Laboratory Diagnosis of Mycobacterium tuberculosis

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Mycobacteriology

The genus Mycobacterium comprises more than 150 species. The most familiar species are *M. tuberculosis* and *M. leprae*, the causative agents of tuberculosis and leprosy. Both diseases have long been associated with chronic illness and social stigma.
General Characteristics

- Mycobacteria are slender, slightly curved or straight rod-shaped organisms 0.2×0.4 to 10 μm in size. They are nonmotile and do not form spores. The cell wall structure has an extremely high lipid content; thus mycobacterial cells resist staining with commonly used basic aniline dye at room temperature.
Mycobacteria do take up dye with increased staining time or application of heat; however, they resist decolonization with up to 3% hydrochloric acid, and some also resist decolonization with 95% ethanol. These characteristics, referred to as acid fastness and acid alcohol fastness.
Physiology of *M. tuberculosis*

- Mycobacteria are **strictly aerobic** and growth more slowly than most bacteria pathogenic for humans. Most mycobacteria associated with disease require **2 to 6 week** on complex media at specific optimal temperature.

- The most **rapidly growing** species generally grow on simple media in **2 to 3 days** at temperature **20°C to 40°C**
The growth of *M. tuberculosis* is enhanced by an atmosphere of **5% carbon dioxide** and a growth medium with pH 6.5 to 6.8. One of the mycobacteria pathogenic for humans *M. leprae* fails to grow in vitro.
M. Tuberculosis
Most important mycobacteria

- More than 95% of all human infections are caused by six species:
  - *M. tuberculosis*
  - *M. avium-intracellulare*
  - *M. kansasi*
  - *M. fortuitum*
  - *M. chelonae*
  - *M. leprae*
Table 1 - Classification of Important Mycobacteria

<table>
<thead>
<tr>
<th>Mycobacteria</th>
<th>Clinical Significance</th>
<th>Pigmentation*</th>
<th>Rate of Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unclassified Mycobacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.leprae</td>
<td>Strict Pathogen</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>M.tuberculosis</td>
<td>Strict Pathogen</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>M.bovis</td>
<td>Strict Pathogen</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>M.ulcerans</td>
<td>Strict Pathogen</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Clinical Significance</td>
<td>Pigmentation*</td>
<td>Rate of Growth</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>RUNYON GROUP II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. scrofulaceum</td>
<td>Rarely pathogenic</td>
<td>Scoto</td>
<td>Slow</td>
</tr>
<tr>
<td>M. szulgai</td>
<td>Strict pathogen</td>
<td>Scoto</td>
<td>Slow</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>Rarely pathogenic</td>
<td>Scoto</td>
<td>Slow</td>
</tr>
<tr>
<td>M. gardonae</td>
<td>Nonpathogenic</td>
<td>Scoto</td>
<td>Slow</td>
</tr>
<tr>
<td>M. flavescens</td>
<td>Nonpathogenic</td>
<td>Scoto</td>
<td>moderate</td>
</tr>
</tbody>
</table>
## Table 1- Classification of Important Mycobacteria (cont)

<table>
<thead>
<tr>
<th>Mycobacteria</th>
<th>Clinical Significance</th>
<th>Pigmentation*</th>
<th>Rate of Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUNYON GROUP III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.avium-intracellulare</td>
<td>Usually pathogenic</td>
<td>No</td>
<td>Slow</td>
</tr>
<tr>
<td>RUNYON GROUP IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.fortuitum</td>
<td>Rarely pathogenic</td>
<td>No</td>
<td>Rapid</td>
</tr>
<tr>
<td>M.chelonae</td>
<td>Rarely pathogenic</td>
<td>No</td>
<td>Rapid</td>
</tr>
</tbody>
</table>
Levels of Laboratory Service

ATS Recommendations

- **Level I**
  - Collect, smear & transport specimens
- **Level II**
  - Smear & culture; ID & sensi testing of Mtb
- **Level III**
  - Smear & culture; ID & sensi testing of all mycobacterial isolates
Safety consideration

- The serious nature of tuberculosis disease and the usual airborne route of infection require that specific safety precautions be used by anyone handling mycobacterial specimens.
Safety in the Mycobacteria Lab

- **Active TB in Mycobacteriologists**
  - 100 x General Population
  - 3 x Other Laboratory Workers

- **Skin Test Conversions in TB Labs**
  - 8-30%
Safety in the Mycobacteria Lab (continued)

- **Behavioral risk factors for infection**
  - Lack of adequate safety guidelines
  - Noncompliance with guidelines
- **Aerosol production**
  - Pouring, pipetting & mixing liquids
  - Homogenizing & cutting tissues
  - Dropping or spilling culture flasks
  - Preparing smears
- **Needle sticks**
Safety in Mybacteriology Lab.

