



## Module 10

### Quality Assurance of Sputum Microscopy

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<b>Purpose</b>	To provide you with an understanding of quality assurance and external quality assurance of AFB smear microscopy
<b>Prerequisite Modules</b>	Modules 1–9
<b>Learning Objectives</b>	<p>At the end of this module, you will be able to</p> <ul style="list-style-type: none"><li>▪ Describe the elements of Quality Assurance</li><li>▪ Explain why internal Quality Control is important to assessing laboratory performance</li><li>▪ Describe the three components of External Quality Assessment (EQA)</li><li>▪ Prepare for a supervisory visit</li><li>▪ Describe the process of sampling slides for blinded smear rechecking.</li></ul>
<b>Content Outline</b>	<ul style="list-style-type: none"><li>▪ What is Quality assurance?</li><li>▪ Quality Control in the TB laboratory</li><li>▪ EQA and why it is important</li><li>▪ EQA Components<ul style="list-style-type: none"><li>• Panel Testing</li><li>• On-Site Evaluation</li><li>• Blinded Rechecking</li></ul></li></ul>
<b>Handout and Exercises</b>	None
<b>Appendix</b>	None

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## Module 10: Quality Assurance of Sputum Microscopy

For many countries with a high burden of TB, direct smear microscopy remains the most cost-effective tool for the laboratory diagnosis of patients with infectious TB (smear-positive pulmonary disease). However, if the laboratory diagnosis is unreliable, then patients with infectious TB may not be diagnosed, resulting in ongoing transmission of disease in the community and more severe disease in the individual. Alternatively, patients without TB may be treated unnecessarily. Therefore, quality assurance of AFB sputum smear microscopy is essential.

### WHAT IS QUALITY ASSURANCE?

Accuracy and reliability of laboratory testing are critical to the success of TB control programs. All parts of the testing system must be monitored to ensure the quality of the overall process, to detect and reduce errors, and to improve consistency between testing sites. To ensure reliability and to reduce errors, a quality system must address all parts of laboratory testing.

Quality Assurance (QA) is a system designed to improve the reliability and efficiency of laboratory services. WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) have defined several components for a quality assurance program for AFB smear microscopy:

- **Quality Control (QC):** A systematic internal monitoring of work practices, technical procedures, equipment, and materials including quality of stains.
- **External Quality Assessment (EQA):** A process to assess laboratory performance. EQA includes onsite evaluation of laboratories, panel tests, and blinded smear rechecking.
- **Quality Improvement (QI):** A process by which the components of smear microscopy diagnostic services are analyzed with the aim to identify and permanently correct any deficiencies. Data collection, data analysis, and creative problem solving are skills used in this process.

### QUALITY CONTROL IN THE TB LABORATORY

QC helps to ensure that the results produced by a laboratory are accurate, reliable, and reproducible. The QC program should be performed regularly, and to be effective, the process must be practical and readily included in standard laboratory reporting practices. All laboratory technicians are responsible for performing, recording, and reporting results of QC.

Many components of QC are either performed in conjunction with routine testing or as part of the regular management of the laboratory.

For laboratories performing sputum smear microscopy, QC is usually divided into the following:

- Laboratory arrangement and administration
- Laboratory equipment
- Specimens and request forms
- Reagents and stains
- Staining and smear examination
- Reporting and administration

#### **Laboratory arrangement and administration**

- Ensure that doors into the laboratory are always closed. Work areas, equipment, and supplies should be arranged for logical and efficient workflow.
- Work areas should be kept free of dust. Benches should be cleaned at least daily with an appropriate disinfectant.
- Use laboratory procedures that comply with NTP guidelines
- Every procedure performed in the laboratory must be performed as written.
- The procedures must be kept in the laboratory and be readily available.
- Any changes to procedures must be dated and initialed by the laboratory supervisor.
- Staff should have appropriate training and have their performance monitored.

#### **Laboratory equipment**

- The operating manual and cleaning instructions for all equipment must be readily available.
- Dated service records must be kept for all equipment.
- Microscope and balance must be monitored regularly to ensure consistent performance.

#### **Specimens and request forms**

- Perform microscopy only upon written request of authorized persons. Do not allow oral requests without a follow-up completed request form.
- Insist on adequately completed request forms and proper labeling of specimens. This ensures positive identification of patients.
- Reject specimens that cannot be properly identified, leaking, or in broken containers, request a repeat specimen.
- Record the date specimens arrive in the laboratory. Document on the request form any delays in the delivery of specimens to the laboratory.
- Evaluate the quality of sputum specimens. Record and monitor the number of salivary specimens received by the laboratory.
- Keep laboratory request forms separate from specimens. Forms that have been contaminated during transportation or otherwise by specimens should be discarded after autoclaving, burning, or burying. Accurately make duplicate form from the original form before discarding.

