

## Gram Stain and Quantitative Sputum Culture in Bacteriological Assessment of Lower Respiratory Tract Infection

Joseph A. ODHIAMBO<sup>1</sup>, Kazunori OISHI<sup>2</sup>, Kiwao WATANABE<sup>2</sup>  
Tsuyoshi NAGATAKE<sup>2</sup>, Hiroshi SUZUKI<sup>3</sup>, and Keizo MATSUMOTO<sup>2</sup>

- 1) *Respiratory Diseases Research Center, Kenya Medical Research Institute, Nairobi 47855*
- 2) *Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki 852, Japan*
- 3) *Aino Memorial Hospital, Aino-Cho, Nagasaki Prefecture, Japan*

**Abstract:** We evaluated the clinical role of Gram-stained smears and quantitative culture in examining sputum specimens from patients with respiratory infections. Fresh sputum specimens (75 prospectively and 86 retrospectively) from elderly patients with predominantly chronic pulmonary disorders were submitted to this study. The respiratory pathogenic organisms were isolated more frequently from high quality sputum defined by a modified sputum screening system on Gram-stained sputum smears. In this study, 34.8% of specimens examined were qualified for rejection. A significantly positive correlation between the number of dominant bacteria on Gram-stained smears and the subsequently isolated pathogens was demonstrated. The modified sputum screening system used in the present study is recommended for routine laboratory use to save technical and financial resources, and to enhance the diagnostic value of sputum specimens.

**Key words:** Gram stain, Quantitative culture method, Sputum cytology, Lower respiratory tract infection

### INTRODUCTION

The clinical usefulness of Gram-stained sputum smears and sputum culture has remained a matter of considerable debate due to the confounding effect of oropharyngeal contamination upon expectorated specimens (Martin *et al.*, 1978). Some authorities have even suggested that little is gained by examining sputum specimens (Lentino and Lucks, 1987). In the less developed parts of the world where the subject of cost-effectiveness and the burden of respiratory infections remain issues of critical concern, the potential available alternative means of enhancing the diagnostic value of sputum samples should be explored.

---

Received for publication, August 15, 1990.

Contribution No. 2393 from the Institute of Tropical Medicine, Nagasaki University.

The use of initial microscopic examination have been suggested as a significant method of saving technical time and financial resources besides providing useful clinical information for clinicians (Matsumoto *et al.*, 1978; Martin *et al.*, 1978). Several microscopic screening systems for sputum have been devised (Wong *et al.*, 1982). Some of these systems, however, appear rather unrealistic for routine use by service-oriented clinical laboratories especially those handling large numbers of specimens against limited technical and financial resources. In this regard, the present study was designed to evaluate a modified, simple screening system using Gram-stained sputum smears and quantitative sputum culture in the bacteriological assessment of lower respiratory tract infections.

#### MATERIALS AND METHODS

*Sputum samples:* Fresh sputum specimens submitted to the Department of Internal Medicine (Institute of Tropical Medicine, Nagasaki University) from adult patients with potential lower respiratory infections were investigated. The expectorated sputum specimens were prospectively (February to July, 1989) and retrospectively (January to June, 1988) assessed.

*Gram-stained smear and sputum quality:* The method for Gram staining of sputum specimens was based on Hucker modification (Sonnenwirth, 1980). The sputum quality was defined by the combined numbers of squamous epithelial cells (SEC) and polymorphonuclear cells (PMNC) observed at lower magnification ( $\times 100$ ) on Gram-stained smears. At least one third of each smear was observed. The quality of sputum specimens were defined as below; Good quality: PMNC $>25$  and SEC $>10$ , Borderline quality: PMNC $>25$  and SEC $>10$ , Poor quality: PMNC $>25$  and SEC $>10$ . The morphologic characteristics of bacteria on Gram-stained smears were observed under  $\times 1000$  oil immersion objective, and recorded. The presence of various bacteria was recorded semi-quantitatively based on a four-category scale: 0=none, +=rare, ++=moderate, +++=numerous. Moderate or numerous bacteria observed on Gram-stained smears were evaluated to be dominant.

*Sputum quantitative culture:* To each ml of sputum specimen, 0.2ml of N-acety-L-cysteine (176.2mg/ml, Senju Pharmaceutical Company, Japan) was added and vortexed. Serial ten-fold dilution of sputum sample was made in sterile saline. Using a flame-sterilized calibrated (0.01 ml) wire loop, one loopful of each dilution was inoculated on the corresponding portions of trypticase soy agar (Becton Dickinson Microbiology Systems, USA) containing 7% rabbit blood. Plates were incubated for 18-24 hours at 35°C under 5% CO<sub>2</sub>, and colony count were done. For specific identification, appropriate tests were done using standard procedures.

*Clinical informations:* Clinical data corresponding to specific date of sputum collection was obtained from patient case records at Nagasaki University Hospital, Nomozaki Municipal Hospital and Tagami Hospital.

