

Component 1: Laboratory Evaluation–Acid-Fast Bacilli Microbiology (AFB) Tool

Why the laboratory component of tool?

Laboratories form the foundation for diagnosing TB. A well functioning laboratory plays a crucial role in TB control. By responding quickly and providing quality service, the laboratory will enable early diagnosis, hence decreasing spread, ensuring appropriate treatment, and minimizing possible complications, including death (4).

Who should do this evaluation?

The evaluator needs to have a good understanding of acid-fast bacilli (AFB) smear microscopy and good laboratory practice (GLP). Methods assessed include specimen collection, smear preparation, acid-fast stain procedure, reading, and reporting. The evaluator must understand appropriate methods and ensure they are being followed. The use of standard laboratory reference texts is encouraged and should be observed (4,5). As always, guidelines from the World Health Organization (WHO) and National TB Program (NTP) in the host country and the refugees' country of origin should be used and followed.

Further explanation of tool

There are 3 parts:

1. Evaluation worksheet for evaluator to complete
2. Scoring guide that provide suggested scores, rating, and comments and recommendations section
3. Explanation worksheet that explains importance the of each item scored, including references

In completing the evaluation worksheet, the evaluators should watch three to five patients coming for various stages of treatment. The point values are assigned from the experience gained during the pilot testing and are only suggestions. If you, as the evaluator, believe the scoring should be different that is appropriate, your experience along with the tool should direct your scoring. Resulting scores (suggested or locally adapted) of sub-sections of this component would be important to share with the program because the component covers a broad range of topics and different sub-sections may have different levels of competencies. In addition, you may want to give partial points. Partial item point values should be explained and recommendations given in the **Comments and Recommendations** section after the score guide. If major deficiencies are observed in any sub-section during the evaluation, the evaluator should intervene to improve the program where needed.

Part 1: Laboratory Evaluation Worksheet

Site _____ Country _____ Date _____
dd/mm/yy

Write point score in last column if item passed. Write "0" if item failed. Write N/A if "not applicable" or N/E if "not evaluated."

Item No.	Point Value	Description (explanations of these items are on the next sheet)	Suggested Score
Record Keeping: Evaluator Observes			
1	4	Laboratory log/register is legible (dates of collection, quality of sputum sample [i.e., saliva], and results) <i>(Give 1 point each for legible dates, sputum quality, and smear results)</i>	
2	3	Slides labeled permanently with identification number or patient's name (if frosted, may use pencil)	
3	2	Lab uses one result form per patient and labels form with identification number or name <i>(Give 1 point each for each element)</i>	
4	2	Lab keeps smears for at least 3 months (one quarter) and stores them appropriately <i>(Give 1 point for each element)</i>	
<p>For item 5: Arbitrarily select 3 different pages of register (preferably from different months), average each page for percentage of samples that were saliva, then take average of the 3 pages. If ≤10% saliva, give 5 points; if 11%-20%, give 4 points; if 21%-30%, give 3 points; if otherwise, give 0 points. If specimen quality not indicated in register/log, ask separately, 3 different staff (if possible), what percent of accepted specimens are saliva and average the three percentages.</p> <p>Indicate the source of information of this worksheet (√ the box): <input type="checkbox"/> register <input type="checkbox"/> staff</p>			
5	5	Lab encourages sputum, not saliva	
Sputum Collection: Laboratory staff			
6	4	Adequate supply of clean sputum containers with wide mouth and screw top (enough for 3 months of patients)	
7	4	Tell patients <u>how</u> to give adequate sputum specimen (see Appendix A)	
8	4	Obtain at least one morning sputum <i>(Ask technician to explain sputum collection and timing; do not ask yes or no questions)</i>	
9	4	Collect sputum outside away from others <i>(Ask to see location to ensure away from others)</i>	
Smearing Method Measures: Laboratory staff			
10	2	Uses new, clean slides and have an adequate supply (enough for 3 months of patients)	
11	2	Uses clean applicator (stick, pipette, or wire loop [if loop, remove sputum from prior specimen before flame]) for smearing	
12	2	Air-dry slide <i>(Ask the laboratory technician about drying times, do not ask as yes or no question)</i>	
13	2	Heat-fix slide (with flame or slide warmer to 65-75°C) <i>(Ask the technician whether heat fixing is performed. If yes, ask him or her to explain what is done [do not ask as yes or no questions but ask for his/her explanation of process])</i>	
<p>For items 14-16: Arbitrarily select 10 different stored slides (preferably from different months and at least 5 different months). If 9-10 slides meet criteria below, give full points; if 7-8 slides meet criteria, give 4 points, if 5-6 slides meet criteria, give 3 points; if otherwise, give 0 points.</p>			
14	5	Uses appropriate smear size (1-2 cm x 2-3 cm)	
15	5	Uses appropriate evenness (even throughout)	
16	5	Uses appropriate thickness (once dry, able to read print through thick film at 4-5 cm)	