### **Staining Reagents**

- All staining reagents should be labeled with the name, date of preparation, and date first opened.
- If staining reagents are prepared in another laboratory, indicate date received.
- Any material found to be unsatisfactory should be recorded as such. Remove this material from the laboratory immediately so it is not used.
- Limit stocks to six months' supply. Rotate stock to ensure that oldest material is used first.

### **Staining and smear examination**

- Filter carbol fuchsin regularly.
  - Do not stain more than twelve slides at a time.
  - Include positive and negative controls at least weekly.
  - Read control slides before patient smears.
  - Unacceptable control slides include the following:
    - positive control is not stained red
    - negative control remains red after decolorization
    - background is not properly decolorized.
- Resolve any problems with control smears before reporting patient smears. Some problems may require repeating patient smears in a failed staining batch.

### **Recording and Reporting**

- Send microscopy results out as soon as they are available, preferably within 24 hours after the sputum specimen is received. Monitor any delays or turn around time in delivery on the report form.
- Analyze microscopy results on a monthly basis to detect changes which may indicate a problem.
- All microscopy results must be recorded in standard format in laboratory register.
- All records should be retained for at least two years.

## **EXTERNAL QUALITY ASSESSMENT (EQA) AND WHY IT IS IMPORTANT**

The intent of EQA is to help laboratories identify errors and improve practices for better performance. Effective EQA is a collaboration of laboratories at every level.

EQA does not identify individual slide errors nor validate individual patient diagnoses. Involvement in an EQA activity should not be seen as a threat, but rather as an opportunity to strengthen skills. Most laboratory technicians want to provide accurate testing. Good performance in EQA activities reassures them that their results are contributing to TB diagnosis and control.

Three methods can be used to evaluate laboratory performance:

- Onsite evaluation
- Panel testing
- Blinded smear rechecking

## **Onsite evaluation**

As part of an EQA program, laboratories at every level are visited and evaluated.

Every laboratory performing sputum smear microscopy should receive an onsite evaluation by an experienced laboratory technician at least once a year. These visits allow the worker to be observed under actual working conditions. The condition of equipment, laboratory safety, and the adequacy of supplies are also assessed. During these visits, the technician observes the process for specimen collection, smearing, staining, reading, recording, and reporting. Stained smears can be reviewed during the visit. When problems are detected, solutions can be suggested and potentially implemented immediately.

It is the responsibility of the peripheral laboratory to assist the visiting supervisory staff. The laboratory should make records available, demonstrate routine performance, and provide information. The laboratory should let clinic staff know about scheduled visits so that patients won't have to wait too long for results.

The visiting supervisory staff uses a standard checklist of measures that follow country guidelines for AFB microscopy. These visits provide an opportunity to learn from a colleague about standards, techniques, and methods. Ways to improve performance can be discussed.

DOTS require a quarterly visit by a district supervisor. These visits provide an opportunity for basic supervision including assessment of laboratory supplies, basic procedures, and performance of internal QC. District supervisors need to be sure that a functional microscope is available. The supervisor can collect slides for rechecking, deliver slides for panel testing, or deliver results of EQA activities.

## **Panel testing**

This is a countrywide system for sending stained and/or unstained slides from the central laboratory to the peripheral sites for reading and interpretation. A panel test (PT) exercise usually involves sending a PT with an identical composition (of negatives and positives) to many laboratories at the same time. It is useful as a first measure of the current level of laboratory performance, as well as to determine critical priorities for expanding EQA. It measures the ability of a technician to stain and/or read smears but does not assess routine laboratory performance.

It is the responsibility of the laboratory technicians to read the PT slides in the same way they read patient slides. They also need to take the same amount of time as for routine smears. If slides are sent to the laboratory, the results should not be shared with other technicians to compare results. Panel testing is an opportunity to compare performance with other laboratories, and reassures the technician that he or she can attain the same results as other laboratorians.

The number of slides included in a set must be sufficient to make the exercise meaningful, but not so many that they are a burden to the laboratory undertaking the panel test. Ten slides are generally considered an acceptable number; this represents about half the maximum number of slides that a technician can examine without losing quality. The PT must include slides with differing grades of positivity in order to evaluate the ability of technicians to correctly grade

positive slides. It is important to send the same batch to all laboratories so that the total performance of all participating laboratories can be compared.

