Lab dx - microscopy misc

Factors Affecting the Clinical Value of Microscopy for Acid-Fast Bacilli

Benjamin A. Lipsky, JoAnn Gates, Fred C. Tenover, and James J. Plorde

From the Departments of Medicine and Laboratory Medicine, University of Washington School of Medicine; and the Seattle Veterans Administration Medical Center, Seattle, Washington

In order to assess the clinical value of microscopy for acid-fast bacilli (AFB), the results of 3,207 clinical specimens submitted for mycobacterial smear and culture were analyzed. Mycobacteria grew from 176 (5.5%) of the specimens, 95 (54%) of which were *Mycobacterium tuberculosis*. Although the overall sensitivity of the smear was low (33%), 65% of respiratory specimens yielding *M. tuberculosis* had positive AFB smears. Furthermore, 96% of patients with pulmonary tuberculosis from whom more than one specimen was processed had at least a single positive AFB smear. Smear sensitivity correlated well with quantitative growth; 89% of specimens yielding ≥50 colonies per slant were smear positive. Specificity of the AFB smear was high; 89% of smear-positive specimens had positive cultures. After the results from culture-negative patients known to have active tuberculosis were eliminated from the analysis, the specificity of a positive smear rose to 98.3%. When the results of all specimens from each patient were considered in toto, the AFB smear had a predictive value of ≥96%.

A definitive diagnosis of mycobacterial disease rests upon the recovery and subsequent identification of the causative organism from a patient's secretions, body fluids, or tissues. Currently employed cultural methods require extended periods for completion [1]. Consequently, the initial management of individuals suspected of having a communicable or life-threatening mycobacterial illness is frequently based on the results of microscopic examination of the submitted clinical specimens. Considerable controversy exists, however, regarding the predictive value of this procedure. Two studies published in 1975 [2, 3] reported that acid-fast bacilli (AFB) were seen on fluorescent smear in <25% of specimens from which mycobacteria were cultured. Moreover, half of all specimens demonstrating AFB microscopically were subsequently shown to be culture negative. A number of subsequent studies have found the AFB smear to be more helpful [4–14]. In an effort to help clarify the factors that influence the sensitivity and specificity of microscopy for AFB, we performed a detailed analysis of our experience at the Seattle Veterans Administration Medical Center (SVAMC) from 1977 through 1980 in conjunction with a review of the literature.

Received for publication July 5, 1983, and in revised form September 20, 1983.

Presented in part at the 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy, October 4, 1982, Miami Beach, Florida.

Please address requests for reprints to Dr. B. A. Lipsky, MCCU (136B), Seattle VAMC, 4435 Beacon Avenue South, Seattle, Washington 98108.

Materials and Methods

Mycobacteriologic techniques. All body fluids and tissue homogenates submitted for mycobacterial culture were checked initially for contamination with rapidly growing bacteria. If the results were negative, an aliquot of the body-fluid sediment or tissue homogenate was used to prepare smears and inoculate solid media as described below. The remaining material, including the fluid supernatants, was placed in Middlebrook's 7H9 broth (Difco Laboratories, Detroit, Mich.).

Contaminated specimens and all sputa and bronchial washings were treated for 15 min with equal volumes of 0.5% *N*-acetyl-L-cysteine containing 2% NaOH and 1.45% sodium citrate before centrifugation (1,800 *g* for 15 min). An aliquot of the sediment was used to prepare smears of ~1 × 2 cm. These were heat-fixed at 70 C for 2 hr, stained with 0.1% auramine O for 15 min, decolorized with 0.5% HCl (concentrated in 70% ethyl alcohol) for 2 min, then treated with 0.5% potassium permanganate. The slide was scanned for AFB at low power (100×) three times lengthwise and nine times widthwise, a total of 30 fields. Positive smears were confirmed with a high power