Part 1: Laboratory Evaluation Worksheet

Item No.	Point Value	Description (explanations of these items are on the next sheet)	Suggested Score
Stain/Reagent Preparation: Laboratory staff			
For item 17: Arbitrarily select 5 positive slides (preferably from different months and at least 3 different months). If all good, give 4 points; if 4 good, give 2 points; if 3 or less, give 0 points. NOTE: some slides for items 14-16, might be appropriate for item 17			
17	4	Stain the slides so AFB are not faded, even after 3 months (AFB stain red)	
18	2	Uses stains without precipitate (<i>probably need to assess by examining slides or filter stain</i>). Filtering stain before use can reduce precipitate.	
19	4	Uses reagent grade stains (commercial or prepared on site)	
20	1	Stores stains at room temperature and away from bright light or heat source (<i>Give ½ point for each element</i>)	
If stains prepared on site, answer items 21-25, then skip to 27			
21	1	Uses colorless or white crystal phenol and store it in refrigerator or cool area (<i>Give ½ point for each element</i>)	
22	2	Uses clean water (preferably distilled, <u>not</u> tap water)	
23	1	Uses balance to weigh 0.1 gram of stain powders	
24	4	Uses approved stain formulas	
25	1	Records dates of when stains prepared and determines expiration date	
If commercial stain used, answer items 26			
26	9	Uses commercial stains within expiration date	
27	2	Uses stains (commercial or prepared on site) within 12 months of opening/preparing (preferably within 6 months) (<i>look for record of date when the stain was prepared or opened [usually on bottle]</i>)	
Staining Procedure: Laboratory Staff			
28	5	Uses approved staining procedure	
29	3	Uses approved times	
30	2	Uses timer for staining procedure	
31	1	Stains individual slides to prevent cross contamination	
32	1	Changes solution in bottles used to stain slides every 2 weeks and records change (<i>Give ½ point for each element</i>)	
Microscopy and Reading: Laboratory Staff			
33	2	Has 100X magnification (plus 10X eye piece)	
34	1	Uses clean oil for slide and remove oil from slide before storing with absorbent paper (reduces risk of fungus) (<i>Give ½ point for each element</i>)	
35	4	Reads each slide for 5 minutes or 100-150 fields (<i>Ask technician amount of time needed to determine slide is negative; do not ask yes or no questions. If only observed for 2 minutes, at 100x oil immersion before reporting as negative, give 2 points</i>)	
36	2	Uses microscope in good working order, i.e., has mechanical stage that moves freely in both axes and well maintained	
37	1	Has microscope area with appropriate lighting (good ambient light on cloudy days) and sufficient seating space and without distraction or vibration (<i>Give ½ point for each element</i>)	
38	3	Uses positive control smear at least every week and after new reagent (<i>Give 2 points, if only every two weeks</i>)	
39	1	Uses negative control smear at least every week and after new reagent	
40	4	Performs external proficiency testing and results observed by evaluator (<i>If no results observed, give 0 points</i>)	
41	1	Processes >15 specimens each week (about 65 each month)	
42	2	Uses internationally accepted grading system for reporting results	
43	1	Performs second reading on all positive slides	
44	1	Reports results within 24 hours from specimen receipt	

