Laboratory diagnosis of pulmonary tuberculosis

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MBS 240

12-07-2024

Objectives

- Know the causative agent of pulmonary TB
- Understand specimen collection and processing
- Understand the Laboratory methods for detection/diagnosis
- Understand recording and reporting of results

What is pulmonary tuberculosis?

- Tuberculosis is a bacterial infection caused by *Mycobacterium tuberculosis*
  - And other species of MTB complex including *M. bovis*, *M. africanum, M. canettii*
    - also known as tubercle bacilli or AFB.
  - Infection can be pulmonary or extrapulmonary
    - Can occur concurrently .
- Spread via airborne transmission from person to person in confined environment.

Aerosol Formation: Spread of droplets from infected persons

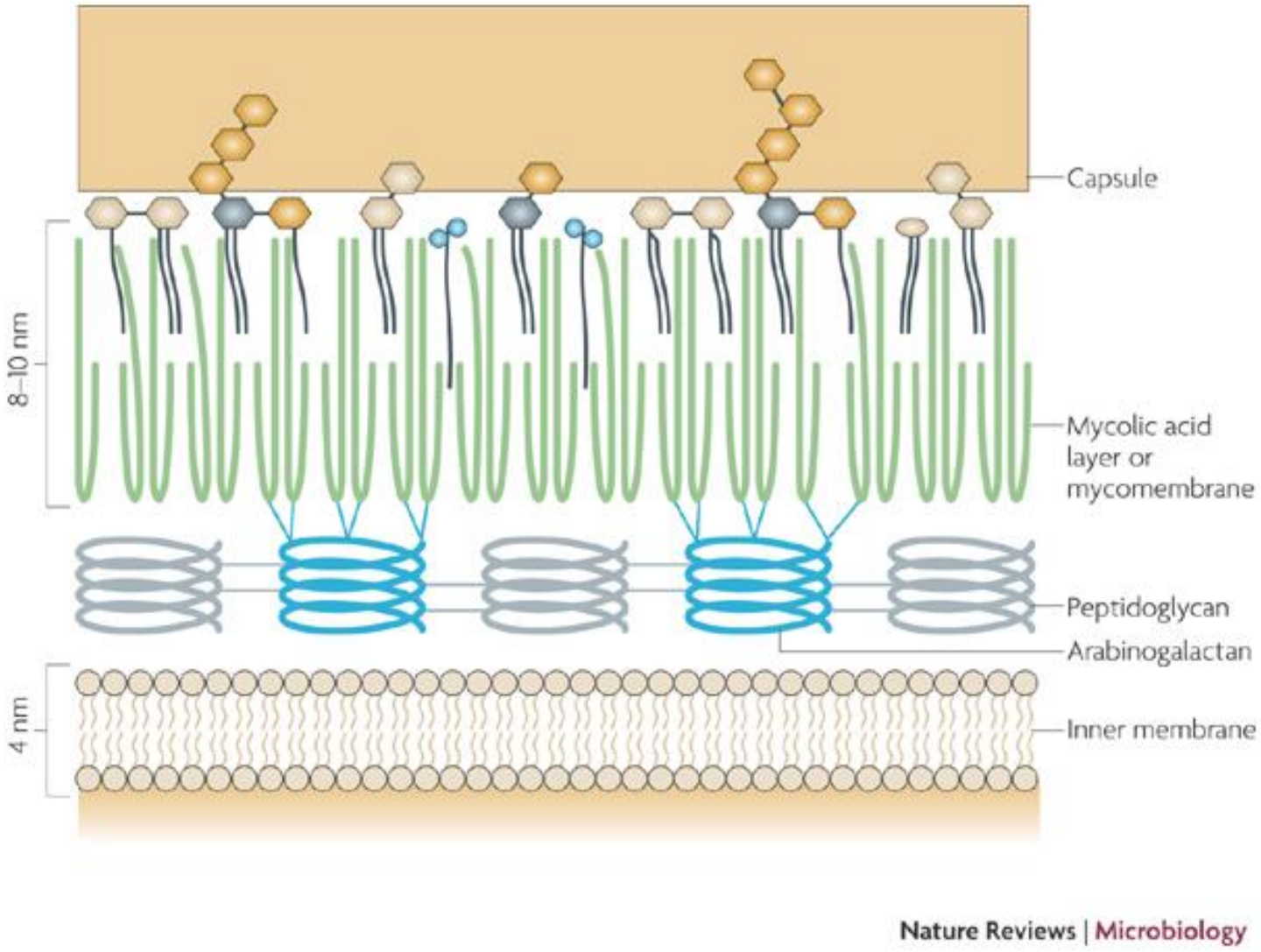
- Coughing
- Singing
- Sneezing
- Talking

What sets *Mycobacterium tuberculosis* apart from other bacteria?