RM 5993

9. Strumpf IJ, Tsang AY, Sayre JW. Re-evaluation of sputum staining for the diagnosis of pulmonary tuberculosis. Am Rev Respir Dis 1979;**119**:599-602
10. Rickman TW, Moyer NP. Increased sensitivity of acid-fast smears. J Clin Microbiol 1980;**11**:618-20
11. Murray PR, Elmore C, Krogstad DJ. The acid-fast stain: a specific and predictive test for mycobacterial disease. Ann Intern Med 1980;**92**:512-3
12. Smithwick RW, Stratigos CB. Acid-fast microscopy on polycarbonate membrane filter sputum sediments. J Clin Microbiol 1981;**13**:1109-13
13. Sathianathan S, Khalil A. A simple diagnostic culture method for use in a tuberculosis control programme. Bull WHO 1981;**59**:919-21
14. Petersen KF, Urbanczik R. Mikroskopische und kulturelle Methoden für die Laboratoriums-diagnose der Tuberkulose. Kurzer historischer Überblick. Zentrabl Bakteriol Mikrobiol Hyg [A]1982;**251**:308-25
15. Vestal AL. Procedures for the isolation and identification of mycobacteria. Publication no. CDC-76-8230. Washington, DC: US Department of Health and Human Services, 1976:65-90
16. Toman K. Sensitivity, specificity and predictive value of diagnostic tests. Bull Int Union Tuberc 1979;**54**:275-6
17. Strumpf IJ, Tsany AY, Schork MA, Weg JG. The reliability of gastric smears by auramine-rhodamine staining technique for the diagnosis of tuberculosis. Am Rev Respir Dis 1976;**114**:971-6
18. Greenbaum M, Beyt BE Jr, Murray PR. The accuracy of diagnosing pulmonary tuberculosis at a teaching hospital. Am Rev Respir Dis 1980;**121**:477-81
19. Truant JP, Brett WA, Thomas W Jr. Fluorescense microscopy of tubercle bacilli stained with auramine and rhodamine. Henry Ford Hospital Medical Bulletin 1962; **10**:287-96
20. Narain R, Rao MSS, Chandrasekhar P, Pyarelal. Microscopy positive and microscopy negative cases of pulmonary tuberculosis. Am Rev Respir Dis 1971;**103**: 761-73
21. Runyon EH, Karlson AG, Kubica GP, Wayne LG, Sommers HM, McClatchy JK. Mycobacterium. In: Lennette EH, ed. Manual of clinical microbiology. 3rd ed. Washington, DC: American Society for Microbiology, 1980:155-64
22. Kalich R, Bennert G, Fieldler T, Kubin M, Mezensky L, Turzova M, Ulber H. Comparison of auramine-rhodamine B and acridine orange for staining of acid-fast bacteria. J Hyg Epidemiol Microbiol Immunol 1979; **23**:307-17
23. Warring FC Jr, Sutramongkole U. Nonculturable acid-fast forms in the sputum of patients with tuberculosis and chronic pulmonary disease. Am Rev Respir Dis 1970; **102**:714-24
24. Parrot R, Marchal G, Deshais B, Augier J, Gillet P. Les bacilles tuberculeux non cultivables vus en microscopic en fluorescence. Revue de Tuberculose et de Pneumologic 1971;**35**:197-210
25. Kvach JT, Veras JR. A fluorescent staining procedure for determining the viability of mycobacterial cells. Int J Lepr 1982;**50**:183-92
26. Hobby GL, Holman AP, Iseman MD, Jones JM. Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. Antimicrob Agents Chemother 1973;**4**:94-104
27. Wayne LG. Microbiology of tuberculosis. In: Fishman AP, ed. Pulmonary diseases and disorders. New York: McGraw Hill, 1981:1255-63
28. Krasnow I, Lawrence GW. Comparison of methods for tuberculosis bacteriology. Appl Microbiol 1969;**18**: 915-7
29. Sommers HM. The laboratory diagnosis of mycobacterial disease. In: Youmans GP, ed. Tuberculosis. Philadelphia: WB Saunders, 1979:404-13
30. Rothlauf MV, Brown GL, Blair EB. Isolation of mycobacteria from undecontaminated specimens with selective 7H10 medium. J Clin Microbiol 1981;**13**:76-9
31. Kubica GP, Gross WM, Hawkins JE, Sommers HM, Vestal AL, Wayne LG. Laboratory services for mycobacterial diseases. Am Rev Respir Dis 1975;**112**:773-87
32. Mitchison DA. Examination of sputum by smear and culture in case-finding. Bull Int Union Tuberc 1968;**41**: 139-47
33. Weinstein RA, Stamm WE, Anderson RL. Early detection of false-positive acid-fast smears. An epidemiological approach. Lancet 1975;**2**:173-4
34. Mitchison DA, Keyes AB, Edwards EA, Ayuma P, Byfield SP, Nunn AJ. Quality control in tuberculosis bacteriology. 2. The origin of isolated positive cultures from the sputum of patients in four studies of short course chemotherapy in Africa. Tubercle 1980;**61**:135-44
35. Aber VR, Allen BW, Mitchison DA, Ayuma P, Edwards EA, Keyes AB. Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 1980;**61**:123-33
36. MacGregor RR, Clark LW, Bass F. The significance of isolating low numbers of *Mycobacterium tuberculosis* in culture of sputum specimens. Chest 1975;**68**:518-23
37. Gruft, H. Evaluation of mycobacteriology laboratories: the acid-fast smear. Health Laboratory Sciences 1978; **15**:215-20
38. Tsukamura M. Clinical significance of casual isolation of acid-fast organisms from sputum of tuberculosis patients. Am Rev Respir Dis 1973;**108**:1429-30