Part 1: Laboratory Evaluation Worksheet

Site _____ Country _____ Date _____
dd/mm/yy

Item No.	Point Value	Description (explanations of these items are on the next sheet)	Suggested Score
Safety Measures: Laboratory			
<i>If specimen not centrifuged, answer 45 (Give 1 point) and skip to 47</i>			
45	1	Does not centrifuge specimen	
<i>If specimen centrifuged, answer item 46</i>			
46	1	Dilutes specimen to be centrifuged with equal volume of 5% Na hypochlorite	
47	2	Uses biological safety cabinet (BSC). If no BSC, performs smear processing in separate area with good ventilation (open window) <i>(Give 1 point for good ventilation)</i>	
48	2	Has well ventilated airflow through laboratory from less-contaminated to more-contaminated areas	
49	1.5	Has a hand washing facility with soap <i>(Without soap, give 1 point) (Ideally, evaluator will observe hand washing technique with brisk rubbing of one hand over the other)</i>	
50	4	Disposes of contaminated material appropriately (especially sputum and used smear-making materials) <i>(Need to see incinerator, area of burning or burial)</i>	
51	2	Cleans bench tops before and after smear preparation and immediately after any spills	
52	1	Restricts access to laboratory	
53	1	Stores flammable reagents in flammables storage cabinet	
54	1	Has standard operating procedures readily available in laboratory	
55	1	Uses standard operating procedures	
56	1	Performs administrative laboratory work in separate room from processing	
57	0.5	Provides continuing education training program for laboratory personnel	
58	0.25	Provides proof of training (degree, certification, license or credit)	
59	0.25	Provides annual chest X-ray (or tuberculin skin testing) for laboratory personnel	
Cultures: Laboratory			
60	4.5	Has access to cultures and drug sensitivity testing (DST), at least for relapsed or continued smear-positive patients	
A		Score Achieved (add score achieved for items 1-22)	
B		Value of All N/A OR N/E Responses	
C		Suggested Total Score Possible (65 points possible minus value in line B, above)	

Part 3: Laboratory Explanation Worksheet

Item No.	Explanation
Record Keeping	
1-4	Good laboratory practices to reduce risk of confusing slides of different patients are labeling and keeping slides and records for at least 3 months. National TB (NTP) Programs usually provide request forms; if not, the program is responsible for making its own. Slides must be stored appropriately, preferably in slide boxes. If slides boxes are not available, store with tissue paper between each slide.
5	Examine sputum for thick, mucoid quality, pieces of purulent material, blood, and volume (3-5 mL for a good specimen) (3). Clear saliva and nasal discharge are not suitable—request repeat specimen, if possible (but even saliva can yield positive result).
Sputum Collection	
6	The use of dirty sputum containers increases risk of artifact on smear. A wide-mouth opening and screw-cap container will minimize risk of contaminating outside and top, if leakage occurs.
7-8	Three early mornings sputum samples give highest yield of obtaining a positive smear. For practical reasons, this might not be feasible or followed by NTP guidelines. Because of laboratory workload in countries where external quality assurance exists, two sputum samples (one morning) are now endorsed by WHO (6). Instructions to patients may be given by lab or clinical staff; either way, specific instructions need to be given (see Appendix A)
9	Minimize risk to other persons; this procedure has greatest exposure risk to laboratory personnel.
Smearing Method Measures	
10	Reduce risk of artifact leading to interpreting result as positive finding (false-positive result).
11	With applicator, collect pieces of thick, purulent material.
12-13	Do not dry smear with sunlight or ultraviolet (UV) light. To keep smear on slide, heat fix air-dried smear to slide by passing slide with smear side up 2-3 times over flame for 2-3 seconds or by placing slide on 65°-75°C electric slide warmer for >2 hours. Do not leave the slide in an unprotected area where it could be damaged.
14-15	Smear size (1-2 cm x 2-3 cm), thickness (once dry, able to read print through thick film at 4-5 cm), and even across whole smear.
Stain/Reagent Preparation	
17	Color retention of white cells and AFB are indications of quality of stain/reagent.
18	Increase quality of stain and reduce risk of artifact—dirt or precipitate can lead to false-positive result. Probably need to assess by examining slides or filter stain.
19	Use only reagent grade stains.
20	Reagents must be stored at room temperature or precipitates may form, leading to false-positive result. Reagents must be kept in the dark and away from bright light to ensure they do not break down. Place them in a cabinet or in brown bottles away from bright light.
<i>If stains prepared on site, answer items 21-25, then skip to 27</i>	
21	Phenol for making fuchsin-phenol stain must be colorless or white crystal. Brown-tinted or liquid phenol is unacceptable. It must be kept in a cool, dark place (preferably a refrigerator) to remain white crystal. Phenol maintained at room temperature might degrade.
22	To reduce risk of artifact (environmental mycobacteria), use freshly prepared distilled/deionized water for staining reagents.
23	Need calibration weight to appropriately know and measure quantities for stain reagents.
24-25	Only approved formulas should be used (4). Good laboratory practice requires all stain reagents be dated at time of preparation. From preparation date determine expiration date.
<i>If commercial stain used, answer item 26</i>	
26	Chemical stock containers should be dated when received and when first opened.
27	Use prepared staining reagents within 6-12 months of preparation and commercial stains within 6-12 months of opening (but not after expiration date).

