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Danger of Sputum Purulence Screens in Culture of *Legionella* Species

JAMES G. INGRAM AND JOSEPH F. PLOUFFE*

Division of Infectious Disease, Department of Internal Medicine, The Ohio State University College of Medicine, Columbus, Ohio 43210

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Previous studies have shown the benefit of sputum purulence screens in routine bacterial culture. We have correlated 944 sputum smear and *Legionella pneumophila* culture results and demonstrated that 47 to 84% of *L. pneumophila*-positive samples would have been discarded by using established sputum purulence screens. We recommend acceptance of all specimens submitted for *Legionella* culture.

To improve quality and reduce cost of sputum cultures, criteria for acceptable sputa for routine bacterial culture have been developed. Before these criteria were used, interpretation of culture results was greatly confused by growth of mixed oropharyngeal flora (2). Elizabeth Barrett-Connor's observation that pneumococci were absent from sputum cultures in 45% of cases of bacteremic pneumococcal pneumonia (1) further led to efforts to improve culture results by grading the purulence of sputum specimens according to the number of basal squamous epithelial cells (BSEs) versus polymorphonuclear leukocytes and culturing only the most purulent samples (2, 4–6, 9, 10). Using the amount of polymorphonuclear leukocytes versus BSEs to indicate relative purulence, these efforts showed that the more purulent specimens grew numbers and types of organisms similar to those grown by random transtracheal aspirates (9, 10) and cultures from purulence groups 4 to 6 (Table 1) had 79% agreement with cultures from paired transtracheal aspirates (4). Thus, rejecting nonpurulent, or BSE-contaminated, specimens has been shown to increase the likelihood of isolating organisms from the lower respiratory tree versus the oropharynx in routine bacterial culture.

Earlier studies have not evaluated using such a purulence screen for the specific culture of *Legionella pneumophila*. The present study used a modification of the Murray and Washington sputum purulence classification system (9) to evaluate the effect of discarding nonpurulent sputum specimens on *Legionella* culture results.

Sputum samples were obtained from adults admitted with pneumonia to 10 different hospitals in Franklin County, Ohio, during an ongoing community-based pneumonia incidence study (8). Sputum samples were processed by the Clinical Microbiology Laboratory at The Ohio State University Hospital. All of the samples were used to make smears which were stored for later Gram staining. The sputum samples were also plated onto two pairs of buffered charcoal-yeast extract agar with alpha ketoglutarate and that agar containing polymyxin B, anisomycin, and vancomycin (Remel). One pair of plates was made from sputum washed with a 0.2 M KCl-HCl solution, pH 2.2. The plates were incubated aerobically at 35 to 37°C in a convection incubator without CO_2 and examined daily for the presence of *Legionella* colonies. Identification was confirmed by direct fluorescent-antibody assay (3).

For this study, 944 sputum smears were Gram stained. The entire slide was scanned microscopically at low power (10× objective), and representative fields were categorized on the basis of the number of BSEs and leukocytes (Table 1). A group 6 that was added to the original classification of Murray and Washington by Geckler et al. (4) was retained. The percentage of *L. pneumophila*-positive sputum samples that would have been discarded with the use of published purulence criteria was calculated.

Of 944 total samples, 19 (2%) were positive for *L. pneumophila*. Positive cultures were obtained from all of the Gram stain groups, with most coming from group 6 sputa (small to moderate numbers of both BSEs and polymorphonuclear leukocytes) (Table 1).

Previous purulence screens of sputum specimens submitted for routine bacterial culture have been shown to be useful in minimizing waste of laboratory resources (2), improving the percentage of lower respiratory tract pathogens isolated from culture, and interpretating culture results (4–6, 9, 10). Our data do not support the use of purulence screens for specimens submitted for *Legionella* culture. Table 2 lists criteria for acceptance of sputum samples for routine bacterial culture recommended by three different sets of authors and the percentage of positive *L. pneumophila* cultures that would have been missed by using each criterion. Culturing only group 5 specimens as suggested by Murray and Washington (9) would have resulted in missing 84% of cultures positive for *L. pneumophila* in our series. Following the recommendations of Van Scoy (10) would have missed 68%, and following those of Geckler et al. (4) would have missed 47% of the positive *L. pneumophila* cultures. Although it is important to ensure that laboratory resources are not wasted, we have shown that discarding nonpurulent or BSE-contaminated samples submitted for *Legionella* culture will unacceptably reduce the yield of positive results.

The specimens that were positive for *L. pneumophila* were found in all six groups of purulence, with the second-highest percentage coming from group 1, which is clearly heavily contaminated with oropharyngeal secretions. *L. pneumophila*-positive cultures without marked purulence (<25 leukocytes per low-power field) were more common (13 of 19) than positive cultures with purulence (6 of 19). Kirby et al. reported in their review of 61 Legionnaires' disease cases that the cough is initially dry but may become

* Corresponding author. Mailing address: N-1135 Doan Hall, OSU Medical Center, Columbus, OH 43210. Phone: (614) 293-8733. Fax: (614) 293-4556.

TABLE 1. Grouping of 944 sputum smears by purulence groups and *Legionella* culture results

Culture group	No. (per low-power field) of:		*Legionella*-positive cultures		*Legionella*-negative cultures	
	BSE cells	Leukocytes	No. of slides	%	No. of slides	%
1	>25	<10	4	21	95	10
2	>25	10–25	2	11	51	6
3	>25	>25	2	11	118	13
4	10–25	>25	1	5	107	12
5	<10	>25	3	16	279	30
6	<25	<25	7	37	275	30
Total			19	101	925	101

productive of nonpurulent or minimally purulent secretions. In addition, small numbers of polymorphonuclear leukocytes in sputum or transtracheal aspirate specimens have been reported to be consistent with Legionnaires' disease (7). We have demonstrated that many of the samples from which *L. pneumophila* was isolated were nonpurulent and BSE contaminated.

Discarding nonpurulent or BSE-contaminated samples will lose an unacceptable 47 to 84% of samples that would grow *L. pneumophila*. Previous reports suggest that the use of selective media and acid washing in *Legionella* culturing effectively control oropharyngeal contamination (3). All sputum specimens submitted for *Legionella* culture should be cultured regardless of the purulence of the specimen.

TABLE 2. Purulence grouping of *Legionella*-positive samples and the percentage of positive specimens discarded by the use of three purulence criteria

Author(s) (reference)	Recommended group(s) for culture	No. of smears		% Discarded
		Purulent	Nonpurulent (discarded)	
Murray and Washington (9)	5	3	16	84
Van Scoy (10)	3–5	6	13	68
Geckler et al. (4)	4–6	10	9	47

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