Module 5

Collection, transport and receipt of specimens

Purpose	To provide you with the knowledge and skills for proper collection, transport and receipt of specimens for *Mycobacterium tuberculosis* culture.
Prerequisite modules	Modules 2 and 3
Module time	1 hour 5 minutes
Learning objectives	At the end of this module, you will be able to: – explain the proper collection of the specimens; – explain how to select suitable containers for the collection of different specimens; – explain the proper labelling of specimens to be submitted to the TB laboratory; – explain the proper transportation of specimens; – list features of a good specimen; – fill in the laboratory register.
Content outline	• Procedure for specimen collection • Suitable specimen containers • Transport of specimens • Receipt of incoming specimens • Evaluation of specimen quality • Specimens and request forms: quality assurance
Exercise	• Collection transport and receipt of specimens.
Annexes	5.1 Laboratory request form 5.2 Laboratory register

INTRODUCTION

In terms of the effectiveness of the TB laboratory, nothing is more important than the appropriate selection, collection, transportation and handling of specimens for microbiological diagnosis.

Specimens should be refrigerated if there is likely to be any delay in their processing, unless they are mixed with CPC as a preservative (mixed with an equal volume of 1% cetyl pyridinium in 2% sodium chloride). Improper handling and transport of paucibacillary (contain few bacilli) specimens can cause the death of mycobacteria and lead to false-negative culture results. Unless specimens are collected with the utmost care and promptly transported to the laboratory under the proper conditions, the advantages of culture will not be fully realized.

Safety during transportation of potentially infected specimens is also critical: incorrect packaging or handling of specimens may be dangerous as it can cause spread of tuberculosis infection.

Exposure to possible sources of contamination, such as tap water, must be avoided during specimen collection, since the presence of environmental mycobacteria may result in false-positive smear and/or culture results.

STRATEGY FOR SPECIMEN COLLECTION

More than 85% of TB disease in high-prevalence countries is pulmonary, but every part of the body can be affected. Extrapulmonary TB is often paucibacillary and thus smear-negative. For this reason microscopy is inadequate as a diagnostic tool, and culture is the only means of obtaining a definitive diagnosis.

A laboratory that performs culture will receive different kinds of specimens and different collection and processing procedures may therefore be needed.

Sputum sample

Most specimens received by the laboratory are sputum samples. It is critical to explain clearly to the patient how to collect the sputum specimen, using simple and easily understood words, and also to provide written instructions.

Cough of more than 2 weeks' duration is a distinctive symptom of pulmonary TB. Sputum specimens from any patient presenting this symptom must be investigated by the laboratory.

For the diagnosis of pulmonary TB, the international policy on TB case detection recommends the examination of three sputum smears within two consecutive days: "SPOT – MORNING – SPOT". The first sample is collected at the time of the first visit (spot specimen). The second sample is collected at home early the following morning in a container provided by the laboratory, and should be delivered as soon as possible to the laboratory (morning specimen). When the patient returns to deliver the morning specimen, he or she is asked to provide the third specimen (spot specimen).

A smear-positive case is defined as follows [references 1, 2]:

> *Tuberculosis in a patient with at least two initial sputum smear examinations (direct smear microscopy) positive for acid-fast bacilli (AFB+)*.

A systematic review of 37 eligible studies clearly showed that most TB cases (average 85.8%) were detected with the first sputum specimen. With the second sputum specimen, the average incremental yield was 11.9%; with the third specimen, when the first two were negative, the incremental yield was 3.1% [reference 3].

It is expected that microscopic analysis of two sputum smear samples will improve case-finding through better quality of service, reduced time for diagnosis and initiation of treatment, and fewer patients dropping out of the diagnostic pathway.

Thus, a reduction – from three to two – in the number of specimens to be examined for screening TB cases has been accepted in places with high workloads and limited human resources, provided that quality assurance (QA) programmes are implemented.

If the national TB programme (NTP) adopts the two-specimen policy, one specimen must be collected at home.