Part 3: Laboratory Explanation Worksheet

Item No.	Explanation
	Staining Procedure
28	Fuchsin-phenol must be applied, heated until steaming, then rinsed with tap water and drained; decolorized with acid-alcohol (25% sulfuric acid or 3% hydrochloric acid), then rinsed with tap water and drained; and methylene blue applied, then rinsed, drained, and dried at room temperature.
29	<p>Times may vary, one suggestion:</p> <ul style="list-style-type: none"> • Fuchsin-phenol for 5-10 minutes, then rinsed; • Acid-alcohol for 2-3 minutes, then rinsed; and • Methylene blue for 1 minute, then rinsed.
30	Too difficult to ensure appropriate times without a timer. Inappropriate times will lead to false answers.
31-32	Staining jars should not be used because of cross-contamination. Best practice is to stain slides individually. The staining bottles should be changed every 2 weeks and documented. Good laboratory practice (GLP) should include maintenance and cleaning of equipment.
	Microscopy and Reading
33	Use 100X magnification (plus 10X eye piece) to scan smear and count any AFB seen.
34	Oil must be clean, clear, and low in viscosity (not wood oil) to ensure optimum optical conditions. Oil must be wiped off objective lens with lens or fine tissue paper at the end of each working day. Remove oil from slides with tissue paper before storing to reduce risk of artifact and fungus.
35	At 100X magnification, use side-to-side or up-and-down sweeps of smear, taking care not to scan the same area twice. Observe 100-150 fields or for 5 minutes before calling a smear negative for AFB.
36	To systematically observe slide (side-to-side or up-and-down pattern), stage must operate in both axes. To correctly read slides, microscope needs to be clean of dust that might interfere with identification of bacilli or give erroneous positive results. Use of known positive and negative controls can assist in determining quality of microscope. After cleaning, cover microscope with vinyl or cotton cloth and store in secure place free from moisture and dust. Keep microscope and slides away from dust and dirt to increase life of microscope and reduce artifact.
37	Microscope area should have appropriate lighting and seating. Comfortable seating aids the technician's attention span. Microscope area free from distractions or vibrations allows for greater attention span of technician and more accurate observation of slide.
38-39	Use a positive control smear containing AFB and a negative control smear containing no AFB stained at least once a week, daily recommended. However, each new stain solution must be tested with a positive control and negative control smear before staining of patients' smears.
40	National or International Proficiency Testing Programs provide clinical specimens or slides to be tested by other laboratories to determine the tested laboratory's ability to give an accurate report. If a laboratory has established such a link, it indicates the potential for a high degree of proficiency for the laboratory personnel. Results of proficiency testing must be seen.
41	Laboratories preparing and observing >15 AFB smears per week or 65 AFB smears per month are better able to maintain proficiency in this process (7,8). However, each technician should not examine >25 smears per day.