The Mycobacterial Cell Wall

- Waxy cell wall composed of mycolic acid
- Outer coating serves as an efficient barrier and can endure unfavorable conditions
  - Disinfectants
  - Many drugs (antibiotics)
  - NaOH treatment during specimen processing
  - Staining procedures e.g. gram stain

Mycobacterial cell wall



Factors contributing to high tuberculosis infection rates

- High incidence of TB driven by:
  - HIV/AIDS
  - Poverty
  - Mis-use of anti-tuberculosis drugs
  - Development of drug resistant TB
  - Inadequate TB infection prevention and control (IPC) measures

Impact of poverty on TB

- Crowded living conditions facilitate transmission
- Lack of education about symptoms
- Lack of infection control in homes and health care facilities
- Dependence on traditional healers
- Lack of access to health care sites
- Cost of health care
  - TB diagnosis & treatment is free in Zambia!

Multidrug resistant tuberculosis (MDR-TB)

- First line drugs used for treatment of TB
  - Isoniazid, rifampicin, pyrazinamide & ethambutol
- MDR-TB is resistance to the most potent 1st line drugs
  - Isoniazid & rifampicin
- Treatment after failure with 2nd line drugs
  - Extensively drug resistant (XDR-)TB

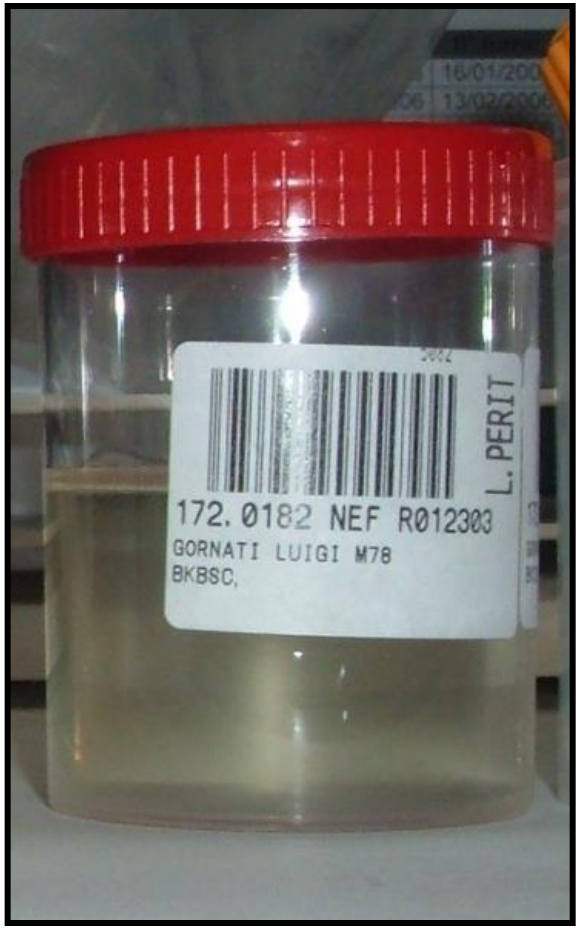
Specimen collection and processing

Patients must be instructed properly

- Rinse mouth with clean water (boiled water).
- Open container but do not touch inside container or cap.
- Take 3–4 deep breaths, holding breath for 3-5 seconds before exhaling.
- Cough after the last exhalation.
- Empty sputum into container.
- Return specimens promptly to the lab.

Containers for Sputum Collection

- Strong, unbreakable
- Leak proof, screw-capped with a water-tight seal
- Sterile
- Single-use
- Up to 50 ml capacity
- Translucent or clear material
- Easily-labeled walls



Specimen containers must be labelled clearly

- Patients name, date of collection
- Complete request form to accompany specimen

Specimens must be stored adequately

- Specimens must be promptly transported to lab
- Processed as soon as possible upon arrival at lab
- Stored in refrigerator (4˚C) if not processed immediately