However, a reduction – from three to two – in the number of specimens examined for screening TB patients should be recommended only in settings with a well-established laboratory network, a fully functional external quality assessment (EQA) programme for smear microscopy (including on-site evaluation with feedback mechanism), and where the workload is very high and human resources are limited [reference 4].

Specimen collection

It is very important that patients are clearly informed about:

- the importance of sputum examination for diagnosis of TB or follow-up of TB treatment;
- the need for collecting real sputum, not saliva (although the laboratory should never reject a salivary specimen);
- how to open and close the containers;
- how to produce good sputum (i.e. by repeated deep inhalation and exhalation of breath followed by cough from as deep inside the chest as possible);
- how to avoid contamination of the exterior of the container (i.e. by carefully spitting and closing the container);
- how to collect and safely deliver the morning sputum to the laboratory; and
- the need for three sputa to improve the diagnostic yield.

Other specimens

Extrapulmonary specimens may be divided into two main groups according to the extent of contamination:

- aseptically collected specimens, usually free from other microorganisms (sterile);
- specimens contaminated by normal flora or specimens not collected aseptically (not sterile).

Body fluids (spinal, pleural, pericardial, synovial, ascitic, blood, pus, bone marrow) should be aseptically collected in sterile containers by the physician, using aspiration techniques or surgical procedures. For fluids that may clot, sterile potassium oxalate (0.01–0.02 ml of 10% neutral oxalate per ml fluid), or heparin (0.2 mg/ml), or sodium citrate (two drops of 20% sodium citrate for every 10 ml of fluid) should be added as an anticoagulant for culture. .

Specimens should be transported to the laboratory as quickly as possible (within 1 week). Blood samples should be discouraged: the diagnostic yield is low and samples are susceptible to contamination during the particular technique required

The physician should place aseptically collected tissues in sterile containers *without fixatives or preservatives*. If specimens are to be shipped, they should be protected from drying by adding sterile saline and maintaining a temperature of 4–15 °C. Specimens should be transported to the laboratory as quickly as possible.

Urine is the most common of the specimens expected to be contaminated. To minimize excessive contamination of urine specimens, external genitalia should be washed before specimen collection. Once received in the laboratory, a urine sample must either be processed immediately or centrifuged and the pellet refrigerated. As excretion of tubercle bacilli is intermittent, three consecutive early-morning midstream specimens must be collected.

Swabs are always suboptimal specimens and are not recommended because of the risk of infection for the specimen collector. They may be useful in children and patients who cannot produce sputum or may swallow it. A sterile absorbent cotton swab should be used for collection;

the best time is the early morning before food and drinks are taken. The addition of 0.9% saline solution to the container helps to keep the swab wet.

Other respiratory specimens that can be submitted to the laboratory for mycobacteria culture are bronchial secretion (minimum volume 2–5 ml) and bronchial alveolar lavage (minimum volume 20–50 ml). Transbronchial and other biopsies taken under sterile conditions should be kept wet during transportation by adding 0.5–1 ml of sterile 0.9% saline.

Pleural effusion is a suboptimal specimen: tubercle bacilli are mainly in the pleural wall and not in the fluid. The minimum volume for pleural effusion is 20–50 ml.

In children, who produce little if any sputum, aspiration of the early-morning gastric content can be used for TB diagnosis. The gastric aspirate should be transported immediately to the laboratory and neutralized by adding 100 mg of sodium bicarbonate.

SUITABLE SPECIMEN CONTAINERS

As a general rule, specimens should always be collected and submitted in sterile, leakproof, disposable, appropriately labelled, laboratory-approved containers without any fixatives. Waxed containers must not be used because they may yield false-positive AFB smear results. If centrifugation is used for culture, the use of collection containers suitable for centrifugation can be considered. It allows decontamination and centrifugation in the collection container avoiding transferring to another container.

