CHAPTER XIV
QUALITY ASSURANCE IN BACTERIOLOGY

Need for quality assurance and SOPs in Microbiology

Microbiological investigations are important in the diagnosis, treatment, and surveillance of infectious diseases and policies regarding the selection and use of antimicrobial drugs. It is

therefore essential that test reports:– are reliable, standardized,

provide the information that is required at the time it is needed and



Figure. Quality assurance system.

Providing appropriate, reliable and affordable microbiological services

Laboratory personnel, clinicians, community health officers and sanitary officers must work closely together in deciding the microbiological services that are required and ensuring the services provided are appropriate, reliable, and affordable.

This involves identifying:

- The infectious diseases that require laboratory investigation

(priority pathogens).

- Role of district laboratories in surveillance work and the investigation of epidemics.
- Techniques (SOPs) to be used to collect specimens, identify pathogens and perform antimicrobial susceptibility tests.
- Most appropriate systems for reporting and recording the results of microbiological
- investigations, collating and presenting data for surveillance purposes.

- Quality assurance.
- Training requirements, supervision, and ongoing professional

support.

- Equipment and microbiological supplies needed and systems

for distribution of supplies.

- Costs involved.

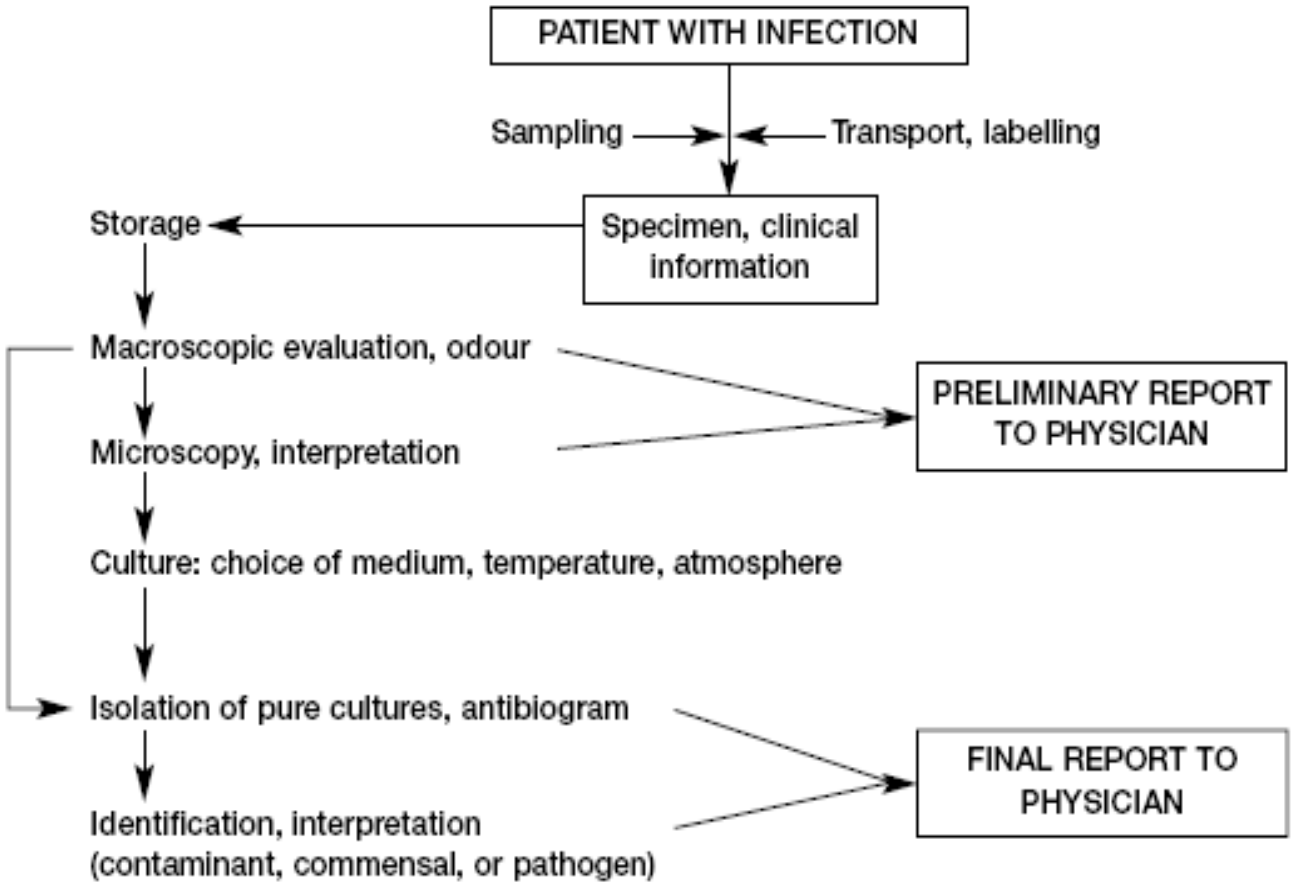


Figure. Steps in the laboratory investigation of an infected patient.

Sputum specimen to diagnosis TB (CDC protocol)

Specimen Collection Container: Specifications

- 50 ml capacity
- Translucent or clear material
- Single-use combustible material
- Screw-capped with a water-tight seal
- Easily-labeled walls



Specimen Collection

- Three (3) specimens optimal for identifying infectious cases of tuberculosis
- Make collection convenient and efficient for both patient and laboratory worker

Timing of Specimen Collection

Spot–Morning–Spot

WHO/IUATLD Recommendation

- Spot — initial visit to the clinic
- Early morning — first sputum in the morning
- Spot — second visit to the clinic

Spot-Morning-Spot

Advantages

- Requires only two visits (convenience to the patient)