Transporting specimens

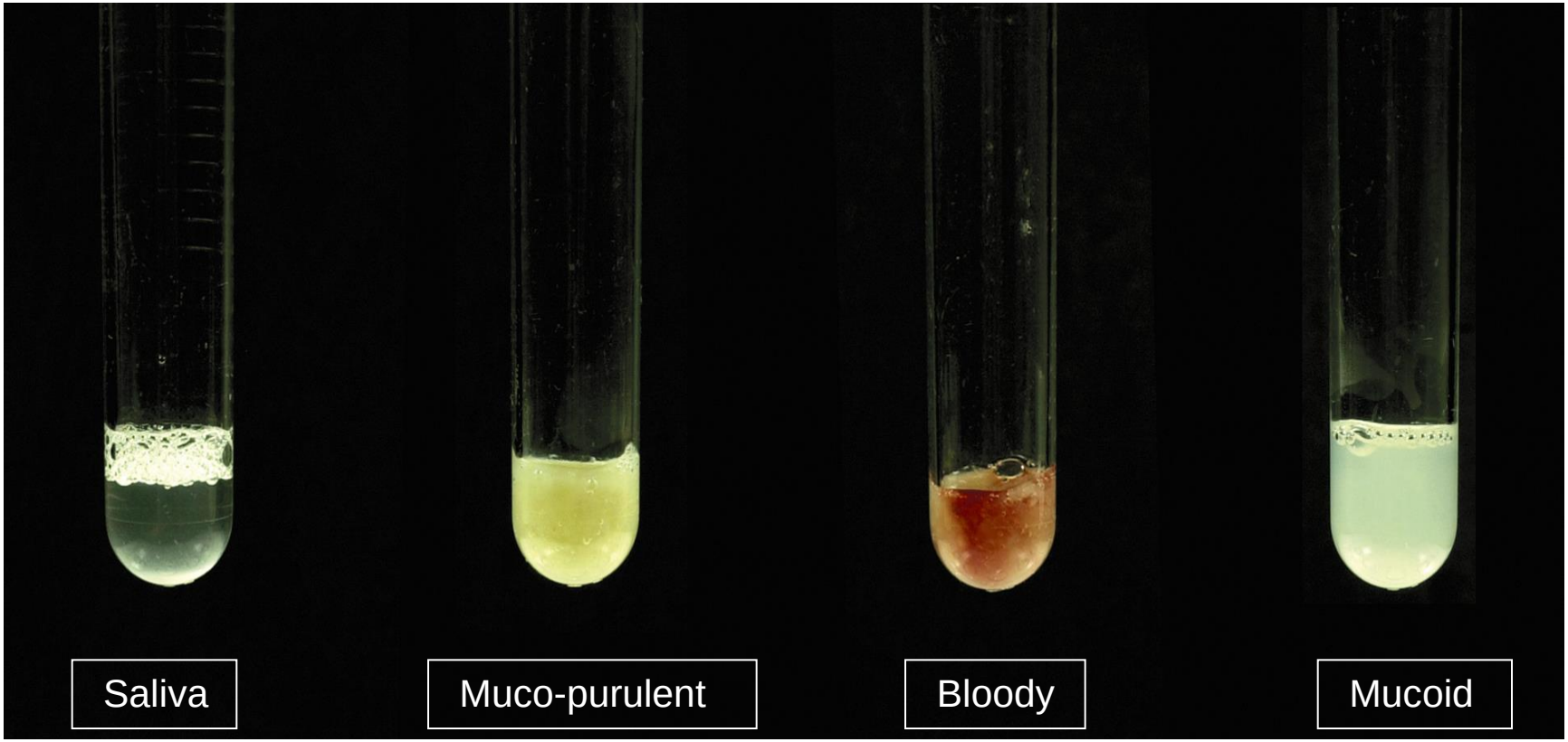
- Specimens must be transported in a containment system
  - Primary containment
    - Collection container with screw cap top
  - Secondary containment
    - Specimen container in a sealable, biohazard bag
    - Place requisition in outside pouch of biohazard bag
  - Tertiary containment
    - Specimens in bags are placed in transport box (Styrofoam with fibreboard, plastic, or metal)
    - cold chain transport and keep specimens protected from light