- Personnel Safety
- Proper ventilation
- Proper use of biologic safety cabinet
- Use of proper disinfectant.
Proper Disinfectant in Mycobacteriology Lab

- Phenol – Soap mixture
- Sodium hypochloride
- Formaldehyde
- Phenol 5%
Specimen Collection

- Mycobacterium may be recovered from a variety of clinical specimens.
- The successful isolation of mycobacteria from clinical specimens begins with properly collected and handled specimens. Whenever possible, diagnostic specimens should be collected before initiation of therapy.
Specimen collection

- All specimens should be transported to the laboratory and ideally should be processed as possible after collection. If immediate transport is not possible, the specimen should be refrigerated, but no longer than overnight. Delay in processing lead to false-negative cultures and increased bacterial contamination.
Acceptable specimens

Respiratory specimens:
Sputum
Normal saline nebulized, induced sputum
Transtracheal aspirate
Bronchoalveolar lavage
Bronchoalveolar brushing
Laryngeal swab
Nasopharyngeal swab
Continue...

Body Fluid
Pleural fluid
Pericardial fluid
Joint aspirate
Gastric aspirate
Specimens

- Peritoneal fluid
- Cerebrospinal fluid (CSF)
- Stool
- Urine
- Pus
Specimen

Body Tissue
Blood
Bone marrow biopsy/aspirate
Solid organ
Bone
Skin
Sputum and other Respiratory secretions

- Sputum and bronchial aspirates are the most common specimens. The sputum samples should be 5-10 mL
Sputum Specimen Collection

- Collection of good specimen is critical
  - First morning expectoration
  - Rinse mouth with water
  - Produce specimen by deep coughing
  - Expectorate directly into lab-provided tube
  - If specimen inadequate, induce sputum
- Dispatch immediately to lab
  - Containers to be closed and sealed
  - Refrigerate if not processed immediately
AFB Stains

- Sensitivity on expectorated sputum
  - M. tuberculosis 65%
  - MOTT 20%

- Cum Sensitivity in series of 3 sputa
  - M. tuberculosis 80%
AFB Stains

- Cum sensi of smears in culture series that yield both a (+) culture for M. tuberculosis and one or more positive smears
  - First specimen 86%
  - Second specimen 95%
  - Third specimen 95%
  - Fourth specimen 100%
Gastric aspiration

- gastric aspirates are used to recover mycobacteria that may have been swallowed during the night. Use of this type of specimens should only be used for patients who fail to produce sputum by aerosol induction, children under 3 years of age, and nonambulatory.
Gastric aspirates

- Gastric aspirates should be obtained in the morning after an overnight fast. Three specimen should be collected within 3 days.
- Sterile water, 30 to 60 ml, is instilled either orally or via nasogastric tube aspiration. Prolonged exposure to gastric acid kills mycobacteria and diminishes culture yield.
- Specimen processing should be done expeditiously, or the specimen should be neutralized with sodium carbonate or another buffer salt to pH 7.
Urine

- For examination of urine first morning midstream specimen is preferred. The entire volume of voided urine, or minimum 15ml., is collected in a sterile container. A specimen may be collected through an indwelling catheter with a sterile needle and syringe. *Urine specimen should be refrigerated during the interval between collection and processing.* Specimens should be processed promptly.
Urine

- As a general rule, pooled specimens collected over 12 to 24 hours are not recommended. Such specimens are more subject to contamination and may contain few viable tubercule bacilli.
Stool

- Examination of stool specimens for the presence of acid-fast organisms can be useful in identifying patients (such as individual with AIDS) who may be at risk for developing disseminated mycobacterial disease resulting from *M. avium* complex (MAC).
It has been reported that 68% of MAC culture-positive stool specimens are acid-fast smear negative. Stool specimens should be collected in clean containers without any preservative, and send directly to the laboratory for processing. If processing within a few hours is not possible, the specimens should be kept frozen at -20 until processing time.
Blood

