Appendix C

Examination of Sputum for Acid-Fast Bacilli

Sputum Collection

A. General

1. For the visa medical examination, all applicants with abnormal chest x-rays compatible with tuberculosis (TB) must have 3 sputum specimens examined for acid-fast bacilli.

2. Sputum specimens may be collected in the panel physician's office or at a laboratory facility. In either case, the area where the sputum is collected should be well ventilated and precautions should be taken to ensure that health care workers and others are not exposed to infectious aerosols and materials. Contaminated materials should be disposed of in accordance with standard biosafety procedures.

3. Specimens must be obtained under the direct supervision of a health care worker (i.e., specimens are not to be collected at home and brought to the physician's office or laboratory).

B. Procedure for Sputum Collection

1. Sputum must be collected in clean containers that are free from paraffin and other waxes or oils. Specimen containers should have an opening at least 2 cm across and should have at least a 20 cc capacity. The container should be clear so the specimen can be visualized without opening the container, and it must have a leakproof closure.

2. Sputum collection containers should be labeled with the name or identifying number of the applicant. The label should be on the side of the container rather than on the lid.

3. Instructions for the patient/applicant:

   (a) Sputum should be collected in the morning, before the patient has eaten.

(b) Have the person rinse his or her mouth with water before starting to collect the specimen to remove contamination such as food particles and bacteria. Patients with postnasal discharge should clear these passages before beginning sputum collection.

4. Instruct the patient as follows:

(a) Cough deeply. The lungs are like a sponge, containing moisture as well as air. Try to squeeze some of this moisture out of the lungs by coughing. Saliva from the mouth or mucous from the nasal or throat areas are not acceptable.

(b) Take a deep breath, hold your breath for a few seconds, and then expel the air slowly. Do this twice. The third time, inhale deeply, hold your breath, and then forcefully blow the air out through your mouth. The fourth time, inhale deeply and cough.

(c) Hold the sputum container to your lower lip and gently release the specimen from your mouth into the container. After coughing, clear your throat to avoid swallowing the specimen.

5. The person supervising the sputum collection may rap gently and firmly on the applicant's back to help induce coughing and sputum production.

6. Patients should be instructed to continue coughing until at least 5 ml of specimen has been obtained. Patients may need to rest in between coughing to avoid dizziness.

7. The person assisting with collection should inspect the specimen to ensure that it contains sputum and not saliva. Sputum is frequently thick and mucoid, but it may be fluid with fine chunks of dead tissue that show up like solid flakes. The color may be dull white or dull light green. Bloody specimens will be red or brown. **Saliva is thin and nearly clear and is not an acceptable specimen.**

8. The specimen container should be sealed and the label verified as correct before being transported to the laboratory.

Note: Children under six years old and disabled adults may not be able to follow these instructions. It is acceptable to take a throat swab if sputum cannot be collected from a patient using these instructions, but the use of a throat swab specimen must be marked on the medical report form.

Instructions for Acid-Fast Microscopy

Slide Preparation

A. New, clean glass slides should be prepared. An identification number should be marked on the slide. Slides should not be reused for acid-fast microscopy.

B. Use a wire loop, an applicator stick, or a small disposable pipette to transfer a small droplet of sputum from the collection container to the slide. Attempt to collect pieces of the light-colored dead tissue that are sometimes found in sputum. Spread the droplet evenly over an area of about 1 x 2 cm.

C. Dry the smears without heat (air dry) and then fix the dried smears to the slides with heat by holding each one, with the smear side up, over the flame of a burner for 2 or 3 seconds. When heat fixing the slide, tilt it slightly upward, away from the hands, to deflect the heat away from the fingers. **Do not dry the slides with heat, sunlight, or ultraviolet light, or leave them in an unprotected area where they may be damaged.**

Ziehl-Neelsen Acid-Fast Stain Procedure

Reagents

A. Fuchsin-phenol: Solution A - Dissolve 0.3 g. basic fuchsin in 10 ml 90% to 95% ethanol or methylated spirits. Solution B - Mix 5 g phenol crystals in 95 ml distilled water. Mix solution A with 90 ml of solution B. NOTE: Use colorless phenol crystals only. Brown-tinted phenol or phenol that is liquid at room temperature is unacceptable.

B. Acid-alcohol: Add 3 ml concentrated hydrochloric acid to 97 ml 90% to 95% ethanol or methylated spirits.

C. Methylene blue: Dissolve 0.3 g methylene blue chloride in 100 ml distilled water.

Note: Consult standard laboratory references for further information on reagent preparation.

Procedures

A. Check to ensure that the identification marked on each slide is the same as that on the laboratory report form.

B. Stain a smear that is known to contain acid-fast bacilli (AFB) at least once a week. This is the AFB positive quality control smear. Also, always test a new stain solution with a positive control smear before staining the applicant's smears.

C. Place the slides on the staining rack and cover each smear with a 2 x 3 cm piece of absorbent paper. Add enough fuchsin-phenol solution to cover the paper and have some stain solution remain over the paper, about 5 drops. Do not use staining jars or pans for staining or rinses. AFB could be transferred from positive to negative smears.

D. Apply heat (with flame or electric slide warmer) to the underside of the slides until steam is seen rising from them. Do not boil the stain or allow it to dry. Add more stain solution if needed.

E. After 5 minutes of staining, remove the paper with forceps.

F. Rinse with tap water, then drain off the excess water.

G. Flood the smear with acid alcohol to destain for 2 minutes.

H. Rinse with tap water and drain. Destain again with acid-alcohol if the smear remains pink, then proceed to the next step.

I. Flood the smear with the methylene blue solution and counterstain for 1 to 2 minutes.

J. Rinse, drain, and dry at room temperature.

Reading and reporting the smears

A. Observe the quality control smear first to determine the quality of the staining reagents and the procedure and to ensure a properly functioning microscope.

B. Put a drop of immersion oil on the smear either before or after the slide is positioned on the mechanical stage. Do not touch the smear with the tip of the dropper. Wipe oil from the objective lens after observing each smear.

C. Scan the smear at 800X to 1,000X magnification. Use a pattern of side-to-side or up-and-down sweeps of the smear. Take care not to scan the same area twice. Observe 300 fields before you report the smear as negative for AFB. If AFB are found, count until you have seen 20 or 30 AFB or you have reached the 300 field limit. Use the reporting scheme below:

Number of AFB found	Report as:	or Report as:
0	Negative for AFB	-
1-2/300 fields	Number AFB seen/smear	+
1-9/100 fields	Number/100 fields	1+
1-9/10 fields	Number/10 fields	2+
1-9/field	Number/field	3+
>9/field	>9/field	4+

The use of magnification of less than 800X should be clearly stated on the report. One or two AFB per smear is not considered positive.

Note: This document summarizes one method of staining and reading slides. Other methods are acceptable. For more information on other methods and for detailed safety information, the panel physician should review an appropriate manual on acid-fast microscopy.