Quality Assessment of Sputum Specimens

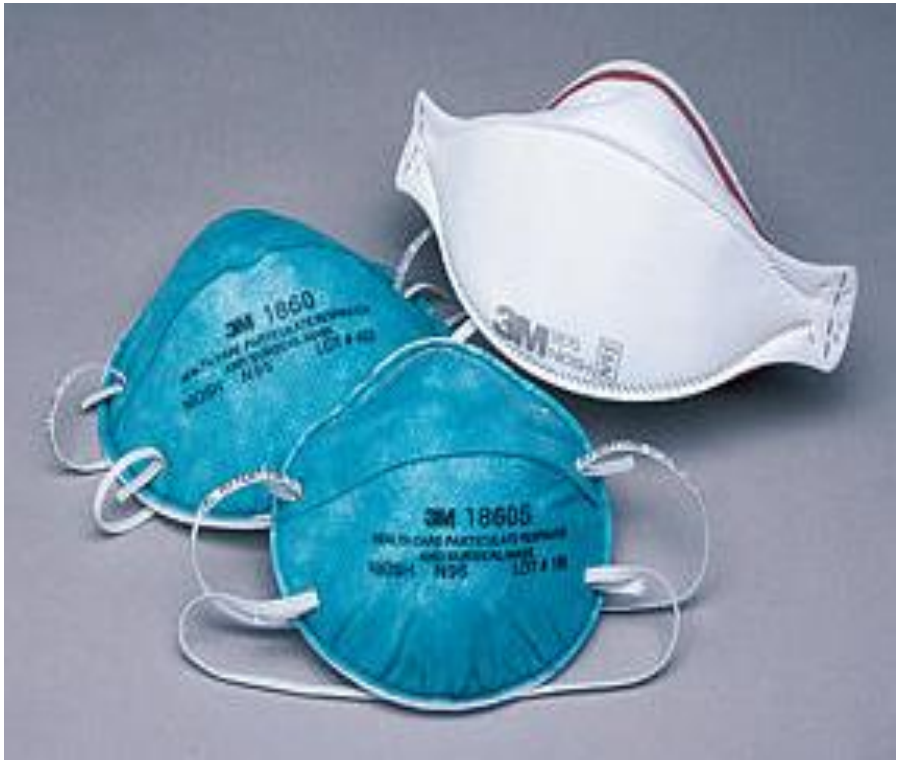
- Characteristics of a good sputum specimen
  - Mucoid or mucopurulent appearance
  - Minimum amounts of saliva
  - Optimal volume: 5ml-10ml
  - Minimum volume: 0.5 ml

  *Record characteristics on form

Assessing Quality of Sputum Specimens



Personal protective equipment (PPE) MUST be worn



N95 masks must be worn

Water resistant gowns and double gloves must be worn

Specimen processing

- All specimen processing should be carried out in a bio-safety cabinet (level 3)
  - Specimens should only be considered safe upon fixing onto a slide.
- Pre-treatment of sputum necessary before culture
  - NALC-NaOH commonly used method.

Principles of processing

- Sputum specimens are viscous materials contaminated with normal flora

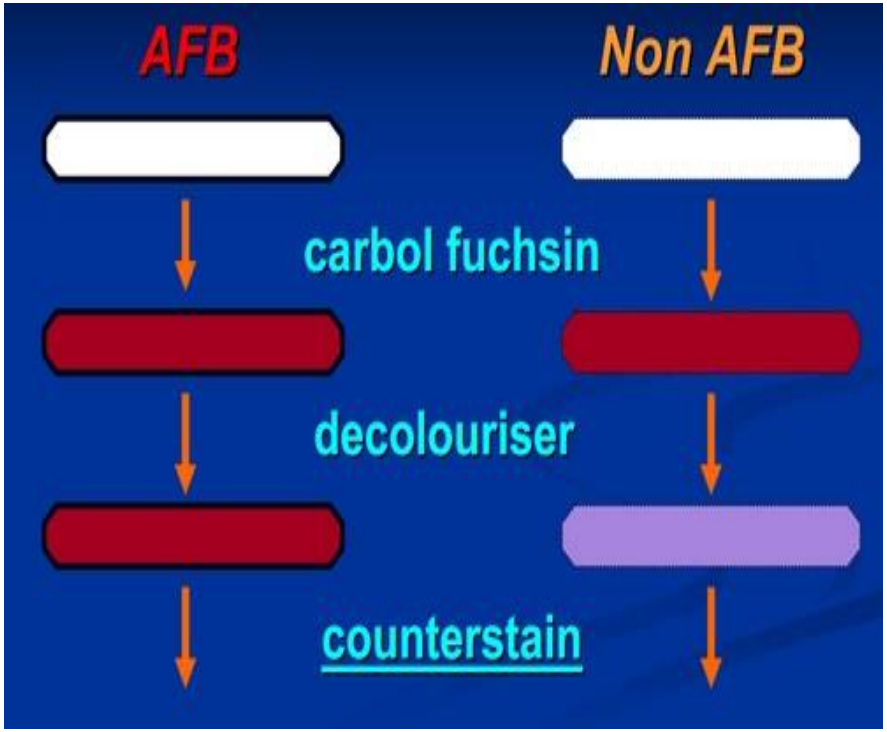
- Processing involves pre-treatment of the sputum specimens
  - Digestion: to free the TB bacilli from the mucus, cells or tissue in which they may be embedded
  - Decontamination: to eradicate normal flora that grow more rapidly than MTB and would interfere with the ability to recover MTB
  - Homogenization of the digested materials
  - Concentration of the TB bacilli by centrifugation before smear preparation and media inoculation