- Mycobacteremia, once considered rare, is now often seen in patients with AIDS but less frequently in other immunocompromised hosts. Majority of the infections are caused by MAC. The isolator lyses-centrifugation system or direct inoculation of blood into BACREC 13A have been shown to be effective for collection and culture of blood samples.
Tissue and other samples

At times, tissue and other body fluid may be noted for microscopic examination and culture. Whenever possible, CSF specimen should be from large-volume spinal tapes to increase diagnostic yield. Diagnosis of tuberculous meningitides is extremely difficult. Peritoneal (ascitic fluid) smears are also rarely positive for acid-fast bacilli.
continue..

- Cultures of large volumes and collection of the specimen into **BACTEC bottles** or other mycobacterial liquid media may help maximize yield in this and other dilute specimens.
Specimens may need to obtain from the lung, pericardium, lymph nodes, bones, joints, bowel or liver. The tissue or fluid should be collected aseptically and placed in a sterile container. If the tissue is not processed immediately, a small amount (10 to 15 ml) of sterile saline should be added to prevent dehydration.
It may be necessary to collect fluid containing fibrinogen (e.g. pleural, pericardial, peritoneal) into container with an anticoagulant. The amount fluid recommended:

- 2 ml for CSF
- 3-5 ml for exudates, pericardial and synovial fluid
- 10-15 ml for abdominal and chest fluid.
To ensure optimal recovery of mycobacteria from clinical specimens, many specimens must be processed before inoculation onto culture media. Each step must be carried out with precision. Specimens from sterile body sites must be simply concentrated if large volume) and inoculated. However, specimens that may contain normal flora bacteria should be contaminated and concentrated.
Most clinical specimens such as sputum contain mucin or organic debris that surrounded the bacteria within the sample. The purpose of digestion and decontamination are:

1. To liquefy the sample through digestion of the proteiaceous material and (2) to allow the chemical decontaminating agent to contact and kill the nonmycobacterial organism.
Digestion and Decontamination

- Specimens that contain mucus and require both digestion and decontamination are sputum, gastric lavage, bronchoalveolar lavage, bronchial washing and transtraacheal aspirates.
Decontamination and digestion

- Voided urine, autopsy tissue, abdominal fluid and any fluid known to be contaminated require decontamination. Specimens from normally sterile sites such as CSF, synovial fluid and biopsy tissue from deep organs do not require decontamination.
Decontamination and digestion agents

Each Laboratory should maintain a proper balance between rate of recovery of mycobacterial and the suppression of contaminating growth. Failure to isolate mycobacteria from patients with signs and symptoms of classic mycobacterial disease may indicate that the decontamination is too harsh. On the other hand, if more than 5% of all specimens cultured are contaminated, the decontamination procedure may be inadequate.
Direct Smear for Acid-Fast Bacilli

- The conventional acid fast staining methods are:
  - Ziehl- Neesen method
  - Kinyoun stain
  - Other methods
  - Auramine or auramine-rhodamine fluorochrome stains-
Desirable Features

- Sensitivity
- Specificity
- Predictive value
- Speed
- Reliability
- Reproducibility
- Cost
- Safety
- Easy to use
- Robustness
- Widest usage

What is new in the diagnosis of TB
AFB smear

AFB (shown in red) are tubercle bacilli
Examination and interpretation of smears

- Smears being examined for acid-fast organism should be carefully examined with minimum of 300 field.
- The overall sensitivity of acid-fast smears varies from 20%-80% depending on the extent of infection.
Aims of sputum microscopy

- Diagnosis of patients with infectious tuberculosis
- Monitoring progress of patients on treatment
Advantages of sputum microscopy

- More reliable than x-ray for the diagnosis of infectious TB
- Simple to perform
- Easy to read
- Minimal infrastructure required
- Inexpensive
- Quick
- Only tool to monitor and declare patients as “cured”
Diagnosis of pulmonary tuberculosis

Three specimens optimal

- Spot specimen on first visit; sputum container given to patient
- Early morning collection next day
- Spot specimen during second visit
Three sputum smears are optimal

