

## ACID FAST BACILLI (AFB) SMEAR, MICROSCOPY

Specimen	<ul style="list-style-type: none"> <li>Early morning sputum (EMS), bronchoalveolar lavage, CSF, gastric aspirates, body fluids, aspirations, tissues etc.</li> <li>Collect specimen in sterile clean screw-capped containers without any preservatives.</li> </ul>
Unacceptable	<ul style="list-style-type: none"> <li>Saliva</li> <li>Stool</li> <li>24 hours pooled urine</li> <li>Specimen found leaking upon receiving</li> <li>Early morning sputum (EMS) containing food particles</li> <li>Volume of EMS less than 3 mL</li> <li>Body fluids or aspirations more than 50 mL</li> <li>Specimen in fixatives or preservatives</li> <li>Specimen taken with swab</li> <li>Empty containers</li> </ul>
Method	<ul style="list-style-type: none"> <li>Smear prepared from N-acetyl-L-cysteine Sodium hydroxide (NALC- NaOH) decontamination and concentrated clinical specimens</li> <li>Screening of fluorochrome bacilli: <b>Auramine O Stain</b></li> <li>Confirmatory of acid-fast bacilli (AFB): <b>Kinyoun stain</b></li> <li>If smear is acid- fast bacilli positive (new case), reflex tests include: Molecular detection of known mutations associated with Isoniazid and Rifampicin resistance – Hain Lifesciences Genotype MTBDR<sub>plus</sub> (and molecular detection of known mutations associated to fluoroquinolones and second-line injectable drugs – Hain Lifesciences Genotype Mtbdrsl, if required)</li> </ul>
Performed	Monday to Thursday & Saturday
TAT	Two (2) working days
Clinical Usage	To detect presence of AFB bacilli in clinical specimens and culture
Reference Range	<ul style="list-style-type: none"> <li>Smear negative (No AFB seen)</li> <li>Smear positive (AFB seen with quantitation reported)</li> </ul>

Quantitation according to World Health Organisation,

NUMBER OF ACID- FAST BACILLI SEEN		Report
AURAMINE STAIN (400X)	KINYOUN STAIN (1000X)	
0	No AFB in 100 fields	No AFB seen
3-24 AFB/ 50 fields	1-9 AFB/ 100 fields	1+
1-6 AFB/ field	10-99 AFB/ 100 fields	2+
7-60 AFB/ field	1-10 AFB/ field	3+
>60 AFB/field	>10 AFB/ field	4+