negative specimens from patients not receiving treatment is unclear; they may represent MOTT of no clinical significance [23, 38]. Among the patients with genuine false-positive smears studied by Boyd and Marr [2], 81% had no roentgenographic or clinical evidence of tuberculosis. Likewise, investigations by Narain et al. [20] showed that the great majority of those with false-positive smears did not develop tuberculosis after prolonged followup. Our one patient with a positive sputum smear and negative culture has shown no evidence of mycobacterial disease in the three years since the specimen was obtained. We conclude that for a patient without a recognizable reason for a false-negative culture or without clinical or roentgenographic evidence of mycobacterial disease a single positive smear may be cautiously disregarded.

A negative AFB smear is more difficult to interpret than one that is positive. It is important to review the results of AFB smears on all specimens submitted from a given patient since, in our experience, more than half of the false-negative smears from patients with tuberculosis were from patients who had other smear-positive specimens. If a patient is suspected of having pulmonary tuberculosis, a series of two or more sputum specimens with negative smears make this diagnosis very unlikely. A negative smear is much less helpful in excluding nonpulmonary or nontuberculous mycobacterioses. Nevertheless, since patients with negative AFB smears of clinical specimens are unlikely to pose a risk of spreading infection to others, very useful information regarding the need for isolation of the patient is gained from the AFB smear.

Summary

The accuracy of the AFB smear may be markedly improved by adhering to certain guidelines. Laboratories interpreting these smears should employ experienced personnel, use appropriate equipment, and process adequate numbers of specimens to maintain their proficiency [1]. Clinicians should notify the laboratory of the method by which a specimen was collected and whether or not the patient had been receiving antituberculous therapy. A series of at least three (but not more than six) sequential sputum specimens should be obtained from patients with suspected pulmonary mycobacteriosis [24]. Nonpulmonary specimens (except voided urine and superficial biopsies) should be collected aseptically, so that digestion and decontamination procedures can be avoided. When needed, these procedures should be accomplished with the mildest agents consistent with avoiding overgrowth and with strict attention to timing. For direct smears, a small amount of the most caseous or purulent portion of a sputum specimen should be selected; this material, or a sputum homogenate, should be thinly smeared and carefully heat-fixed on a new glass slide and properly stained with filtered reagents. Special care must be taken to avoid cross-contaminating specimens. If a sample cannot be processed immediately, it should be refrigerated. The new selective growth media may help in laboratories where contaminated and falsely-negative cultures have been a problem. High-speed centrifuges and polycarbonate membrane filters may be useful for AFB smears on nonrespiratory specimens. The results of this carefully performed AFB smear should then be interpreted in conjunction with the available clinical information. The smear is most useful in evaluating patients suspected of having pulmonary tuberculosis. When a series of sputum smears is obtained from such a patient who has not been receiving antituberculous therapy, the combined results are remarkably helpful in establishing or excluding this disease.

References

1. Sommers HM, McClatchy JK, Monello JA. Laboratory diagnosis of the mycobacterioses. CUMITECH 16. Washington, DC: American Society for Microbiology, 1983.
2. Boyd JC, Marr JJ. Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. Ann Int Med 1975;**82**:489–92
3. Marraro RV, Rodgers EM, Roberts TH. The acid-fast smear: factor fiction? J Am Med Technol 1975;**37**:277–9
4. Fierer J, Merino R. Acid-fast smears and tuberculosis [letter]. Ann Intern Med 1975;**83**:430–1
5. Stottmeier KD. Acid-fast smears and tuberculosis [letter]. Ann Intern Med 1975;**83**:429–30
6. Roland RP. False-positive acid-fast smears [letter]. Lancet 1975;**2**:1063
7. Burdash NM, Manos JP, Ross D, Bannister ER. Evaluation of the acid-fast smear. J Clin Microbiol 1976;**4**: 190–1
8. Pollock HM, Wieman EJ. Smear results in the diagnosis of mycobacterioses using blue light fluorescence microscopy. J Clin Microbiol 1977;**5**:329–31

