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Sampling Variability in the Microbiological Evaluation of Expectorated Sputa and Endotracheal Aspirates

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A five-center study was conducted with the aim of determining how reproducibly expectorated sputa and tracheal aspirates could be sampled when preparing Gram-stained smears and inoculating cultures. With both specimen types, excessive variation was noted among Gram stain results obtained from replicate smears. Less variation was noted among culture results, especially with tracheal aspirates.

Pneumonia remains the sixth leading cause of death in the United States (3). Diagnosis of this disease is primarily dependent on clinical history, physical examination, and radiographic studies. The role and value of microbiological studies of lower respiratory tract secretions is uncertain. Sputum Gram staining and culture are often used as an aid to the diagnosis and management of these infections; however, numerous studies have disputed their usefulness, especially in providing information helpful in establishing a diagnosis of bronchopulmonary infection (13, 14).

Gram stains of sputa have traditionally been used to rapidly obtain presumptive information for guiding initial antimicrobial therapy. Various studies have shown mixed results regarding this practice (6, 8, 14). Some studies have indicated that the Gram stain results significantly correlate with culture results (9), while other studies have demonstrated little association (13, 14). Correlation between Gram stain and culture results is, however, only one criterion for defining the utility of sputum examination in patients with lower respiratory tract infection. Another important consideration is the ability to reproducibly interpret Gram-stained smears. One recent study (4) demonstrated excessive variability when Gram stains of lower respiratory tract secretions were examined by different microscopists.

An even more fundamental concern is the ability to reproducibly sample specimens such as expectorated sputa and tracheal aspirates in the first place when preparing Gram stains and inoculating cultures. Laboratory personnel must choose the most purulent portion of the specimen to process while avoiding saliva. Appropriate sampling is based upon subjective assessment and fraught with logistical difficulties and is, therefore, subject to variability. The question arises, how reproducibly can such specimens be processed?

Our multicenter study examined the degree of variation observed among replicate Gram stains and cultures of expectorated-sputum and tracheal-aspirate specimens. To our knowledge, no prior study has examined this issue.

Ten tracheal-aspirate and 10 expectorated-sputum specimens from each of five hospitals (University of Iowa Hospitals and Clinics, Iowa City, Iowa; St. Lukes Hospital, Houston, Tex.; Henry Ford Hospital, Detroit, Mich.; Veterans Administration Hospital, Boston, Mass.; and the Geisinger Medical Center, Danville, Pa.) were processed and examined at each institution over an 8-week period from February through April 2000. Specimens were randomly selected for inclusion in the study. All specimens were processed within 30 min of receipt in the laboratory.

Using noncotton synthetic swabs to transfer specimens to glass slides, three different medical technologists prepared smears (designated A, B, and C) directly from the same specimen, each attempting to sample the most purulent portion of the specimen. The three smears were Gram stained and examined by the same medical technologist, pathology resident, or microbiologist. The smears were examined at low power (×100) for the quantity of squamous epithelial cells (SEC) and polymorphonuclear cells and under oil (×1,000) for the presence of bacteria or fungi. Quantitation of these parameters was accomplished using the following criteria: (0, none seen; 1+, 1 to 5; 2+, 5 to 10; 3 +, 11 to 25; and 4+, >25 per field). Microorganisms were described based on morphology and staining reaction (e.g., gram positive or negative, cocci or bacilli, etc.). No attempt was made to assign genus or species designations to individual morphotypes.

The three technologists who initially prepared Gram stains also inoculated three separate sets of cultures (designated A, B, and C). Again, using noncotton synthetic swabs, an attempt was made to sample the most purulent portion of the specimen. MacConkey, chocolate, and 5% sheep blood agar plates were inoculated and incubated at 35°C in 5 to 7% CO_2 for 48 h. After 24 and 48 h of incubation, bacterial and/or fungal growth on each culture plate was quantitated by a medical technologist using the following criteria: (0, no growth; 1 +, 1 to 10 colonies; 2+, 11 to 30 colonies; 3+, growth into the second quadrant with >30 colonies; 4+, growth into the third quadrant). Standard microbiology techniques were used to identify each organism to species level with the following exceptions: alpha

