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Gram Stain Evaluation of the Quality of Sputum Specimens for Mycobacterial Culture

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A group of 34 mycobacteria, consisting of 25 *Mycobacterium tuberculosis* and nine strains of three other species, was isolated from 400 expectorated sputum specimens submitted on 148 patients from county-wide sources. Eight strains (24% of the total) were isolated from specimens evaluated by Gram stain to be oropharyngeal fluids. The remaining 26 strains were isolated from ungradable specimens and those primarily of lower respiratory origin. It was concluded that the random examination of sputum by Gram stain to determine the specimen's quality for mycobacterial isolation is not necessary.

In recent years, there has been increased emphasis placed upon the relevance of clinical microbiology. This has included preliminary evaluation of specimens to determine their value and quality for culturing pathogenic microorganisms. Sputum specimens submitted for bacteriological culture have been evaluated by Gram stain to determine the extent of oropharyngeal contamination in the specimen as compared with fluids that originate from the lower respiratory tract (1). Specimens that contain at least 10 leukocytes with mucus but less than 25 squamous epithelial cells per low-power field (×100) are considered suitable for bacterial culture (1). This rationale has the potential of eliminating a considerable number of unsuitable specimens from the laboratory workload. The degree of orophyaryngeal contamination in sputum specimens for mycobacterial, fungal, mycoplasmal, or viral respiratory infection is considered to be less important (3).

Despite the fact that mycobacteria can be isolated from sputum as well as saliva (5), a retrospective Gram stain evaluation was conducted on sputum specimens submitted for isolation of acid-fast bacilli. The objective was to determine to what extent the Gram stain criteria used to evaluate the quality of sputum for bacterial isolation also applied to the isolation of mycobacterial species. We felt this was particularly worth investigation in a public health laboratory, because sputum specimens are submitted on a countywide basis from inpatient and outpatient hospital services, public health clinics, private laboratories, offices of private physicians, and public health nurses in the field investigating possible tuberculosis-related disease.

Sputum specimens were processed using the *N*-acetyl-L-cysteine-NaOH digestion procedures (2, 4). Smears of digested sediment were prepared for staining with the Auramine-Rhodamine technique and, when positive, were confirmed by Ziehl-Neelsen stain. Isolation and identification methods for mycobacteria were performed by conventional techniques (2, 4) approved by the Microbial Diseases Laboratory of the California State Health Department, Berkeley. Gram stain evaluation of sputum was based upon the criteria of Murray and Washington (3). Undiluted, untreated expectorated sputum was spread on glass slides with wooden applicator sticks, air-dried, heat-fixed, and Gram stained. Several drops of immersion oil were spread over the smear, which was then examined with the microscope at ×100 magnification. The entire smear was observed and scored from 1 to 5 based upon the numbers of squamous epithelial cells and leukocytes with mucus that were seen per field. Specimens scored 1 or 2 are salivary in origin and not suitable for bacterial culture. Scores of 3, 4, or 5 denote specimens suitable for culture of material from the lower respiratory tract. These show, by Gram stain, decreasing numbers of squamous cells and increasing numbers of leukocytes. A score of 0 was added to the scheme for smears not sufficient in cellular debris or mucus to make a judgment. At least two individuals independently evaluated each smear to determine agreement in scoring.

Mycobacterial species were isolated from 34 (8.5%) of 400 sputum specimens submitted on 148 individuals (Table 1). Twenty-five strains of *M. tuberculosis* were isolated from 11 patients, none of whom was receiving antitubercular

TABLE 1. *Relationship between isolation of mycobacteria from sputum specimens and the quality of the specimens*

No. of specimens	Score[a]	Mycobacteria isolated	
		M. tuberculosis	Other[b] mycobacteria
70	0	2	1
115	1	3	1
29	2	3	1
77	3	5	4
39	4	6	2
70	5	6	0
400		25	9

[a] Gram stain evaluation of untreated specimen (see text for criteria).

[b] One *M. gordonae*, one *M. fortuitum*, and seven *M. avium* complex.

drugs at the time of culture. Six *M. tuberculosis* strains were isolated from specimens graded 1 or 2 that were submitted on four patients who were previously identified as having reactivation tuberculosis. *M. tuberculosis* was isolated from six other specimens graded 0, 3, or 5 on these same four patients during the study. The nine atypical mycobacteria, isolated from seven different patients not known to have previously produced cultures positive of *M. tuberculosis*, consisted of seven strains of *Mycobacterium avium* complex, one *Mycobacterium gordonae*, and one *Mycobacterium fortuitum*. The latter two species were isolated from specimens graded 1 or 2.

Two strains of *M. tuberculosis* and one *M. avium* complex were isolated from 70 specimens graded 0 (scant cells or debris, ungradable). Excluding the 0 group, eight mycobacteria were isolated from specimens graded 1 or 2, whereas 23 mycobacteria were isolated from specimens graded 3, 4, or 5 ($\chi^2 = 2.51; P = 0.11$). The digested concentrates of 10 specimens that yielded *M. tuberculosis* and two specimens that yielded other mycobacteria were also positive by Auramine and Ziehl-Neelsen stains. None of these 12 specimens was graded 1 or 2 by Gram stains.

The 400 specimens used in this evaluation represented approximately 25% of the current mycobacterial sputum culture workload derived from a county population of 582,000. The data derived indicate that whereas 36% of the specimens submitted were scored 1 or 2 by Gram stain, 24% of the acid-fast bacilli isolated came from these groups. One-half of the positive specimens were submitted from hospital laboratories, whereas the remainder came from offices of private physicians and public health clinics. We conclude that the random examination of sputum by Gram stain to determine the specimen's quality for mycobacterial isolation is not worthwhile or acceptable. Since physicians are primarily responsible for submitting proper specimens to a laboratory, the use of the Gram stain might be of some value in isolated instances where it might possibly be helpful to know whether or not specimens from patients suspected of having mycobacterial disease, but who have consistently negative smears and cultures, are of lower respiratory origin.

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