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High Sensitivity and Specificity of Acid-Fast Microscopy for Diagnosis of Pulmonary Tuberculosis in an African Population with a High Prevalence of Human Immunodeficiency Virus▽

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Laboratories in low-income countries report that acid-fast microscopy is insensitive and nonspecific. We demonstrate that for a Ugandan population with high prevalences of tuberculosis and human immunodeficiency virus infection, acid-fast microscopy is highly sensitive (93.1%) and specific (100%) when performed by trained technologists in a carefully controlled manner using established techniques.

There were 9.2 million new cases of tuberculosis worldwide in 2006, with the highest rates of disease in African countries. Despite efforts to control tuberculosis and reduce the rate of infections, the lack of accurate laboratory diagnosis hinders these efforts. Although broth culture techniques and the introduction of nucleic acid-based tests can improve laboratory diagnosis (7), these procedures are not widely available in most low-income countries. Instead, attempts to confirm the clinical diagnosis most commonly rely upon acid-fast microscopy. Unfortunately, the majority of laboratories in low-income countries report that this test is insensitive and nonspecific. One explanation offered for the poor performance of microscopy is that many patients, particularly those with human immunodeficiency virus (HIV) infection, have paucibacillary disease. However, this explanation seems to be unlikely because most patients in low-income countries present with advanced disease. We believe that a more likely explanation for the problems with microscopy is that the preparation of specimens and the performance and interpretation of staining are controlled inadequately. We report the results of acid-fast microscopy for specimens obtained from a Ugandan population with high prevalences of tuberculosis and HIV infection.

Our institutions have a long-term, collaborative relationship with Mulago Hospital and National Tuberculosis and Leprosy Programme Clinic in Kampala, Uganda. As part of an evaluation of a direct nucleic acid amplification assay for mycobacteria (1, 13), sputa for the diagnosis of pulmonary tuberculosis were collected from a cohort of Ugandan patients with a high prevalence of HIV infection (38.2%). In addition to the processing of specimens for molecular diagnostic testing, culture and acid-fast microscopy analyses of all specimens were performed at the National Institutes of Health Clinical Center Mycobacteriology Laboratory. This approach allowed us to assess the sensitivity and specificity of acid-fast microscopy under well-controlled conditions.

Subjects with histories of ≥3 weeks but <6 months of cough or dyspnea were included if they were ≥18 years of age, produced an expectorated sputum specimen, and provided consent. Subjects receiving mycobacterial treatment were excluded. The Joint Clinical Research Centre Institutional Review Board; the University of California, San Francisco, Committee on Human Research; and the Uganda National Council for Science and Technology approved the protocol.

Specimens were liquefied using 10% dithiothreitol and concentrated by centrifugation at 3,000 × *g*, and the pellet was frozen at −20°C within 8 h of collection. Specimens were stored for up to 7 months (median duration of storage, 110 days; range, 7 to 195 days) and shipped on dry ice to the National Institutes of Health Clinical Center Mycobacteriology Laboratory.

Upon receipt in the lab, specimens were thawed, decontaminated for 15 min with an equal volume of 4% NaOH with 0.5% *N*-acetyl-L-cysteine, and centrifuged at 3,000 × *g* for 15 min at 4°C. Specimens were decanted following centrifugation, and the sediments were resuspended in the remaining supernatant. The resuspended pellets were stained using the auramine-rhodamine fluorochrome method and examined with a fluorescence microscope at a magnification of ×250, and the results were recorded according to the CDC guidelines (4). All smears were interpreted by the same technologist, and smear results were recorded before culture results were available. Culture was performed by inoculating the pellet onto a plate of Middlebrook 7H11 agar (0.3 ml; Remel) and into an MGIT broth (0.5 ml; Becton Dickinson) supplemented with 0.8 ml of a mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. 7H11 plates were incubated for 6 weeks at 37°C in 8 to 10% CO_2, and MGIT broths were incubated at 37°C in the BACTEC MGIT 960 system. The

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TABLE 1. Summary of fluorochrome acid-fast stain results

Fluorochrome stain result[a]	No. of samples with culture-grown colonies quantified as[b]:				
	None	Rare	Few	Moderate	Abundant
Negative	32	5	0	0	0
1+ Positive	0	7	4	2	1
2+ Positive	0	4	12	12	3
3+ Positive	0	1	4	7	10

[a] Microscopy results were quantified using the CDC guidelines (4) as follows: negative, no AFB or 1 or 2 per slide seen at a magnification of ×250; 1+ positive, 1 to 9 AFB/10 fields; 2+ positive, 1 to 9 AFB/field; and 3+ positive, ≥10 AFB/field.

