Preparation of Acid-Fast Microscopy Smears for Proficiency Testing and Quality Control

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A method is presented for preparing smears for proficiency testing and quality control in acid-fast microscopy. The work was prompted by the increased demand for acid-fast bacilli positive smears with characteristic microscopic appearance and among-smear uniformity.

Since the passage of the Clinical Laboratories Improvement Act of 1967 (1), laboratories must be licensed if they receive interstate shipments of specimens for clinical examination. The requirements for licensing in mycobacteriology include participating in proficiency testing programs and using quality control smears for acid-fast microscopy. Proficiency test smears are used to evaluate the ability of microscopists to properly stain, scan, and report on acid-fast stained specimen smears. Quality control smears are not only used in determining the quality of the staining reagents but are useful in detecting microscope malfunctions and in providing a reference for microscopists who seldom see smears from clinical specimens which are positive for acid-fast bacilli (AFB). All smears used for any of these purposes must have a consistent concentration of AFB, the desired number of AFB per smear, and an authentic appearance of the AFB and the background material.

Although a liquid suspension of a mycobacterial culture could be used to prepare smears, most mycobacterial cultures contain many bacterial clumps and have few isolated cells. While some mycobacterial cultures can easily be suspended, frequently all the bacilli are not acid-fast, do not stain well, or have morphology that is not characteristic of the tubercle bacilli seen in sputum smears. Therefore, sputum containing AFB from a tuberculosis patient is the most desirable material for preparing both proficiency testing and quality control smears.

Digestion and decontamination methods for isolation of mycobacteria from sputa produce satisfactory material for quality control smears but may be undesirable for proficiency testing because AFB may not be killed when the smears are heat fixed. The few AFB that remain alive present a health hazard if they are rubbed from the smears. People who prepare proficiency test

smears prefer to use material containing only dead AFB because they cannot prevent the subsequent improper handling of smears in test laboratories.

All microorganisms, including AFB, are killed when sputum is mixed with an equal volume of 5% sodium hypochlorite (NaClO) (2). Household chlorine bleach usually contains 5 to 6% NaClO and can be used. Sputum is liquefied quickly by NaClO, and the solution is sterile within 10 min. This method of processing specimens for preparing smears is recommended for laboratories which limit examination to acid-fast microscopy, without culturing for isolation and identification (3, 4).

Smears prepared from sediment obtained by centrifuging an NaClO-treated specimen may contain outlines of crystals after staining and have only a few human cells because the rest are destroyed by the NaClO. Therefore, the background in these smears is not characteristic of sodium hydroxide (NaOH)-processed specimens. However, if the sediment of an AFB-negative, NaOH-processed specimen is mixed with that of an NaClO-processed specimen, smears which contain dead AFB that stain well by all acid-fast staining methods can be prepared. The resulting smears have AFB and background debris which appear as those seen in sputum smears from clinical specimens.

For preparation of these smears, collect AFB-positive and -negative sputa. Select the amount of material needed and freeze the remainder for later use. Place 10-ml portions in 50-ml screw-cap centrifuge tubes. Add 10 ml of 5% NaClO to the AFB-positive sputum, cap tightly, and gently agitate the mixture so that the sputum is liquefied; the inside of the tube should be covered by the decontaminating solution. Let the tube stand at room temperature for 10 min of decontamination. Add deionized water to the 50-ml mark

or to the neck of the tube, reseal the tube, and centrifuge at about $2,000 \times g$ for 15 min. Decant the liquid and resuspend the sediment in deionized water. Centrifuge and decant it to remove the residual NaClO which would leave crystal impressions in the dried smears. Resuspend this sediment in 1 ml of deionized water or aqueous 0.2% bovine albumin solution.

Add 10 ml of a 4% NaOH solution to the tube of AFB-negative sputum (or liquefy and decontaminate by any similar method). Gently agitate the liquid in the tube until the sputum is liquefied. After 15 min of decontamination, add deionized water to the 50-ml mark or to the neck of the tube. Reseal the tubes and centrifuge at about $2,000 \times g$ for 15 min. Decant the liquid and resuspend the sediment in 3 to 5 ml of deionized water or aqueous 0.2% bovine albumin solution.

Mix equal volumes of the AFB-positive and -negative material, and prepare several smears by spreading about 0.01 ml over a 2-cm² area on a glass microscope slide. Heat fix at 65 to 70°C for at least 2 h or over a flame as for other bacteriological smears. Stain the smears by an acid-fast procedure (3) and observe them to determine an average number of AFB per field. Then dilute the mixture with the appropriate amount of AFB-negative material to produce

the desired concentration of AFB. Test by staining to determine if the mixture is satisfactory. Add appropriate suspensions to the mixture if necessary and prepare final smears for use.

This method produces good quality smears for controls as well as proficiency testing. About 100 smears can be prepared from 1 ml of liquid preparation, and any unused material can be frozen until needed. Heat-fixed smears can be stored for at least a year.

The Tuberculosis-Parasitology Laboratory of The Georgia Department of Human Resources used a similar procedure for preparing smears in a successful proficiency testing program. The procedure described above should be useful for preparing satisfactory smears for proficiency testing and quality control in acid-fast microscopy.

LITERATURE CITED

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