Cumulative Positivity

First: 81
Second: 93
Third: 100
A good smear

- Made from mucopurulent sputum
- Spread evenly
- 3 cm x 2 cm in size
- Not too thick
- Thin enough to read newsprint through
- Air dried before fixing
## Ziehl Neelsen

<table>
<thead>
<tr>
<th>Number of AFB examined</th>
<th>Number of fields* examined</th>
<th>What to report</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB in 100 fields</td>
<td>100 fields</td>
<td>No Acid Fast Bacilli detected</td>
</tr>
<tr>
<td>1–9 AFB in 100 fields</td>
<td>100 fields</td>
<td>Record exact figure (1 to 9 AFB per 100 fields)</td>
</tr>
<tr>
<td>10–99 AFB in 100 fields</td>
<td>100 fields</td>
<td>1 +</td>
</tr>
<tr>
<td>1–10 AFB in each field</td>
<td>50 fields</td>
<td>2 +</td>
</tr>
<tr>
<td>More than 10 AFB in each field</td>
<td>20 fields</td>
<td>3 +</td>
</tr>
</tbody>
</table>

* Oil immersion fields
Consequences of false negative smear results

- Patients with TB will not be treated, resulting in suffering, spread of TB and death
- Intensive phase of treatment will not be extended for the required duration, resulting in inadequate treatment
- Patient may lose confidence in the programme
False positive results

- Food particles
- Precipitated stains
- Saprophytic AFB
- Spores of *B. subtilis*
- Fibres and pollen
- Scratches on slide
- Contamination through carry over of AFB from one smear to another
Consequences of false positive results

- Patients are started on treatment unnecessarily
- Treatment is continued longer than necessary, in follow-up examinations
- Medications will be wasted
- Patients lose confidence in the programme
Some important notice

Because of the potential of a smear as positive for acid-fast organism, laboratory professional must realize that organisms other than mycobacterium may stain at least partially acid-fast. *Nocardia* spp., *Legionella* (*micdadei* and *Rhodococcus* sp.) may all appear acid fast.
Notice

- Because of the potential cross contamination of acid-fast bacilli from one smear to another, careful attention should be paid to the staining. Technique. Staining jars should not be employed. Smears should not contact in contact with one another or should be air dried or blotted.
Notice

- Use laboratory made stain
- All positive smear should be checked by laboratory director
- 10% of negative smears should re-checked by another technician
- Maintain the result of positive 2 years. smears until.
Indications for Culture in DOTS

- Failures of re-treatment cases
- Seriously ill cases;
  - extra-pulmonary cases
  - smear negative cases
  - childhood TB & HIV-TB
- For DRS

- Not for New Smear Positive Cases
MYCOBACTERIAL CULTURE

Advantages:

- Increases number of cases found
- Detects cases among smear negative patients
- Establishes viability of organisms
- Distinguishing between Mycobacterial species
- Helps in performing DST
- Helps in diagnosing cases of failure

Limitations:

- Expensive
- Require enriched media
- Require considerable expertise
- Time consuming
Decontamination Procedures

- 1915 – Petroff's NaOH
- 1946 – Trisodium Phosphate
- 1955 – Pancreatin Desogen
- 1958 – Pancreatin + 1% cetrimide
- 1962 – Zephiran Trisodium PO₄
- 1963 – N-acetyl L-cysteine + 2% NaOH
- 1969 – Swab culture technique + 1% cetrimide
- 1975 – CPC + NaCl₂
PETROFF’S METHOD

Advantages:
- Simple, inexpensive & control the growth of contaminants
- Twenty samples can be processed in 2 Hrs, with centrifuge capacity being the limiting factor
- Sterilized NaOH can be kept for several weeks

Limitations:
- The specimen exposure times must be strictly followed to prevent over kill of tubercle bacilli. The initial kill is independent of additional contributory factors such as heat build-up in the centrifuge and centrifugal efficiency
If delay of more than 48 hours between collection and processing is anticipated, the sputum should be collected with 1% CPC and 2% NaCl₂.

