Letters to the Editor

Is a Large Number of Sputum Specimens Necessary for the Bacteriological Diagnosis of Tuberculosis?

The recent increase in tuberculosis (TB) incidence (7) has further focused interest on efforts to design more effective diagnostic procedures to improve classical diagnostic methodologies for the disorder (1, 2, 5, 6). The recent paper by Nelson and coworkers (3), concerning the contribution of the number of specimens to the efficiency of TB diagnosis, prompted us to retrospectively review results of acid-fast bacillus (AFB) smears and cultures of patients diagnosed with culture-proven pulmonary TB at the Institute of Respiratory Diseases of Policlinico San Matteo, University of Pavia, Italy, in the period from 1989 to 1998, in order to assess the diagnostic benefit of analyzing three or more sputum or other respiratory specimens for the diagnosis of pulmonary TB.

AFB were detected microscopically in sputum concentrates with Ziehl-Neelsen stain, and sputum sediments were inoculated onto Löwenstein-Jensen medium slants (4). Cultures were incubated at 37°C for up to 8 weeks, and isolates of *Mycobacterium tuberculosis* complex were identified by using the GenProbe hybridization assay.

Between 1989 and 1998, our mycobacteriology laboratory received a total of 4,173 respiratory specimens for AFB smear and culture, 359 (9%) of these being positive for *M. tuberculosis* complex. The positive cultures were obtained from sputum specimens (78%), bronchoalveolar lavage specimens (17%), pleural fluid specimens (4%), and lung biopsy specimens (1%). During this 10-year period, 192 individuals were diagnosed with culture-proven pulmonary TB. For 127 patients (66%), *M. tuberculosis* was isolated from one or more sputum specimens. The remaining 65 cases (34%) were diagnosed by recovering the microorganism in bronchoalveolar lavage specimens (52 cases), pleural fluid specimens (10 cases), and lung biopsy specimens (3 cases). Among the *M. tuberculosis* culture-positive subjects, for 76 (40%) a single respiratory specimen was submitted to the laboratory, with 43% positive by AFB smear (33 of 76). For 32 persons (17%) two specimens were examined for mycobacteria, with at least one positive AFB smear for 50% of patients (16 of 32), and for 84 patients (44%) three or more respiratory specimens were sent for examination (Table 1). In this group, AFB smear was at least once positive for 67% of patients (56 of 84). The first culture proved to be positive was the first specimen for 64 of 84 (76%) patients either smear positive or smear negative. The first positive culture was obtained from the second and the third specimen for 16% (13 of 84) and 8% (7 of 84) of either smear-positive or smear-negative patients, respectively.

These findings substantially agree with those shown by Nelson et al. (3) and confirm that the first and the second specimen enable *M. tuberculosis* isolation from a majority of patients (92%), while the third or a subsequent specimen col-

TABLE 1. Frequency distribution of the first positive specimen for patients for whom three or more specimens were collected for AFB smear and culture

First culture-positive specimen	No. of patients (%) that were:		
	Smear positive ^a	Smear negative	Either†
First	46 (82)	18 (64)	64 (76)
Second	5 (9)	8 (29)	13 (16)
Third or later	5 (9)	2 (7)	7 (8)

^a At least one specimen was smear positive.

^b Both smear-positive and smear-negative specimens.

lected is of little diagnostic relevance (8%). Processing and examining these additional specimens likely do not compensate for the increased per patient cost for TB diagnosis (a total of about \$40 per sputum specimen at our institution). Nevertheless, a careful analysis of data from different institutions is required to establish the adequate number of specimens which should be collected.

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