- A spot specimen is available in case the patient does not return with the morning specimen

Disadvantages

- Two out of three specimens may be of poor quality
- High risk of missing a case if only the first specimen is properly examined

Collection Considerations

- Yield decreases rapidly after three specimens
- Morning specimens on average better
  - Collect three morning specimens from hospitalised patients
- Two good examinations may be most efficient with high workload
- Follow NTP guidelines for exact specimen collection strategy

Follow-up Specimens for Monitoring Treatment

- Collected during and at the end of treatment
- Early morning specimen
- Consult NTP guidelines for exact collection frequency

Specimen Collection: Safety

- The patient is a greater danger to staff than the specimen!
- Instruct patient to cover the mouth when coughing
- Never collect sputum in the laboratory!
  - Collect OUTSIDE
  - Collect away from other people
- Do not stand near patient during specimen collection

Specimen Collection Guidelines

- Explain clearly to patient
- Why sputum is needed
- Three samples required
  - Spot–morning –spot
- What is a good sample and how to obtain it
- Opening and tight closing of containers
- Not to soil the exterior of the container
- Transport of sputum containers
- The need to return to the clinic

Patient Education: Collection

- Best specimen comes from the lung
- Saliva or nasal secretions are unsatisfactory
- Remove dentures and rinse mouth with water
- Need for three sputum samples for optimal diagnosis

Patient Instructions: Collection

- Inhale deeply 2–3 times, breathe out hard each time
- Cough deeply from the chest
- Place the open container close to the mouth to collect the specimen

Slide Referral

- Smears require less safety precautions for packaging
- Peripheral centres must be trained in collection and smear preparation

Disadvantages

- Risk of poor specimen and poor smear
- Slides are more fragile

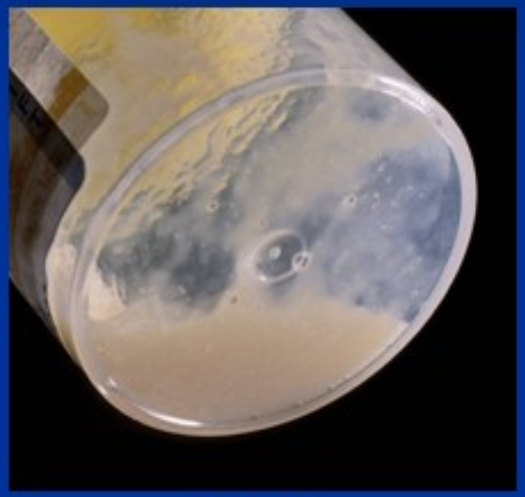
Labeling Specimen Container



Specimen Receipt at Laboratory

- Check specimens for quality:
  - Volume (at least 3–5 ml)
  - Describe sputum consistency (mucoid, purulent, bloody, or watery)
- Register the specimen and allocate a laboratory serial number