cultures is the use of overly harsh procedures for specimen decontamination. Designed to selectively destroy rapidly growing bacterial contaminants, they may, if not properly controlled, adversely affect the viability of mycobacteria, especially the nontuberculous species [16, 27–29]. In general, if <5% of a laboratory's mycobacterial cultures become overgrown with bacterial or fungal contaminants, the processing is probably overly harsh and may be inhibiting mycobacterial growth [27]. False-negative cultures may also result when inadequate decontaminating procedures allow overgrowth of the medium with nonmycobacterial organisms. The use of selective antibiotic-containing media may allow the reduction or elimination of decontaminating procedures, resulting in less specimen contamination and, also, fewer false-negative cultures [30]. On the other hand, defective culture media may fail to support growth of mycobacteria present in clinical specimens. Our experience confirms that at least one egg-base medium (e.g., Lowenstein-Jensen) should be used to maximize recovery of mycobacteria [1]. False-negative cultures may also occur if there are inordinate delays between specimen collection and processing that allow progressive dying-off of organisms [26, 31, 32]. If a specimen cannot be plated on the day it is collected, it should be stored at ≤4 C [31]. Last, false-negative cultures may result when specimens are not held for a full six to eight weeks. Some organisms, particularly drug-resistant strains, may require extended periods of incubation to produce visible growth.

When false-negative cultures are excluded, a residual number of genuine false-positive smears still remain. The diagnostic importance of these false-positive smears is directly related to the prevalence of active tuberculosis in the tested population. As the prevalence decreases, the ratio of true-positive to false-positive smears decreases, lowering the predictive value of a positive smear (Bayes' theorem) [2]. Nevertheless, even in areas of low disease prevalence, genuine false-positive AFB smears are uncommon in well-supervised laboratories. There was only one in our four-year study. The experience of Murray and co-workers has shown that the invocation of Bayes' theorem is never appropriate until a vigorous attempt to improve laboratory performance has failed to improve the specificity of the AFB smear [11]. The preventable causes of false-positive smears are multiple. Mycobacteria often contaminate the water or solutions used in the staining process [8, 21, 33]. Transfer of organisms from a specimen harboring mycobacteria to one that does not may occur via mucolytic agents, microscope immersion oil, specimen flakes, or wire loops [21, 34–36]. A series of studies conducted in laboratories in Africa and London revealed that ~1% of positive smears could be attributed to such organism transfer and another 1% to various clerical errors [35]. False-positive smears have also been reported to result from failure to properly filter and regularly replace reagents, from staining of artifacts present in the preparation, from retention of acid-fast stain by pleomorphic cell wall-deficient forms of nonmycobacterial genera, and from insufficient destaining of non-acid-fast organisms [3, 21]. Some studies have reported that auramine staining procedures produce a higher false-positive rate than do carbol fuchsin-based procedures [6], presumably because auramine may stain nonviable AFB or blood platelets [37]. This possibility should be considered when processing specimens from patients taking antituberculous drugs or specimens that are grossly bloody.

Careful quality control of laboratory procedures with immediate supervisory review of all positive AFB smears can markedly decrease the incidence of false-positive smears [11]. When these are caused by specimen contamination, they may be detected early by employing epidemiologic analyses, such as those described by Weinstein and colleagues [33] for suspicious situations. We attribute our extremely low incidence of false-positive smears in large part to our use of such measures.

Clinical interpretation of results of AFB smears. How, then, should results of AFB smears be interpreted by clinicians? If a specimen from a patient with a clinical illness compatible with tuberculosis and who is not receiving antituberculous drugs is positive by AFB smear, treatment may be started with the knowledge that cultures (in an appropriately supervised laboratory) will almost always be positive. Reviewing all specimens from a given patient will also help detect "false-positive smears," which, in fact, represent false-negative cultures. By this method, Fierer and Merino [4] were able to correct their rate of false-positive smears for two different studied populations from 22% to 11% and from 38% to 13%.

The cause of isolated smear-positive, culture-

Table 7. Comparison of published analyses of paired fluorescent acid-fast smears and cultures.

Reference	No. of specimens	Percentage of specimens					Smear sensitivity (%)	Smear specificity (%)	Overall correlation (%)
		Culture positive	True (+)	Absolute false (+)	Relative false (+)	Absolute false (−)			
2	4,570	2.6	45	0.7	55	78	22	99.3	97.3
3	1,983	2.9	58	0.5	42	76	24	99.5	97.3
4*	4,885†	NA	NA	0.5	22 (11)‡	NA	NA	NA	NA
	5,987†	NA	NA	2.7	38 (13)‡	NA	NA	NA	NA
5	13,433	4.1	88 (95.4)§	0.2 (0.07)§	12 (4.5)§	55	34	99.8	97.1
6	4,669†	0.9	48	0.50	52	52	48	99.5	99.0
7	6,199	3.7	93 (98.9)	0.1 (0.017)	7 (1.0)	57	43	99.9	97.8
8	6,906	5.8	90 (92.5)	0.4 (0.24)	10 (7.5)	50	50	99.8	98.3
9*	1,825†	3.2	88	0.3	12	22	78	99.7	98.9
	1,413†	3.3	83	0.4	17	49	51	99.6	NA
10‖	14,509	7.1	96	0.09	4	75	25	99.9	94.6
	9,727	11.2	82	1.1	18	60	41	98.9	92.4
	14,631	11.6	81 (98.9)	2.6 (0.12)	19 (1.1)	18	82	97.4	95.7
11	10,468	3.9	95.2 (98.8)	0.08 (0.02)	5 (1.3)	61	39	99.9	97.6
12*	916†	8.3	96 (97.7)#	0.2 (0.1)	5 (2.3)	45	55	99.8	96.1
			85 (96.2)**	1.0 (0.2)	15 (3.8)	41	59	99.1	95.7
			70 (93.9)††	3.0 (0.6)	31 (6.1)	25	75	97.0	95.2
13	10,026†	9.3	96.7	0.02	3.3	38	62	99.8	96.2
14	23,511	NA	NA	NA	10	NA	53	99.2	NA
Present series	3,207	5.5	89 (98.3)	0.23 (0.03)	10.7 (1.7)	67	33	99.9	96.3