- CPC acts as homogenizing and decontaminating agent.
- It helps in retaining viability of Tubercle bacilli up to 7 days.
- These specimens should not be treated with NaOH (Petroff’s).
Culture Media and isolation methods

- Mycobacteria are strictly aerobic and grow more slowly than most bacteria pathogenic for humans. The generation time is more than 12 hours (12-18h). The mot rapidly growing species generally grows on simple media in 2 to 3 days at temperatures of 20-40 °C. Most mycobacteria associated with disease require 2 to 6 weeks.
The growth of M tuberculosis is enhanced by an atmosphere of 5 -10\% Co2 for the few first weeks of incubation. Mycobacteria require a pH between 6.5-6.8 for the growth medium and growth better at high humidity.
Culture media

- Culture media for mycobacterium spp divided in three groups:
  - 1-Egg_based Media
  - 2- Serum-or Agar Based media
  - 3-Liquid Media
Egg-Based media

1- Lowenstein - Jensen (LJ)
2- Petagnani

American Thoracic Society
Colony Morphology of M. tuberculosis

- Dry wrinkled warty growth.
- Eugonic
Serum –or Agar –Based Media

- Middle–brook 7H10
- Middle–brook 7H11
- In contrast to egg-based media these media are clear and can be examined using a dissecting microscope for early detection of growth and colony morphology.
Liquid based Media

- Mycobacterium spp grow more rapidly in liquid media. Middlebrook 7H9 broth is a nonselective liquid medium. Used for subculturing stock strains, picking single colonies, and preparing inoculum for in vitro testing.
Liquid medium

- The most sensitive and rapid primary isolation liquid media are Midlebrook 7H12 and 7H13 (BACTEC 12B and BACTEC 13B Becton Dickinson diagnostic system). The BACTEC system is an automated system for detecting growth of mycobacteria spp.
A number of studies have shown that the radiometric BACTEC isolation method significantly improves the recovery time compared with conventional isolation media. The BACTEC vials should be read 4 days of incubation. Negative vials should be retested every 3 to 4 days for the first 2 weeks and then once weekly for the remaining time. With BACTEC method, mycobacteria may be detected in clinical specimens in less than 2 weeks.
Reading and Reporting

Characteristics of Tubercle bacilli

- Growth of Primary culture takes 2 – 4 weeks to obtain visible colonies
- Colonies are buff colored and rough, having the appearance of bread crumbs or cauliflower
- Not easily emulsified but give a granular suspension
- Microscopically frequently arranged in serpentine cords of varying length or show linear clumping
Other Culture Methods

- Septi-check AFB
- MGIT 960
- Backtec/MB/Bact

- Microscopic Observation of Broth Culture
- MODS: Micro Colony Detection System
Biochemical tests

- Biochemical tests to identify mycobacteria include niacin accumulation, nitrate reduction, iron uptake, urease, catalase (semi-quantitative, 68ºC), Tween 80 hydrolysis, tellurite reduction, arylsulfatase, growth on thiophene-2-carboxylic acid hydrazide (TCH), and tolerance to 5% NaCl.
Continue

- These tests are well standardized, reproducible, and inexpensive; but they have 2 major limitation:
First, they are reliable only for species that have been widely studied, so they may not correctly identify newly recognized species. Second, some of these tests are time-consuming, and this can delay definitive results for up to 4 weeks. For these reasons, alternative methods that provide faster and more definitive results now the conventional tests.
Alternative Methods

- Chromatography
- Nucleic acid hybridization
- Nucleic acid amplification (PCR)
Antigens used in serological diagnosis of TB

- Mycobacterial sonicates
- Extracted glycolipids
- PPD
- Ag5 (38KDa Ag)
- A60
- 45 / 47 – KDa Ag
- Ag Kp 90
- 30 KDa Ag
- P32 Ag
- Cord Factor (trehalase dimycolate)
- LAM

What is new in the diagnosis of TB

Chan, E.D., Heifets, L., Iseman, M.D., Tubercle & Lung Dis., 2000, 89, 131-140
Current Recommendation

- Neither the conventional methods nor the newer alternative methods alone satisfy all requirements of definitive identification, rapid results, and cost effectiveness. Also, even if M. tuberculosis is identified by an alternative method such as nucleic acid amplification, the organism must still be cultured for susceptibility testing.
For these reasons, laboratories now use a combination of conventional and alternative methods to achieve the best balance. Although protocols vary, experts generally recommend that laboratories include a fluorochrome stain for smears, a broth-based or microcolony method for culture, and DNA probes or chromatography for identification.
Drug Susceptibility Testing