Panel testing should be done at regular intervals when it serves as the primary method of EQA. In the absence of a rechecking program, it is recommended every 3-6 months and no less than once a year. Panel testing may also be used intermittently as a supplement to rechecking.

Unfortunately, no system for developing test panels and distributing them to peripheral sites is entirely without problems; these may include:

- Technical difficulties in preparing individual slides
- Errors in the initial reading of a smear at the reference laboratory
- Incorrect recording of expected results
- Fading of stained smears during transport to peripheral sites

Any system for PT must include a mechanism to resolve discrepant results. This may require returning slides to the reference laboratory for rereading or sending a technician from the reference laboratory to the peripheral laboratory for onsite evaluation and rereading of PT slides with the peripheral laboratory technician.

### **Blinded slide rechecking**

Blinded rechecking is the best method for evaluating performance and motivating staff to improve. Rechecking programs are intended to assess overall laboratory performance, not to confirm any individual patient's diagnosis.

This process occurs when controllers at a higher-level laboratory reread a sample of routine smears from the peripheral and intermediate laboratories. A countrywide program for blinded rechecking of slides at regular intervals should be the long-term goal for optimal EQA. However, it is the most resource-intensive and expensive EQA process. Resources required for a rechecking program depend upon the sample size and statistical validity.

Critical components of an accurate and practical rechecking system include:

- A sufficient number of randomly selected slides to represent work performed
- Slides blinded from their initial test results (the supervising laboratory, called the controller, should do this)
- The inclusion of minor errors, representing false positive or false negative interpretations of 1-9 AFB/100 fields, with major errors (the smaller sample size aids implementation and sustainability of rechecking programs)
- A system to provide prompt, continual feedback and improvements to the laboratories that are supervised

Discrepant results must be resolved by a second controller.

Rechecking program results should include a true sample of routine laboratory performance. Therefore, the sample collected must be random and representative of all the smears read by the technicians in the laboratory.

### Technician's responsibilities are:

1. **Store slides in a way that allows retrieval of every slide identified for the rechecking sample.** They must be stored in the slide boxes in the same order as they are listed in the laboratory register. Two blank spaces should be left behind the first slide from a patient with suspected TB. This allows the second and third slides to be added after they are read. This process will keep the results consistent with the laboratory register.
2. **Label slides in a manner consistent with the laboratory register** to ensure that the correct slide can be matched to the result. The labeling must be legible. The result of the smear examination must not appear on the slide.
3. Prior to placing slides in the storage boxes, **allow excess oil to drain off the slides and soak the oil by using paper** (oil can be removed from smears by either gently placing face down on toilet tissue or wrapping in the tissue paper and leaving overnight). Store slides in boxes that allow the immersion oil to drip off. Slides should not touch each other (e.g., do not stack or press slides together).
4. Always **store slides in closed boxes away from direct sunlight.** It is not necessary to use xylene to remove oil from the slides.

Rechecking requires motivated and well-trained staff. They must collect slides in a manner to ensure that a random sample is obtained. To avoid bias, the technician in the peripheral laboratory must never perform the sampling. In many countries the supervisor will collect the sample during the quarterly visit.

To eliminate bias, slides are selected using the laboratory register. This ensures that the technicians keep all slides regardless of result or quality. Slides must not be selected from the slide box.

Slides are collected from the entire set of slides whether the result was positive or negative. Following this approach, four quarterly collections (one year's worth) will produce a sufficient sample size for a statistically precise conclusion.

The supervisor identifies which slides are to be collected on the collection form. The technicians may then collect the slides from the boxes. Technicians should be able to readily retrieve all of the slides. If a slide is missing, the next slide as identified in the laboratory register should be substituted regardless of its result.

## Key messages



- Everybody is responsible for ensuring that laboratory results are accurate and reliable.
- To demonstrate and maintain high-quality results, a laboratory's performance needs to be monitored by the following regular QA activities:
  - Internal Quality Control (QC)
  - External Quality Assessment (EQA)
- QC is the process of effective and systematic internal monitoring of routine laboratory work.
- EQA is the systematic and independent assessment of laboratory performance.



## Module Review: Module 10

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

**What are the components of Quality Assurance?**

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**What is Quality Control?**

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**What is blinded slide rechecking?**

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**For blinded slide rechecking, how does the technician store the slides?**

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**What should you do to make sure that the ZN reagents are working properly?**

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