NOTE. For definitions of + and −, see table 1; NA = not available.

* = Data are from two different populations.

† = Sputum specimens only.

‡ = Data calculated by patients.

§ = Corrected for patients with known tuberculosis or receiving antituberculosis drugs.

‖ = Specimens were centrifuged at different speeds (see text).

\# = Direct smear.

** = After centrifugation.

†† = Polycarbonate membrane filter method (see text).

tures—can dramatically decrease a laboratory's rate of false-negative smears [11].

Smear specificity. When discussing the specificity of a positive AFB smear, a distinction must be made between those instances where mycobacteria present in the specimen fail to grow in culture (i.e., false-negative cultures) and those where the specimen truly does not contain mycobacteria (i.e., false-positive smears). A review of the hospital charts of the patients in our study with culture-negative but smear-positive specimens revealed that six of the seven were known to have tuberculosis. Most of these patients had been receiving antituberculosis therapy. This is consistent with the experience of other investigators who have reported that 66%–95% of the specimens with "false-positive" smears came from patients receiving therapy for culture-proven tuberculosis [4, 7, 8, 10, 11, 23]. Cultures generally become negative within a few weeks of starting therapy, although patients can continue to cough up stainable bacilli for a period of months, even when treated with bactericidal medications [24]. Overall, about 10% of tuberculous patients receiving effective chemotherapy will have positive smears and negative cultures [1]. One suggested means of compensating for this problem is to increase from three to 10 per slide the number of AFB needed to consider a smear positive in patients receiving therapy [10]. Slides with three or fewer bacilli account for up to 85% of false-positive smears [20]. Techniques under development for microscopically differentiating between viable and nonviable mycobacteria should also help in this situation [25, 26].

Among patients who are not receiving chemotherapy, the most important cause of false-negative

Table 6. Quantitative comparison of smears for acid-fast bacilli (auramine) and culture for specimens growing *Mycobacterium tuberculosis*, 1977–1980.

Smear quantitation	No. of colonies on culture				Totals
	1–10	11–50	51–100	>100	
–	24 (7)*	3 (3)*	2 (1)*	1	30
±	3	1	. . .	2 (1)*	6
1+	2 (1)*	5 (1)*	2 (1)*	2	11
2+	. . .	2	1	5	8
3+	2	3	2	6	13
4+	. . .	. . .	2	20	22
Total no. (% smear positive)	31 (13)	14 (71)	9 (78)	36 (92)	90

NOTE. Excludes smear-positive, but culture-negative, specimens and specimens lacking culture quantitation. See text for definition of quantitation symbols.

* Figures in parentheses are number of nonrespiratory specimens in that category.

For both tuberculous and nontuberculous mycobacterioses, the efficiency of the concentration techniques employed directly affects the sensitivity of the AFB smear. The specific gravity of tubercle bacilli ranges from 1.07 to 0.79 [12], however, making centrifugal concentration of many specimens ineffective. In fact, one report documents instances where centrifugal concentration has resulted in a decrease rather than an increase in the smear-positivity rate [10]. In some circumstances this problem may be overcome by the use of higher relative centrifugal force in the concentration procedure. Investigators at the Oklahoma State Department of Health (Oklahoma City, Okla.) improved AFB smear sensitivity from 25.2% to 82.4% by increasing relative centrifugal force from 1250 *g* (standard procedure) to 3800 *g*. Unfortunately, such high-speed centrifugation requires expensive refrigerated centrifuges, rarely available in clinical microbiology laboratories and difficult to vent through a HEPA filter. If high-speed centrifugation is employed, care must be taken to use centrifuge tubes capable of tolerating the higher centrifugal force and sealed aerosol-free safety carriers. Filtration of specimens through polycarbonate membrane filters may prove a satisfactory alternative to concentration by high-speed centrifugation. By use of this technique, Smithwick and Stratigos [12] showed an increase in AFB smear positivity of 35% compared with that for centrifuged specimens and 45% compared with that for direct smears. Both of the above techniques may be of benefit where mycobacterial cultures cannot be prepared or to increase the sensitivity of AFB microscopy for specimens likely to have low concentrations of mycobacteria; they are probably not necessary in most cases of pulmonary tuberculosis.