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TABLE 1. Characteristics of lower respiratory tract specimens examined in this study[a]

Characteristic	Finding	No. of specimens	
		Expectorated sputa (n = 26)	Tracheal aspirates (n = 38)
Laboratory	A	6	6
	B	4	9
	C	5	10
	D	5	5
	E	6	8
Polymorphonuclear cells	≥10/×100 field	17	22
No. of different morphotypes seen on Gram stain[b]	0	8	11
	1	10	40
	2	12	35
	3	18	24
	4	13	4
	5	16	0
	≥6	1	0
No. of different organisms recovered in culture[b]	0	2	7
	1	4	23
	2	3	33
	3	21	14
	4	12	20
	5	11	5
	≥6	25	12
Potential pathogen present[b]	*Haemophilus* spp.	24	8
	S. aureus	17	20
	P. aeruginosa	3	18
	Klebsiella spp.		
	Enterobacter spp.	15	21

[a] Data restricted to specimens judged to be representative based on screening for squamous epithelial cells on Gram stains.
[b] The denominator in these categories was 78 with expectorated sputa and 114 with tracheal aspirates, since triplicate determinations were made with the 26 representative sputa and 38 representative tracheal aspirates.

hemolytic streptococci other than *Streptococcus pneumoniae*, gamma hemolytic streptococci, *Neisseria* spp., *Haemophilus* spp. other than *Haemophilus influenzae*, coagulase-negative staphylococci, aerobic diphtheroids, and *Bacillus* spp. These organisms were identified to the group level based on Gram staining and colony morphology and the results of tests for oxidase, catalase, and coagulase reactivity where applicable.

Representative specimens were defined as those containing <25 SEC/×100 field for expectorated sputa and <10 SEC/×100 field for tracheal-aspirate specimens (9, 11). Among replicate determinations, the number of organisms with different morphologies in Gram stains B and C of each specimen were compared to the first Gram stain (i.e., Gram stain A). Expectorated-sputa and tracheal-aspirate culture results were also examined for quantity and type of organisms, with results obtained from cultures B and C compared to those from reference culture A.

Triplicate Gram stains and triplicate cultures from 50 expectorated sputa and 50 tracheal aspirates were initially evaluated. Thus, a total of 300 Gram stains and 300 cultures were analyzed. With three of the 50 expectorated-sputum samples, one of the three replicate Gram stains revealed >25 SEC; in 5 cases, two of the three Gram stains had this finding; and in 16 cases, all three expectorated sputa had >25 SEC observed on Gram staining. These 24 specimens (48% of the total) were judged to be nonrepresentative and were excluded from further analysis. Among the 50 tracheal aspirates, in 12 instances (24%), >10 SEC were noted on at least one of the triplicate Gram stains (one of three in six cases, two of three in one instance, and all three in five cases). These 12 tracheal aspirates were judged to be nonrepresentative and excluded from further analysis. Thus, a total of 26 expectorated sputa and 38 tracheal aspirates were determined to be representative and were examined further. The Gram stain and culture findings for these specimens are presented in Table 1.

Variation in Gram-staining results was evident with both expectorated sputa and tracheal aspirates; however, it was most pronounced with the former specimens (Table 2). Fifty-six percent of the determinations with expectorated-sputum samples and 39% of the determinations with tracheal aspirates demonstrated variation of at least one morphotype from reference Gram stain A. Twenty-five percent of expectorated-sputum samples and 12% of tracheal aspirates demonstrated variations of two or more morphotypes. The degrees of variability observed among Gram-staining results in individual laboratories were roughly comparable among the five institutions participating in this study; however, the least variation was noted in the laboratories of the Henry Ford Hospital and the Geisinger Medical Center (data not shown).

With respect to variability in culture findings, 38% of expectorated sputa and 22% of tracheal aspirates demonstrated variation of at least one organism from reference culture A (Table 2). Only 4% of expectorated-sputum cultures and 5% of tracheal aspirates revealed variation of two or more organisms.