[b] Colonies grown in culture were quantified as rare (growing in broth only or <20 colonies/plate), few (20 to 100 colonies/plate), moderate (101 to 300 colonies/plate), or abundant (>300 colonies/plate).

7H11 plates were examined when MGIT broths were flagged as positive and at the end of the incubation period. All acid-fast bacillus (AFB)-positive cultures were identified using *Mycobacterium tuberculosis* complex AccuProbe (Gen-Probe).

A total of 72 (69.2%) of 104 specimens (from 104 patients) were culture positive for *M. tuberculosis*. No other mycobacterial species were isolated. The fluorochrome stain was positive for 67 of 72 culture-positive specimens (sensitivity, 93.1%) and negative for all 32 culture-negative specimens (specificity, 100%). Although more than two-thirds of the specimens were culture positive, the high sensitivity of acid-fast microscopy observed in this study would decrease if one or more specimens were compromised by the prolonged storage used in this study. The correlation between smear and culture results is summarized in Table 1. The five false-negative smears were associated with the recovery of few organisms in culture: four specimens grew colonies only in the broth culture; one showed rare growth on the 7H11 plate. Further analysis of the smear-positive specimens revealed that large numbers of organisms were observed by microscopy, with one or more organisms observed per microscopic field for 53 (73.6%) of the 72 culture-positive specimens.

The high sensitivity and specificity of acid-fast microscopy reported for this study population of Ugandan patients contrast with data in previous reports from laboratories in low-income countries. We believe that three factors have an impact on the accuracy of acid-fast stains: specimen processing, the acid-fast staining method, and the implementation of a quality assurance program.

Specimen liquefaction and concentration improve acid-fast microscopy performance. Steingart et al. (10) reviewed 83 studies and demonstrated that the sensitivity of acid-fast microscopy improved significantly when specimens were subjected to liquefaction with bleach, dithiothreitol, or sodium hydroxide-*N*-acetyl-L-cysteine, followed by the concentration of the mycobacteria by overnight sedimentation or centrifugation. Centrifugation at 3,000 × *g* or greater to concentrate liquefied specimens produced optimal results.

Three acid-fast stains are used to detect mycobacteria: Ziehl-Neelsen carbolfuchsin stain, Kinyoun carbolfuchsin stain, and the auramine O or auramine-rhodamine fluorochrome stain. The Ziehl-Neelsen stain is used most commonly in low-income countries, while the Kinyoun and fluorochrome stains are used primarily in high-income countries. Of the carbolfuchsin stains, the Kinyoun stain is technically the easier to use; however, the performance of this stain was reported to be inferior to that of the Ziehl-Neelsen stain in a survey of 75 field clinics in Bangladesh (11), as well as in U.S. laboratories participating in a New York state proficiency program (8). Despite these observations, the fluorochrome stain is currently recommended for use in U.S. laboratories. Steingart et al. (9) reviewed 45 relevant studies and concluded that fluorescent microscopy was more sensitive than and as specific as carbolfuchsin stains. The primary factors limiting more widespread use of fluorescent microscopy are the high cost of purchasing and maintaining equipment and the short life span of mercury vapor lamps. The use of light-emitting diodes as a light source is an attractive, inexpensive alternative to mercury vapor lamps (5).

The final aspect that has an impact on the accuracy of acid-fast microscopy is the quality of training that laboratory personnel receive and the on-going assessment of technical proficiency (12). Well-run laboratories have established quality assurance programs emphasizing the use of standardized methods, periodic staff retraining, routine evaluations of reading and reporting through the exchange of smears between peripheral and reference labs, and random sampling of slides from the district tuberculosis register (2, 3). The implementation of similar control measures in U.S. labs has significantly improved the performance characteristics of acid-fast microscopy (6). Unfortunately, many clinics and laboratories in low-income countries employ a staff with minimal training and may not have a program of systematic, periodic assessment of proficiency. Uganda has recently introduced a nationwide quality assurance program to effect better results, and improvements in test performance are anticipated.

In summary, we have demonstrated that acid-fast microscopy for a population with high prevalences of tuberculosis and HIV disease can be a sensitive and specific diagnostic tool. The technical performance of this test is significantly improved by the use of simple methods for decontaminating and concentrating specimens, the introduction of the fluorochrome stain where fluorescent microscopy is practical, and the implementation of systematic training and proficiency testing.

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We do not have an association that might bias this study.

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