Digestion-Decontamination
(N-Acetyl-L-Cysteine- Sodium Hydroxide method)

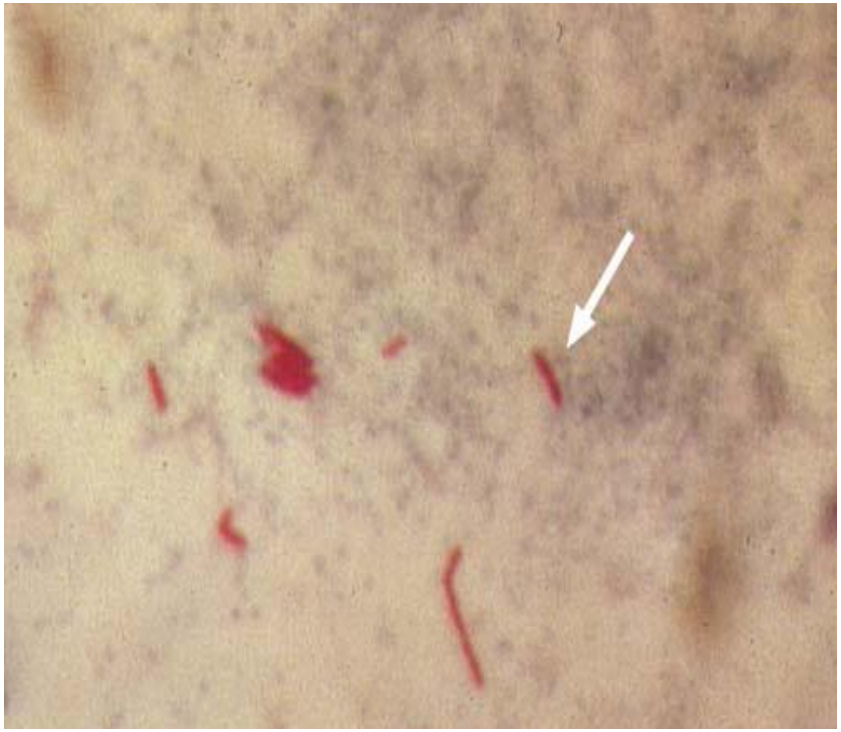
- NaOH
  - Decontaminating agent
- Na Citrate
  - Binds the heavy metal ions that might be present in the specimen that could inactivate the NALC
- N-Acetyl-L-Cysteine (NALC)
  - Digestion agent
  - Mucolytic agent aids in liquefying sputum to release trapped bacteria
  - more efficient decontamination of normal flora and releases AFB

AFB-smear microscopy

- Performed on sputum
- Can be direct or concentrated sputum
- Most mycobacteria are Acid fast bacilli (AFB)
  - Resistant to decolourisation during staining
- Microscopy usually using light microscope
  - Ziehl-Neelsen stain
- Primary means of diagnosis in resource limited settings
- Although ZN microscopy has been phased out and replaced with FM