Another factor influencing smear sensitivity is the staining technique employed. The auramine stain, because it allows rapid, low-magnification screening of the entire smear, is generally acknowledged to be more sensitive than either the Ziehl-Neelsen or Kinyoun procedures [1]. Several studies have found that 15%–18% of all culture-positive specimens have had smears that were positive by auramine stain but negative by Kinyoun or Ziehl-Neelsen techniques [6, 19, 20]. Although some have suggested that the increased sensitivity of the auramine stain is obtained at the cost of specificity, our own experience shows that this is not necessarily true. In fact, specimens with auramine-positive, Ziehl-Neelsen-negative smears have often come from patients with previously diagnosed tuberculosis, most of whom are receiving treatment (J. J. Plorde and G. C. Counts, unpublished observations). It has been suggested that fluorochrome dye may stain nonviable organisms more readily than carbol fuchsin [1].

Regardless of the particular stain used, AFB may be missed if overly thick smears are prepared, decolorization is too vigorous, or poorly contrasting counterstains are used [21]. Finally, it is clear that good training, experience, and supervision are important for reliable microscopic results [1, 18, 22]. The ability to microscopically detect small numbers of AFB depends largely on the alertness and persistence of the technician. Murray and coworkers have shown that careful quality control of laboratory procedures—with reexamination of negative smears from specimens with positive cul-

Table 4. Correlation between the results of smears for acid-fast bacilli (auramine) and those of mycobacterial culture on all specimens, Seattle Veterans Administration Medical Center, 1977 – 1980.

Result of culture	No. of smears that were			Totals
	Positive	Indeterminate	Negative	
Positive	58 (3)	8 (1)	110 (11)	176
Negative	7 (1)*	9 (2)*	3,015 (1,163)	3,031
Totals	65 (4)	17 (3)	3,125 (1,174)	3,207 (1,181)

NOTE. Numbers in parentheses are the number of nonrespiratory specimens in that category.
* Two specimens in each of these categories were from patients known, by other recent positive cultures, to have active tuberculosis.

evaluation of tuberculous patients is still debated. Several recent studies have evaluated the reliability of the acid-fast smear in predicting the presence or absence of viable mycobacteria in clinical specimens (table 7) [2–14]. A few of these studies have investigated some of the variables known to influence the sensitivity and specificity of this procedure, but none have comprehensively evaluated all of these factors and attempted to measure the impact of the results of smears on the clinical management of patients suspected of having mycobacterioses. Our data, in conjunction with an analysis of other published series, allow us to offer some guidance on these issues.

Smear sensitivity. The acid-fast smear is generally considered a relatively insensitive diagnostic procedure. In our laboratory only one-third of smears prepared from concentrates of culture-positive specimens revealed AFB. Other than in reference laboratories, however, it is not generally appreciated that smear sensitivity varies with both the type of specimen and the species of infecting mycobacterium. At SVAMC, AFB smears were positive for 65% of the respiratory specimens from which *M. tuberculosis* was grown. Moreover, of patients who had two or more culture-positive specimens submitted to the laboratory, 96% had at least one positive smear. This high positivity rate appears related to the large number of organisms generally found in specimens from patients with pulmonary tuberculosis, particularly in those with advanced disease [8, 9, 11, 12, 17]. In fact, half of the respiratory specimens growing *M. tuberculosis* in our laboratory had colony counts >50 per slant.

One facet of smear interpretation deserves special emphasis in this regard. Although smears demonstrating three or fewer bacilli per slide (designated indeterminate) are commonly considered equivalent to negative smears, such results should prompt a request for additional studies. In our laboratory, *M. tuberculosis* was isolated from almost three-fourths of such specimens.

In nonrespiratory and nontuberculous mycobacteriosis, both the colony counts and the smear positivity rates have been reported to be significantly lower than those in pulmonary tuberculosis [8, 9, 11, 12, 17, 18]. Among the 81 specimens submitted to our laboratory that grew MOTT, only one had a positive smear and two others had indeterminate results. Of the 69 patients from whom these specimens were obtained, however, only two had any evidence of mycobacterial disease; smears were positive from one of these patients and indeterminate from the other. The other patients were almost all elderly men with acute or chronic bronchopulmonary disease from whom a single sputum specimen grew a few colonies of MOTT. Although the smear is of less value in diagnosing nonrespiratory or nontuberculous mycobacterioses, the former is far less common than respiratory disease and the latter, neither rapidly progressive nor highly infectious. Thus, early diagnosis is less important for these diseases.

