.