Part 3: Laboratory Explanation Worksheet

Item No.	Explanation
42	<p>A reporting scheme of AFB found must be used. One scheme:</p> <ul style="list-style-type: none"> • 0 AFB/100 fields: negative • 1-9 AFB/100 fields: actual number of AFB seen on whole slide • 10-99 AFB/100 fields: 1+ • 1-10 AFB/field in 50 fields: 2+ • >10 AFB/field in 20 fields: 3+ <p>With revised WHO case definition (6), the presence of at least one acid fast bacilli (AFB+) in at least one sputum sample is a sputum smear-positive pulmonary TB case.</p>
43	<p>For good quality assurance, a sample of previously examined slides should be rechecked in a blinded fashion. In addition, recheck positive slides for confirmation should be done. See External Quality Assessment for AFB Smear Microscopy (http://wwwn.cdc.gov/dls/ila/documents/eqa_afb.pdf).</p>
44	<p>For good responsiveness to the needs of physicians and patients (and to minimize exposure), test results should be available within 24 hours of specimen receipt.</p>
Safety Measures	
45	<p>Centrifuging can create infectious aerosols. Do not centrifuge unless appropriate safety precautions are in place.</p>
46	<p>If centrifugation takes place, centrifuge must have an internal cover plus cover for chamber. Dilute specimen with equal volume of 5% Na hypochlorite, place in container with leak-proof cover, and label with patient name or number. Centrifuge at >3000Xg (RCF). Sediment from specimens processed for isolation by culture may be used. Centrifugation slows turnaround time for clinician receiving results and not always worthwhile.</p>
47	<p>Although a Biological Safety Cabinet (BSC) is not required, it is recommended. Simple-to-make cabinets requiring low-level sophistication and materials can be made but proof of their functionality needs assessing.</p>
48	<p>To minimize risk for exposure, ensure good ventilation—wind or fan is blowing over the shoulder and not in direction of others in the area.</p>
49	<p>Good laboratory practices (GLP) is to have hand washing station and to wash hands frequently with soap and water before and after every procedure.</p>
50	<p>Dispose of contaminated material (especially used sputum cups, applicators, and slides) in accordance with standard biosafety procedures; this includes burning (incineration), burying, or autoclaving.</p>
51	<p>GLP includes cleaning of benches and equipment. Use phenolic agents or bleach solution for disinfectant for spills and before and after making smears (bleach solution is good for blood but less effective than phenolic agents against TB). To ensure greater ease with cleaning the benches and reduce risk of trapping infectious materials, it should be a continuous surface.</p>
52	<p>To minimize risk for exposure, access to laboratory should be restricted and the door closed at all times.</p>
53	<p>Flammables (alcohols and organic solvents), strong acids, and strong bases should be stored in a flammables storage cabinet to minimize risk of spills that may cause fires or injuries.</p>

Part 3: Laboratory Explanation Worksheet

Item No.	Explanation
54-55	For a well-functioning laboratory, standard operating procedures (SOP) for procedures used must be written, used, and readily available. Examples can be found on the Internet (Available at: http://www.epa.gov/quality/qs-docs/g6-final.pdf or http://www.fao.org/docrep/W7295E/w7295e04.htm)
56	For a well-functioning laboratory, reduce risk of exposure to infectious materials.
57-58	For a well-functioning laboratory, ensure that professionals staff the lab and that they maintain professional growth.
59	For well functioning laboratory, assess potential of TB disease (although exposure via other aspects of person's life cannot be ruled out in many parts of the world). Aerosols containing <i>Mycobacterium tuberculosis</i> may be produced when handling leaking specimens, opening sample containers, and preparing smears. Ensure that sputum collection occurs away from others.
	Culture
60	Although WHO case definition does not require culture and drug sensitivity testing (DST), in this world of increasing drug resistance this ability should be sought out for patients who are being retreated. Many NTPs have the ability to provide DST, especially for cases with suspicion of drug resistance.