Table 5. Sensitivity of smears for acid-fast bacilli (auramine), by mycobacterial species, Seattle Veterans Administration Medical Center, 1977–1980.

Results of smear	No. (%) of cultures positive for*		Total no. (%)
	M. tuberculosis	MOTT†	
Positive	57 (60)	1 (1.2)	58 (33)
Indeterminate	6 (6)	2 (2.5)	8 (5)
Negative	32 (34)	78 (95)	110 (62)
Totals	95	81	176

NOTE. All specimens are from respiratory sources.
* Numbers in parentheses are percentages of culture-positive specimens with the indicated result on AFB smear.
† Mycobacteria other than *M. tuberculosis*.

Table 2. Specimens having smears for acid-fast bacilli (auramine) and mycobacterial culture, by source, Seattle Veterans Administration Medical Center, 1977–1980.

Source	No. of specimens (%)	No. of positive cultures (%)
Respiratory	2,026 (63.2)	161 (7.9)
Sputum	1,848	153
Bronchial washings	177	7
Gastric aspirate	1	1
Body fluids	892 (27.8)	9 (1.0)
Pleural fluid	71	2
Cerebrospinal fluid	752	7
Other fluids	69	0
Tissues	289 (9.0)	6 (2.5)
Bone marrow	48	0
Other tissues	241	6
Totals	3,207	176 (5.5)

(89%) of these had a positive smear. In contrast, only two (2.6%) of the 77 specimens with MOTT for which growth was quantitated had >50 colonies per slant; one of these two was smear positive. The relationship between the quantitative culture and stain results for *M. tuberculosis* is shown in table 6.

In *M. tuberculosis* infections, the sensitivity of the smear varied with the type of specimen. Among the 80 culture-positive respiratory specimens, 52 (65%) had positive AFB smears. Except for two spinal fluid specimens (29% smear-positivity rate) and three tissues (50% smear-positivity rate), no other culture-positive specimen revealed organisms on microscopic examination of the smear. The sputum specimens that yielded *M. tuberculosis* were from 36 patients; 28 (78%) of these patients had at least one positive AFB smear. Of the 25 patients from whom more than one sputum culture was submitted to the laboratory, 24 (96%) had at least one positive smear.

It is of interest that eight of 17 (47%) of the specimens with indeterminate smear results yielded positive cultures. When the results for six patients with false-negative cultures are removed from the total, the rate improves to eight of 11 (73%).

Smear specificity. The specificity of the AFB smear at the SVAMC was >99.9%. The relative rate of false-positives, a more useful reflection of the specificity, was 11% (seven of 65) for all specimens, and 25% (one of four) for nonrespiratory specimens (table 4). However, six of the seven "false-positive" smears were from patients who had proven active tuberculosis. Five were respiratory specimens; the sixth was a spinal fluid specimen from a patient who had five other positive spinal fluid cultures. At least three of these patients had been receiving antituberculous medications before the time of specimen collection. When these six specimens are excluded, the corrected false-positive rate for all specimens is 1.7% (one of 59). The only legitimately false-positive smear was a respiratory specimen with a low quantitative score (+). Early in this study period, positive auramine stains from 20 specimens that grew *M. tuberculosis* were overstained by the Ziehl-Neelsen method; 17 (85%) were also positive, but three were falsely negative. The two patients from whom these latter specimens were collected were receiving antituberculous therapy.

Predictive value. In our laboratory the predictive value of a positive smear was 89%; this increased to 98% when the data from patients with false-negative cultures were excluded. The predictive value of a negative smear (which includes those with indeterminate results) was 96%.

Discussion

Microscopy for acid-fast bacilli has been performed for more than a century, yet its place in the

Table 3. Positive mycobacterial cultures (*Mycobacterium tuberculosis* and mycobacteria other than *M. tuberculosis*, MOTT), by source, Seattle Veterans Administration Medical Center, 1977–1980.