TABLE 2. Variation among Gram stain and culture results with lower respiratory tract specimens

Specimen	Procedure	No. of instances of variation[a]				
		0	1	2	3	4
Expectorated sputa (n = 26)	Gram staining	23	16	10	1	2
	Culture	32	18	1	1	
Tracheal aspirates (n = 38)	Gram staining	46	21	8	1	0
	Culture	59	13	3	1	

[a] Number of instances in which replicate B or C varied from replicate A with respect to the indicated number of morphotypes observed or bacteria seen.

Differences in the degree of variation among culture results were minimal when the results obtained from individual laboratories were compared.

The most prevalent potential pathogen in both expectorated-sputum and tracheal-aspirate samples was *Staphylococcus aureus*, followed by the *Klebsiella-Enterobacter* group, *Haemophilus* spp., and *Pseudomonas aeruginosa* (Table 1). In 34 of 44 instances (88.6%) where ≥1 pathogen was present in at least one culture of a particular specimen, the same organism(s) was present in both of the other cultures of that specimen. In four cases (9.1%), pathogens were present in two of the cultures but not the third, and in only one case (2.7%), pathogens were observed in only one of the three cultures of a particular specimen.

Clinicians have traditionally utilized expectorated sputa and tracheal-aspirate specimens to diagnose and treat lower respiratory tract infections. In part, this is because these specimens are relatively easily obtained without excessive discomfort to patients.

Sputum Gram stain results are often used by clinicians to serve as a guide for initial selection of antibiotic therapy. Our study, together with the recent findings of Cooper et al., shows that this practice may be inappropriate. They demonstrated excessive variation when different microscopists examined the same Gram stains. We found that the majority of expectorated-sputum samples and a substantial percentage of tracheal-aspirate specimens demonstrated a high degree of variability among the results of replicate Gram stains from the same sample. This lack of sampling reproducibility is probably the result of difficulty in consistently selecting the most representative portion of the specimen for evaluation. Irrespective of the explanation, however, if lower respiratory tract secretion specimens cannot be processed reproducibly, and further, if there exists significant interpretive variability, how can information derived from analyses of such specimens be viewed as definitive?

The utility of the Gram stain has been questioned in other studies. The meta-analysis of Reed et al. revealed marked variation in the sensitivities and specificities of Gram stains compared to a reference standard. They concluded that Gram-stained smears might yield misleading results (14). It should be noted, however, that despite the limitations of sputum Gram stains Heineman et al. concluded that this procedure represents a useful means for evaluating the extent of specimen oropharyngeal contamination (8).

Culture has been extensively used as the "gold standard" in identifying patients with lower respiratory tract infections. The utility of this practice is also controversial (7, 10, 12). Our study indicates that the degree of variation in the number of organisms recovered from replicate cultures was generally minimal and far less than that observed with Gram stains.

Contamination of expectorated sputa with oropharyngeal flora is a major limiting factor. It is perhaps less of a problem with tracheal-aspirate specimens. Geckler et al. noted that tracheal aspirates are superior to expectorated sputa in diagnosing lower respiratory infections (5). Our findings support this assertion. The presence of fewer bacteria in cultures of tracheal aspirates in our study indicates that these specimens may be less contaminated with upper respiratory tract organisms and therefore more indicative of lower respiratory tract infection.

The large degree of variation observed with sputum and tracheal-aspirate Gram stains in our study, together with the recent findings of Cooper et al. (4), leads us to conclude that Gram stains of these specimens are of limited value in providing information useful for the diagnosis and management of patients known or suspected to have lower respiratory infections. Cultures of expectorated sputa and tracheal aspirates may be more useful, particularly the latter specimens.

The findings of this study tend to support the recommendations of the American Thoracic Society, which discourage the use of sputum examination in the diagnostic evaluation of nonhospitalized patients suspected of having community-acquired pneumonia (1). In contrast, the recent community-acquired-pneumonia guidelines of the Infectious Disease Society of America encourage the performance of sputum Gram staining and cultures in nonhospitalized patients (2). In view of the difficulty in reproducibly sampling sputum specimens, as noted in our study, the recommendations of the Infectious Disease Society of America perhaps merit reconsideration.

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