- Drug susceptibility tests should be performed in the following instances:
  - For relapse or re-treatment cases
  - To change the drug regimen when drug resistance is suspected
  - For undertaking drug resistance surveillance studies in a region/country
Types of drug susceptibility testing

Direct

Sputum swab method
Sputum deposit after processing by Petroff’s

Indirect

MIC or Absolute Conc. method
Resistance Ratio Method
Proportion susc. testing
Other Phenotypic Methods

- E Test
- Micro plate tetrazolium reduction assay
- Nitrate reductase assay
- Mycolic acid index susceptibility testing
- Micro colony detection
- Pha B Assay
- Luciferase reporter phage assay
The Quality Assurance Cycle

- Data and Lab Management
- Safety
- Customer Service

Patient/Client Prep
Sample Collection

Sample Receipt and Accessioning

Personnel Competency Test Evaluations

Sample Transport

Quality Control Testing

Record Keeping

Reporting

Pre-Analytic

Post-Analytic
The focus of **EQA** is on the identification of laboratories where there may be serious problems resulting in poor performance, not on the identification of individual slide errors or the validation of individual patient diagnoses.

Three methods to evaluate laboratory performance:
- On-site evaluation
- Panel Testing
- Blinded rechecking
# Laboratory Register Example

## Tuberculosis Programme

### Basic Management Unit TB Laboratory Register

<table>
<thead>
<tr>
<th>Lab. Serial No.</th>
<th>Date specimen received</th>
<th>BMU TB Register No.</th>
<th>Name (in full)</th>
<th>Sex</th>
<th>Age</th>
<th>Complete address (for new patients)</th>
<th>Name of referring health facility</th>
<th>Reason for examination</th>
<th>Microscopy results</th>
<th>Signature Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

1. Only for confirmed TB case registered in the BMU TB register.
2. Facility that referred (send) the patient (or specimen or slides) for sputum smear examination. Use standardized type of referring facilities according to block 2 of the yearly report. Referring facility/provider type is defined as health structure or health providers working in health structure in any of the following TB control functions (DOTS): referring TB suspect/cases, laboratory diagnosis, TB treatment support.
3. Tick or indicate if TB suspect is re-examined just after antibiotics.
4. Indicate month of treatment at which follow-up examination is performed.
5. (NEO): 0 AFB/100 fields; exact number if 1 to 9 AFB/100 fields; (+): 10-99 AFB/100 fields; (++): 1-10 AFB/ fields; (+++): > 10 AFB fields
LABORATORY REQUEST FORM FOR MICROSCOPY

Name of Health Centre ___________________________ Date ________________
Name of patient _________________________________ Age _____ Sex M ☐ F ☐
Complete address: ______________________________________________________

______________________________________________________________

Patient’s register number* __________________________
Source of specimen ☐ Pulmonary
☐ Extra-pulmonary Site ________________________________

Reason for examination ☐ Diagnosis
☐ Follow-up of chemotherapy

Specimen identification number _________________ Date ___________________
Signature of person requesting examination ______________________________

* Be sure to enter the register number for the follow-up of patients on chemotherapy
Laboratory Request Form

Content

- Name of Health Center
- Date of request
- Patient's information (i.e., name, sex, age, address, and register number)
- Source of specimen
- Reason for examination (e.g., diagnosis or follow-up)
- Specimen identification number
- Signature of person requesting examination
**MICROSCOPY RESULTS**

Laboratory serial number: ________________________________

*Visual appearance of sputum*

<table>
<thead>
<tr>
<th></th>
<th>Mucopurulent</th>
<th>Blood-stained</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen 1</td>
<td>ü</td>
<td>ü</td>
<td>ü</td>
</tr>
<tr>
<td>Specimen 2</td>
<td>ü</td>
<td>ü</td>
<td>ü</td>
</tr>
<tr>
<td>Specimen 3</td>
<td>ü</td>
<td>ü</td>
<td>ü</td>
</tr>
</tbody>
</table>

*Microscopy results*

Staining method
- ☐ Ziehl-Neelsen
- ☐ Fluorochrome

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen</th>
<th>Results*</th>
<th>Positive (grading)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>3+ 2+ 1+ 1-9 AFB</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Write negative or positive*

Date: __________________________ Examined by (signature): __________________________