ZN stain of AFB:
AFB retain red dye after Decolourising step



AFB under light microscope Following ZN staining

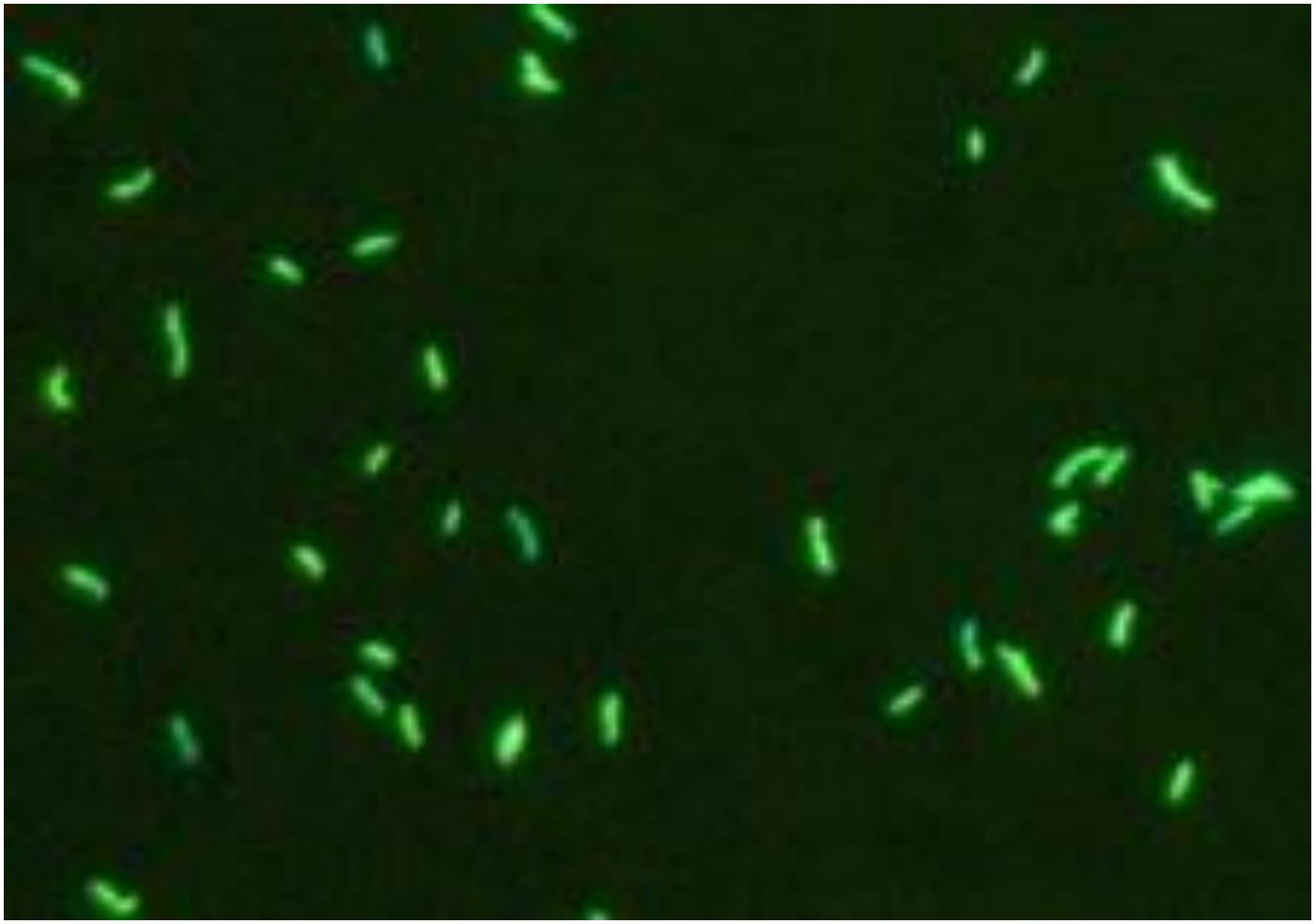
Grading of ZN smear

Number of AFB	Fields	Report
No AFB	In 100 immersion fields	No AFB seen
1-9	In 100 immersion fields	Exact No
10-99	In 100 immersion fields	1+
1-10	Per-field examination	2+
>10	Per-field examination	3+

Fluorescence microscopy

- Requires smears to be stained with fluorescent stain
  - Auramine-rhodamin
- More sensitive than ZN microscopy
  - Will detect small numbers of bacilli
- More slides can be examined compared to ZN
- Lower resolution required than ZN

Fluorescence LED microscopy of Mycobacterium tuberculosis



LED fluorescence Microscopy TB grading

- No AFB: report as “No AFB seen”
- 1-19 AFB/40 fields: actual number recorded
- 20-199 AFB/40 fields: 1+
- 5-50 per field: 2+
- >50 per field: 3+

Culture

- Gold standard for diagnosis
  - more sensitive
- Antigen detection strips specific for MTBC used following culture e.g. Capilia

1. Solid culture
   - Can take up to 6-8 weeks for growth to be observed
2. Liquid culture-BACTEC MGIT system
   - Automated culture system
   - Can take up to 20 days for growth
   - Expensive and only available at referral level

Solid culture

- Lowenstein-Jensen (LJ) for isolation of MTB
- Contains glycerol, potato flour, salts & eggs
  - Glycerol replaced with Na pyruvate for M. bovis
- Malachite green inhibits gram +ve bacteria

Liquid culture

- Performed in automated culture system
  - Mycobacteria Growth Indicator Tube (MGIT)
- BACTEC MGIT system provides rapid detection for TB growth
- Mycobacteria use O_2 during growth
  - O_2 released from indicator in tube bottom
  - Fluorescence indicates growth of bacteria
- Incubates samples at 37˚C

Length of Incubation in MGIT

- Protocol length for growth detection can be from 1 to 56 days
  - Default protocol length is 42 days for detection of TB from processed sputum specimens
  - Positive specimens ~20 days
- Specimens with no growth after 42 days can be reported as negative
  - Subcultures or AFB stains are not required unless flakes of growth are seen in tube