Specimen containers should also:

- be rigid, to avoid crushing in transit;
- be watertight and have screw-caps, to prevent leakage and contamination;
- be wide-mouthed (at least 35 mm in diameter), so that a patient can expectorate easily into a container without contaminating the outside and/or so that materials can be easily placed inside a container without touching the exterior;
- have a capacity of 50 ml;
- be made of transparent material so that specimen volume and quality can be checked without the need to open the container;
- be made of single-use, combustible material to facilitate disposal;
- have easily-labelled walls to allow permanent identification.
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SAMPLE TRANSPORT

- Generally, the robust nature of mycobacteria makes the use of transport media and preservatives unnecessary. However, small quantities of biopsy material may be immersed in a small amount of sterile physiological saline solution. Note that gastric lavages have to neutralized by adding 100 mg of sodium bicarbonate to the gastric aspirate and transport it immediately to the laboratory.

If specimens cannot be transported to the laboratory within 1 hour, it is recommended to keep them at 4 °C. On arrival at the laboratory, specimens should again be refrigerated until they can be processed. Delay between collection and inoculation should not exceed 7 days.

If specimens have to be transported at ambient temperatures, chemical preservation may be considered. The following methods are acceptable:

1. Mixing the fresh specimen with an equal volume of 1% cetyl pyridinium chloride (CPC) in 2% sodium chloride. Tubercle bacilli will survive for up to a week (longer survival has been reported), while the growth of unwanted organisms will be restricted. Remember that CPC has to be removed before culturing: two centrifugation steps are required to achieve this. CPC-

treated specimens cannot be inoculated in liquid media. It may be difficult to make direct smears with CPC-treated specimens because of their viscosity and inability to stick to slides; moreover, CPC interferes with fluorescence, and specimens preserved with this additive therefore cannot be stained with auramine. If CPC is not washed thoroughly, it results in surface decolouration on LJ media, mimicking contamination. It should also be remembered that, to avoid recrystallization of the salt, CPC-containing samples should never be exposed to low temperatures.

2. If cultural examination will begin within less than 24 hours, specimens may be mixed with an equal volume of 10% trisodium phosphate.

For transportation of infectious substances, refer to your country's specific rules. For international transfer of infectious substances, the International Air Transport Association (IATA) should be contacted: www.iata.org.

Specimens and cultures should be packaged in a three component packaging consisting of:
(i) a leak-proof primary receptacle(s);
(ii) a leak-proof secondary packaging; and
(iii) an outer packaging of adequate strength for its capacity, mass and intended use.

For the purposes of transport, infectious substances are defined as substances which are known or are reasonably expected to contain pathogens. (WHO/CDS/EPR/2007.2 Guidance on regulations for the transport of infectious substances 2007). Category A corresponds to an infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. All other infectious substances belong to Category B.

Cultures of *M. tuberculosis* belong to Category A. However, for surface transport, when *M. tuberculosis* cultures are intended for diagnostic or clinical purposes, they may be classified as category B. For surface transport there is no maximum quantity per package. For air transport:
• no primary receptacle shall exceed 1 l (for liquids) or the outer packaging mass limit (for solids)
• the volume shipped per package shall not exceed 4 l or 4 kg.
These quantities exclude ice, dry ice or liquid nitrogen when used to keep specimens cold.

When sending out or receiving specimens, check that:

- request forms are located separately from specimen containers;
- containers are clearly labelled on the side, not on the caps;
- each transport box has an accompanying list that identifies the specimens, the patients from whom they were collected and the collection date;
- the number of specimen containers in the box corresponds to the number given on the accompanying list;
- the identification number on each specimen container corresponds to the identification number on the accompanying list;
- the accompanying list contains the necessary data for each patient;
- the date of dispatch and the particulars of the health centre are on the accompanying list.

RECEIPT OF INCOMING SPECIMENS

For reasons of safety and work-flow, specimens should be received in the office area of the laboratory and delivery boxes should be opened using all the applicable biosafety procedures. To minimize the risk of infection, the following procedures should be applied:

1. Inspect the delivery box for signs of leakage. If mass leakage is evident discard the box without opening by autoclaving or burning.