# NATIONAL MYCOBACTERIA REFERENCE LABORATORY- TEST CATALOGUE

Updated 12/8/2025

Acid- Fast Bacilli (AFB) Culture/ Mycobacterial Culture	
Specimen	<ul style="list-style-type: none"> <li>• Early morning sputum (EMS), bronchoalveolar lavage, CSF, gastric aspirates, body fluids, aspirations, tissues etc.</li> <li>• Blood culture shall be requested through phone. A designated culture bottle vial shall be provided.</li> <li>• Collect specimen in sterile clean screw-capped containers</li> </ul>
Unacceptable	<ul style="list-style-type: none"> <li>• Saliva</li> <li>• Stool</li> <li>• 24 hours pooled urine</li> <li>• Specimen found leaking upon receiving</li> <li>• Early morning sputum (EMS) containing food particles</li> <li>• Volume of EMS less than 3 mL</li> <li>• Body fluids or aspirations more than 50 mL</li> <li>• Specimen in fixatives or preservatives</li> <li>• Specimen taken with swab</li> <li>• Empty containers</li> </ul>
Method	<ul style="list-style-type: none"> <li>• Liquid culture using BBL Middlebrook 7H9 broth (incubated in BACTECT™ MGIT™ 960 instrument, Becton Dickinson, USA) and solid liquid using Lowenstein- Jensen culture</li> <li>• <b>If culture is positive for AFB</b>, reflex tests may include: <ul style="list-style-type: none"> <li>i. Rapid identification of <i>M. tuberculosis</i> complex strain (MTBC) by MPT64 antigen detection.</li> <li>ii. Molecular identification and sub-speciation of MTBC- Hain Lifesciences Genotype MTBC</li> <li>iii. Molecular detection of known mutations associated with Isoniazid and Rifampicin resistance - Hain Lifesciences Genotype MTBDR<sub>plus</sub> (and molecular detection of known mutations associated to fluoroquinolone and second-line injectable drug resistance – Hain Lifesciences Genotype Mtbdrsl, if required)</li> <li>iv. Antibiotic susceptibility testing for MTBC- Isoniazid, Rifampicin, Ethambutol, Streptomycin and Pyrazinamide- BD MGIT liquid system</li> <li>v. For Molecular identification and sub-speciation of non- tuberculous mycobacterium (NTM)- Hain Lifesciences Genotype CM and AS</li> <li>vi. Molecular identification and sub-speciation of <i>M. avium</i> complex (<i>M. avium</i>, <i>M. intracellulare</i>, and <i>M. chimaera</i>), <i>M. abscessus</i> complex (<i>M. abscessus</i> subsp. <i>abscessus</i>, <i>M. abscessus</i> subsp. <i>bolletii</i>, and <i>M. abscessus</i> subsp. <i>massiliense</i>) and <i>M. chelonae</i> with molecular detection of known mutations associated with macrolide and aminoglycoside resistance- Hain Lifesciences Genotype NTMDR, if required</li> </ul> </li> </ul>
Performed	Monday to Thursday & Saturday
TAT	<p>Six (6) to eighteen (18) weeks</p> <ul style="list-style-type: none"> <li>• Receipt of specimen to detection of growth: Six (6) to eight (8) weeks</li> <li>• Reflex tests: Up to additional ten (10) weeks from positive flagged culture, depending on type of species growing, mixed cultures involved or further tests are required</li> </ul>
Clinical Usage	Diagnosis of tuberculosis and other mycobacterial infection
Reference Range	<ul style="list-style-type: none"> <li>• No growth after six (6) weeks incubation</li> <li>• No growth after eight (8) weeks incubation</li> </ul>

# NATIONAL MYCOBACTERIA REFERENCE LABORATORY- TEST CATALOGUE

Updated 12/8/2025

- AFB culture contaminated with other microorganisms
- Mycobacteria species identified. For MTBC, antibiotic susceptibility profile will be reported

## ANTIBIOTIC SUSCEPTIBILITY TESTING, MYCOBACTERIUM TUBERCULOSIS COMPLEX

Specimen	<ul style="list-style-type: none"> <li>• Early morning sputum (EMS), bronchoalveolar lavage, CSF, gastric aspirates, body fluids, aspirations, tissues etc.</li> <li>• Blood culture shall be requested through phone. A designated culture bottle vial shall be provided.</li> <li>• Collect specimen in sterile clean screw-capped containers</li> </ul>
Unacceptable	<ul style="list-style-type: none"> <li>• Saliva</li> <li>• Stool</li> <li>• 24 hours pooled urine</li> <li>• Specimen found leaking upon receiving</li> <li>• Early morning sputum (EMS) containing food particles</li> <li>• Volume of EMS less than 3 mL</li> <li>• Body fluids or aspirations more than 50 mL</li> <li>• Specimen in fixatives or preservatives</li> <li>• Specimen taken with swab</li> <li>• Empty containers</li> </ul>
Method	<ul style="list-style-type: none"> <li>• Liquid culture using BBL Middlebrook 7H9 broth (incubated in BACTEC™ MGIT™ 960 instrument, Becton Dickinson, USA).</li> <li>• Isolate has already been identified as MTBC</li> <li>• Antibiotics to be tested: Streptomycin (1.0 µg/mL), Isoniazid (0.1µg/mL), Rifampin (1.0 µg/mL), Ethambutol (5.0 µg/mL) and Pyrazinamide (100 µg/mL) (and higher concentration of Isoniazid (0.4 µg/mL), if required)</li> </ul>
Performed	Monday to Thursday & Saturday
TAT	Three (3) to eight (8) weeks upon confirmation of MTBC culture
Clinical Usage	<p>Notes:</p> <ul style="list-style-type: none"> <li>• First line drug profile for treatment of <i>M. tuberculosis</i> complex infection</li> </ul>
Reference Range	<ul style="list-style-type: none"> <li>• Sensitive</li> <li>• Resistant</li> </ul>