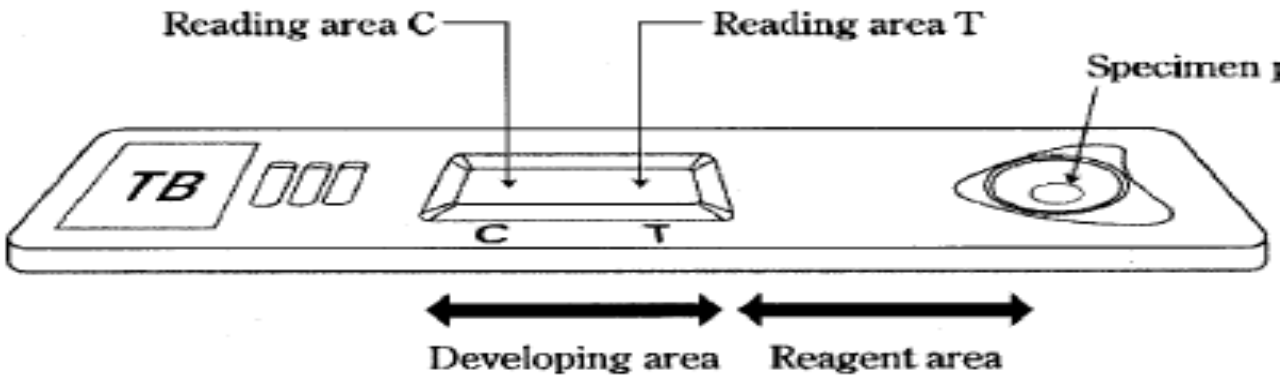
MGIT tubes

BACTEC MGIT machine

- Advantages of culture
  - Detects small numbers of organisms
    - Can detect as little as 10 bacilli/ml
    - Confirms diagnosis of TB in HIV patients
  - Allows species identification
  - Allows for drug sensitivity testing
- Limitations of culture
  - Slow growth, hence long turn-around time
  - Expensive
  - Limited laboratories
    - 3 reference laboratories providing liquid cultures

Antigen detection test for culture

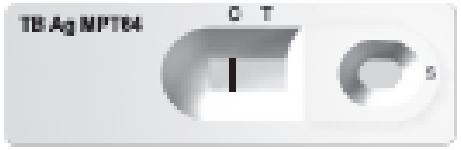
- AFB smear positive actively growing culture
  - Liquid
  - Solid
- Interpretation after 15 minutes



Interpretation

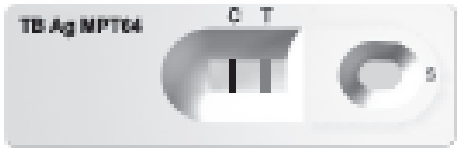
Negative

No *Mycobacterium tuberculosis* antigen



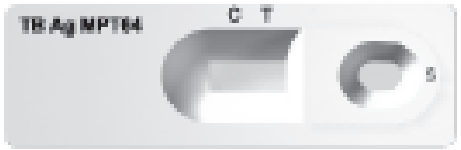
Positive

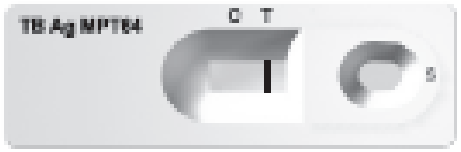
Mycobacterium tuberculosis complex positive



Invalid

It is recommended that the specimen should be re-tested





Antigen Detection Test

Drug susceptibility testing (DST)

- Important for testing sensitivity of TB to anti-TB drugs
- Colonies from solid and liquid culture can be used for testing
- Sensitivity testing carried out at referral level using automated BACTEC MGIT system
- Strains resistant to the first line drugs isoniazid and rifampicin are considered to be MDR

Molecular methods

- Provide definitive identification of MTBC
- Amplifies and detects genomic material
- Rapid detection
- More sensitive than microscopy and culture
- Limitations:
  - Expensive equipment and reagents
  - Requires trained personnel to operate
- Systems for identification include:
  - Line probe assay: identification of different MTBC
  - GeneXpert: RIF resistant marker indicating MDR
  - PCR

GeneXpert

- Automated diagnostic test for ID of MTB
- Cartridge based PCR system
- Detects rifampicin resistance
- Highly sensitive & specific
  - Recommended by WHO for use in HIV-TB co-infection