Source	No. (%) of cultures positive for		Total no. (%)
	M. tuberculosis	MOTT*	
Respiratory	80 (84.2)	81 (100)	161 (91.5)
Sputum	76	77	153
Bronchial washings	3	4	7
Gastric aspirate	1	0	1
Body fluids	9 (9.5)	0	9 (5.1)
Pleural fluid	2	0	2
Cerebrospinal fluid	7	0	7
Other fluids	0	0	0
Tissues	6 (6.3)	0	6 (3.4)
Bone marrow	0	0	0
Other tissues	6	0	6
Totals	95 (54)	81 (46)	176 (100)

* Nonchromogen, 53 (*Avium* complex, 21); chromogenic, 26 (*Mycobacterium gordonae*, 3); rapid growers, 1 (*Mycobacterium fortuitum* complex, 1); *Mycobacterium bovis*, 1.

(630×) dry objective and verified by a senior technician. Organisms were quantitated by the following scale: 1–3 bacilli per slide, ±; 1–9 bacilli per 100 oil immersion fields (OIF), +; 1–9 bacilli per 10 OIF, 2+; 1–9 bacilli per OIF, 3+; >9 bacilli per OIF, 4+. A second aliquot of the sediment was used to inoculate two Lowenstein-Jensen (L-J) slants (Difco Laboratories) and a plate of Middlebrook's 7H11 medium. The remainder of the sediment was diluted 1:10 with sterile saline and spread on a second plate on 7H11 medium. All media were incubated at 35 C in 7.5% CO_2 and examined weekly for eight weeks. Isolated organisms were identified by use of the criteria of Vestal [15]. All organisms were processed sufficiently to determine whether they were *Mycobacterium tuberculosis*. Mycobacteria other than *M. tuberculosis* (MOTT) were speciated only if they grew in appreciable numbers (≥10 colonies per slant), were isolated from more than one specimen, or were judged of possible clinical importance.

SVAMC record review. The smear and culture results for all specimens submitted to the hospital mycobacteriology laboratory from January 1, 1977, through December 31, 1980, were reviewed by one of the authors (J.A.G.). The hospital charts of selected patients were reviewed for pertinent clinical details by two of the authors (B.A.L., J.J.P.).

Statistical analysis. The sensitivity of the AFB smear defines its capacity to correctly distinguish those specimens that will yield positive mycobacterial cultures. Specificity indicates the ability of the smear to properly identify specimens with negative mycobacterial cultures. Predictive value depends both on the sensitivity and specificity of the smear and on the prevalence of mycobacterial disease in the tested population; it defines the probability of either having or not having a positive mycobacterial culture among those specimens with, respectively, positive or negative AFB smears [16]. The methods and terminology for comparison of AFB smears and mycobacterial cultures are shown in table 1.

Table 1. Methods and terminology for comparison of smears for acid-fast bacilli and mycobacterial cultures.

	Result of smear		
Culture	Positive	Negative	Totals
Positive	A	B	T_1
Negative	C	D	T_2
Totals	T_3	T_4	N

NOTE. True (+) = A/T_3; absolute false (+) = C/T_2; relative false (+) = C/T_3; absolute false (−) = B/T_1; sensitivity = A/T_1; specificity = D/T_2; overall correlation = $(A + D)/N$; predictive value of a positive smear = A/T_3; predictive value of a negative smear = D/T_4.

Results

Laboratory data. During the four-year study period, the bacterial contamination rate of mycobacterial cultures varied from 4% to 7%. Analyzable smear results were available on 3,207 specimens submitted from 2,377 patients. As shown in table 2, nearly two-thirds of all specimens were from the respiratory tract. The types of specimens processed next most frequently were CSF (23%) and tissues (9.0%).

Mycobacteria were recovered from 176 (5.5%) of the 3,207 specimens. In 50% of these, growth occurred on both the 7H11 plates and L-J slants; 42% grew on L-J medium only, and 8% on 7H11 only. Ninety-five (54%) of the isolates were identified as *M. tuberculosis* and the remaining 81 (46%) as members of one of the MOTT species. In the great majority of instances in which there was growth on only one medium, MOTT organisms grew in very low numbers. All of the MOTT and 84% of the *M. tuberculosis* isolates were recovered from respiratory specimens (table 3). All but two of the positive cultures were from male patients.

The results of the AFB smears are shown in table 4. Culture-negative specimens were collected from 12 patients who had other recent cultures positive for *M. tuberculosis*. In six of these 12, the smears were positive; in the remaining six, the results were indeterminate (±). In the calculation of smear sensitivity and specificity, all indeterminate smear results were considered negative.

Smear sensitivity. Fifty-eight (33%) of the 176 culture-positive specimens were smear positive. As is apparent in table 5, AFB microscopy was much more sensitive at detecting specimens with *M. tuberculosis* (60% positivity rate) than it was at identifying MOTT (1.2% positivity rate). This discrepancy was likely related to the difference in the relative concentration of organisms in the two groups of clinical specimens. Of the 90 specimens yielding *M. tuberculosis* for which data were available, 45 (50%) had >50 colonies per slant; 40