Specimen Quality

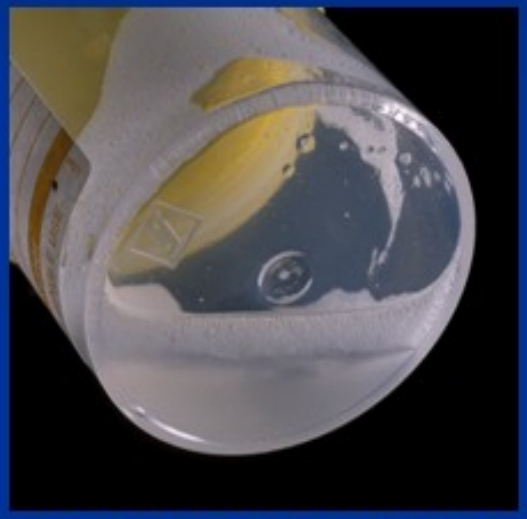


Purulent



Mucoid

Specimen Quality



Saliva or Induced sputum (?)



Blood stained

Skin scraping for leprosy

1. Label the new glass slide with a diamond pencil.
2. Make sure to take the smears from the indicated sites: the right ear lobe, the left ear lobe, any anaesthetic patches or around the right elbow and the left knee.
3. Mark clearly from which site each smear is.
4. Take a fold of skin between your index finger and thumb and squeeze tightly to prevent blood flow.
5. Collect only tissue fluid.
6. If there is lots of blood do not collect the sample.
7. Make a new cut once again squeezing the skin tightly.

- Sputum containers, slides and wooden applicators must be collected in a waste container that is covered with a lid.
- Dig a deep pit of 1 metre, through the waste into the pit and cover the pit with soil when it is about half filled.
- You may also burn all the waste material.
- Do not leave the waste uncovered in the open.
- Do not through the waste into a river.
- Do not reuse slides of Tb and leprosy smears.

Internal Quality Control (IQC) Procedures

A. Gram stain:

1.Follow proper sample collection procedures.

2.Don't overheat the slide when heat-fixing the smear.

3. Filter the crystal violet before use.

4. Discard Lugol's iodine when colour has faded.

5. Decolourise carefully, just few seconds and wash off with

water immediately.

6. Stain known samples of Gram-negative and Gram-positive bacteria once a week and whenever new stains have been prepared.

1. Follow proper sample collection procedures.

2. Label slides with diamond pen, grease pencil will wash off.

3. Do not recycle slides for Ziehl Neelsen stain.

4. Use only new slides for Ziehl Neelsen stain.

5. Don't overheat the slide when heat-fixing the smear.

6. Filter the carbol fuchsin stain onto the slide.

7. Stain known sample of AFB positive sputum once a week or whenever using new carbol fuchsin stain.

8. Store all positive slides in a box for re-checking by an expert.

9. Store all negative slides for two weeks for

C. Urine

1. For urine culture mid-stream urine collected into a sterile container is required to avoid secondary contamination of the urine sample.
2. Collection of mid-stream urine.
3. It is very important to examine the urine Immediately.
4. after collection; delay will increase the number of

bacteria in the urine and give wrong results.

D. Blood

1. Always collect blood for culture before antibiotic treatment has been started.
2. If possible collect the blood at the time when the patient's temperature is raising.
3. Prepare the blood culture bottle for collection.
4. Remove the culture bottle from the fridge and bring to room temperature.
5. Do not use culture bottle if the culture broth appears turbid.
6. Disinfect the skin at the point of collection thoroughly with 70% alcohol. Let the alcohol evaporate do not touch again with your fingers.
7. Collect the blood and under complete sterile condition transfer about 5 ml of blood into the culture bottle. Mix gently.
8. Incubate the culture bottle immediately.

Safe working practices

- Health and safety in district laboratories, including full coverage of microbial hazards, safe working practices, and the decontamination of infectious material and disposal of laboratory.
- The following are some of the important points which apply when working with infectious materials:

1. Never mouth-pipette. Use safe measuring and dispensing devices.
2. Do not eat, drink, smoke, store food, or apply cosmetics in the working area of the laboratory.
3. Use an aseptic technique when handling specimens and culture.
4. Always wash the hands after handling infectious material, when leaving the laboratory and before attending patients.
5. Cover any open wound with a waterproof dressing.
6. Wear appropriate protective clothing when working in the lab.