2. Disinfect the outside of the delivery box using cotton wool or paper towels saturated with a suitable disinfectant (e.g. 5% phenol, 70% alcohol).
3. Carefully check whether there is any leak. Open carefully and check for cracked or broken specimen containers. Any broken specimen container should be autoclaved or incinerated without processing and another specimen should be requested.
4. Check labelling of specimens with individual identification numbers; check that these correspond with the numbers on the accompanying list.
5. Disinfect the inside of the delivery box; wash hands after handling specimen containers.

EVALUATION OF SPECIMEN QUALITY

A good sputum specimen should be approximately 3–5 ml of recently-discharged material from the bronchial tree. It is usually thick and mucoid. It may be fluid and contain pieces of purulent material. Colour varies from opaque white to green. Bloody specimens will appear reddish or brown. Clear saliva or nasal discharge is not suitable as a TB specimen, although saliva should not be rejected. Remember that induced and follow-up sputa resemble saliva, so they should not to be rejected. To avoid contamination or dilution of a good sample, specimens should not be pooled.

Other specimens should be correctly collected and delivered as quickly as possible to the laboratory. Every effort must be made to organize and expedite specimen transportation and processing. Although TB bacilli can survive in sputum for one week in the absence of preservatives, the probability of culturing the bacilli decreases with time and this is especially critical for paucibacillary specimens.

Reject any specimen that is too small or dried to be processed for smear or cultures.

SPECIMENS AND REQUEST FORMS: QUALITY ASSURANCE

For reasons of quality control, tests must be performed only upon written request from authorized persons; oral requests are permitted only with follow-up written instructions. It is also important that specimen request forms are kept separately from the specimens themselves. Forms that have been contaminated by specimens should be sterilized by autoclaving. If mistakes are found in request forms or labelling of specimens, specimens should be registered and rejected.

Document the arrival time of specimens in the laboratory and note any delays in delivery on the report form, particularly for negative/contaminated results. Evaluate the quality of sputum specimens and make a note if a specimen resembles saliva. The report should state “Specimen resembled saliva – interpret a negative result with caution”.

Always register the incoming specimen in the laboratory register: each specimen is given a serial number that should be used to label every test-tube used during testing of that specimen.

Other data that should be reported on the laboratory register are: the date of receipt of the specimen, patient’s name, age, sex, and address, the name of the referring health centre, the reason for DST, the appearance of the specimen. The register must also carry the signature (or name) of the person performing the examination.

REFERENCES

1. *WHO Tuberculosis Programme: framework for effective tuberculosis control*. Geneva, World Health Organization Document 1994 (WHO/TB/94.179:1–7).

2. World Health Organization, International Union Against Tuberculosis and Lung Disease, Royal Netherlands Tuberculosis Association. Revised international definitions in tuberculosis control. *International Journal of Tuberculosis and Lung Disease*, 2001, 5:213–215.

3. Mase S et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *International Journal of Tuberculosis and Lung Disease*, 2007, 11:485–495.

4. *External quality assessment for AFB smear microscopy* [http://wwwn.cdc.gov/dls/ila/documents/eqa_afb.pdf]

5. *Guidance on regulations for the transport of infectious substances 2007–2008*. Geneva, World Health Organization, 2007 (WHO/CDS/EPR/2007.2) available at

http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2/en/index.html).

KEY MESSAGES

- Good-quality specimens are the cornerstone of good-quality TB diagnosis.
- The quality of samples received should be evaluated and recorded.
- Proper specimen collection procedures and containers, adequate specimen volumes and appropriate transport conditions can all affecting TB culture results.
- Packaging of infected specimens that are to be sent by surface or air mail must be carried out according to national biosafety guidelines or international rules.
- Correct labelling of specimens is critical for their identification.
- The number of specimens for TB case diagnosis has been reduced from 3 to 2 in countries with high workloads where microscopy is performed under successful quality assurance programmes.

Module 5: Review

Find out how much you have learned by answering these questions.

Describe the spot–morning–spot strategy

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What are the specifications for suitable containers for collection of sputum specimens?

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Describe the requirements for a properly labelled specimen

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What are the features of good-quality and poor-quality sputum specimens?

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Why and when is it necessary to add preservatives to specimens?

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