Reporting of results

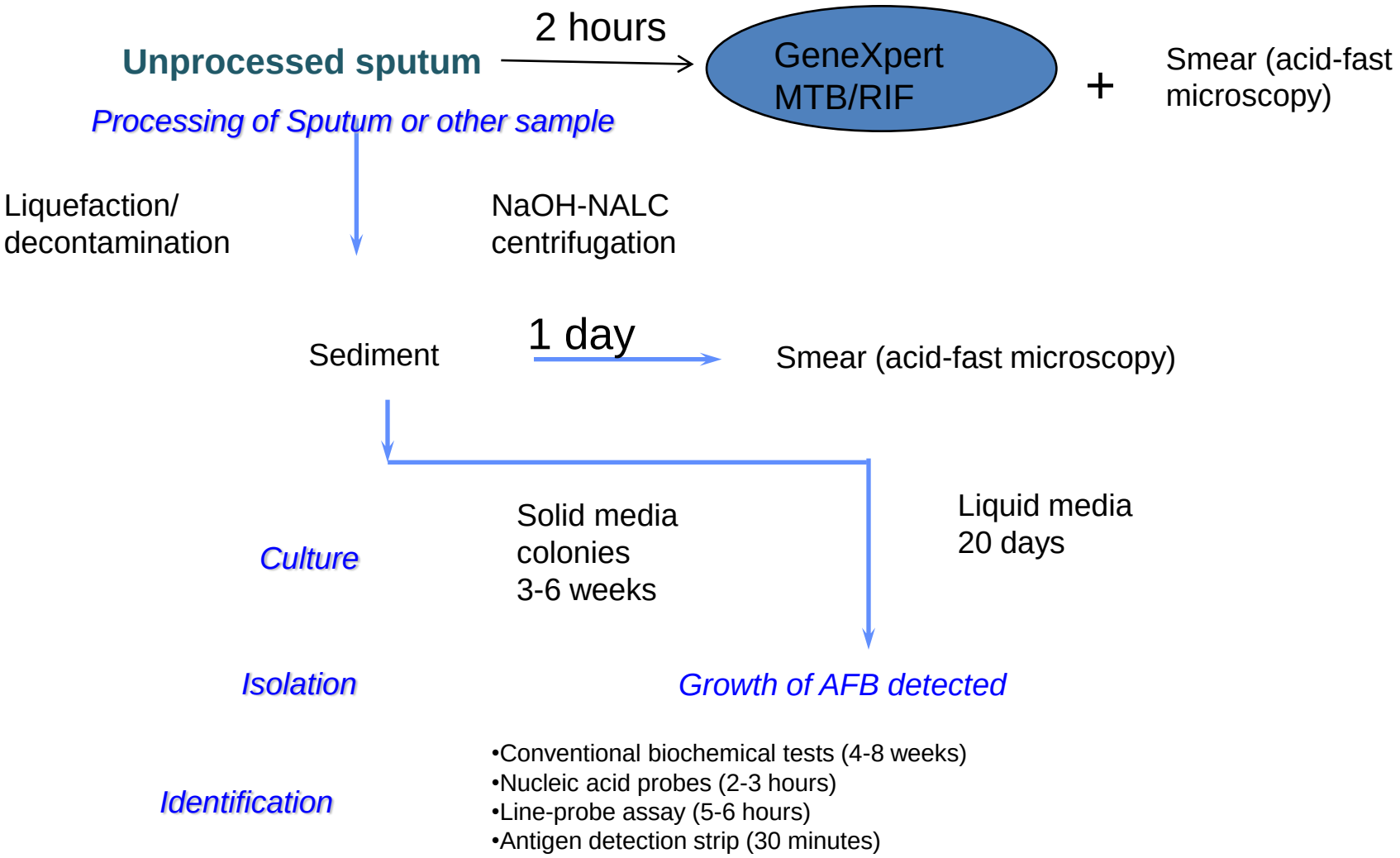
- Laboratory results must be returned to the clinician promptly
- Results should indicate:
  - If MTBC isolated
  - Any other microorganisms isolated
  - Drug susceptibility test results
- Specimen rejection must be reported with reasons
  - Details on container not matching request form
  - missing request form
  - no patient details on specimen

Why is the TB laboratory important?

- Patient and community care
  - Allows for the patient to be placed on appropriate treatment
  - Shortens infectious period
- Diagnosis of TB
  - Allows for timely and accurate diagnosis
  - Patient management for drug therapy
  - Monitoring and treatment of drug resistant TB

In summary

Isolation and Identification of Mycobacteria



CDC / NCID

Further reading

- **Murray, Medical Microbiology** 6th edition.

- **Mims' Medical Microbiology** 4th Ed