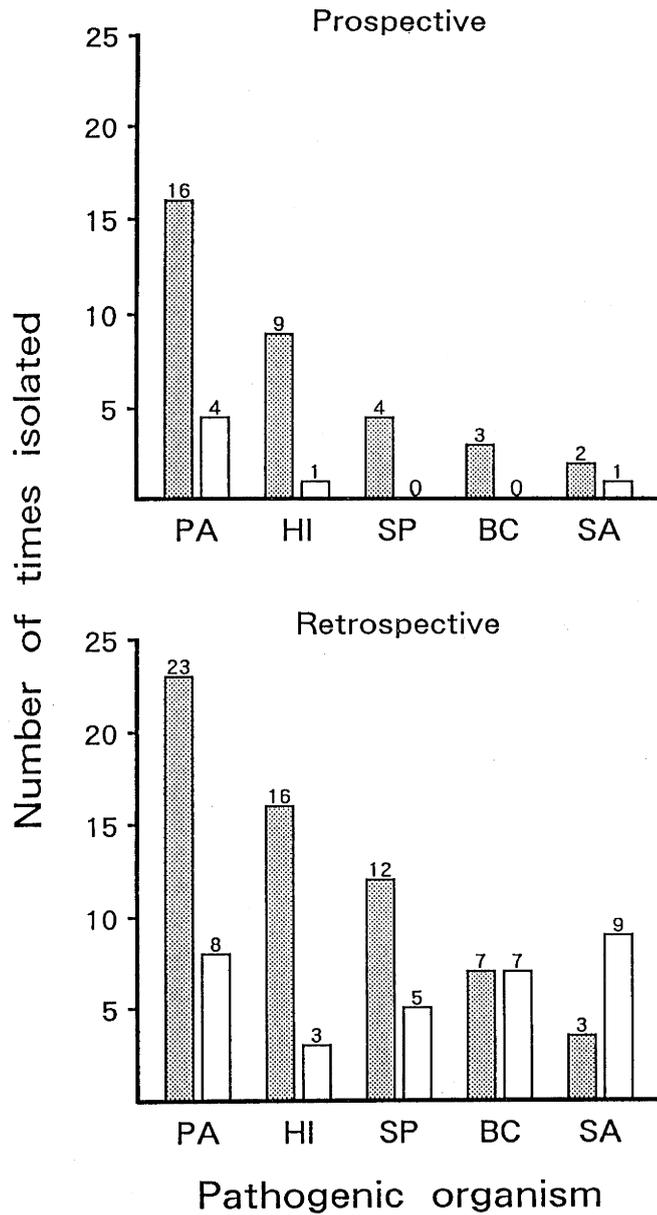
## RESULTS AND DISCUSSION

A total of 161 sputum specimens were examined; 75 prospectively and 86 retrospectively. The patients involved in this study were generally elderly with a mean age exceeding 60 years and an approximately balanced sex ratio. Over 80% of these patients were associated with chronic respiratory disorders; chronic bronchitis, bronchiectasis, diffuse panbronchiolitis and chronic pulmonary emphysema. The distribution of sputum specimens in this study in terms of their quality, as defined by a criteria previously described, is shown in Table 1. Over 60% of the total specimens satisfied the criteria for good quality. However, 34.8% of the total sputum specimens belonged to the criteria for poor quality. The isolated pathogens based on the quantitative culture method in the present study consisted of *Pseudomonas aeruginosa* (21.7%), *Streptococcus pneumoniae* (20.7%), *Haemophilus influenzae* (18.5%), *Branhamella catarrhalis* (16.3%), *Staphylococcus aureus* (14.1%) and others (8.7%). These distribution of respiratory causative organisms corresponded to a previous report (Matsumoto, 1988). We evaluated the frequency of isolation of specific pathogens from good quality or poor quality sputum in the prospective and retrospective study. It was recognized that the frequency of isolation of specific pathogens is generally higher from good quality sputum specimens than from poor ones, with exceptions of *Branhamella catarrhalis* and *Staphylococcus aureus* in the retrospective study (Figure 1). In the prospective study, only 6 specific pathogens including 4 *Pseudomonas aeruginosa* strains were isolated from poor quality sputums. We also examined the relationship between the numbers of dominant bacteria on Gram-stained smears and subsequently isolated specific pathogens. A significantly positive correlation between the number of dominant bacteria on Gram-stained smear and the subsequently isolated pathogens was demonstrated in Figure 2 ( $p < 0.001$ ,  $r = 0.90$ ). The relationship between clinical and laboratory-based parameters (body temperature, C-reactive protein level and white blood cell counts) did not achieve significant levels of correlation.

The management of lower respiratory tract infections is significantly simplified if a specific pathogen is accurately determined. Routine sputum cultures are noteworthy for providing misleading results due to oropharyngeal contamination and irregular distribution

**Table 1.** Distribution of sputum quality defined by a modified criteria on Gram-stained smears in prospective and retrospective study

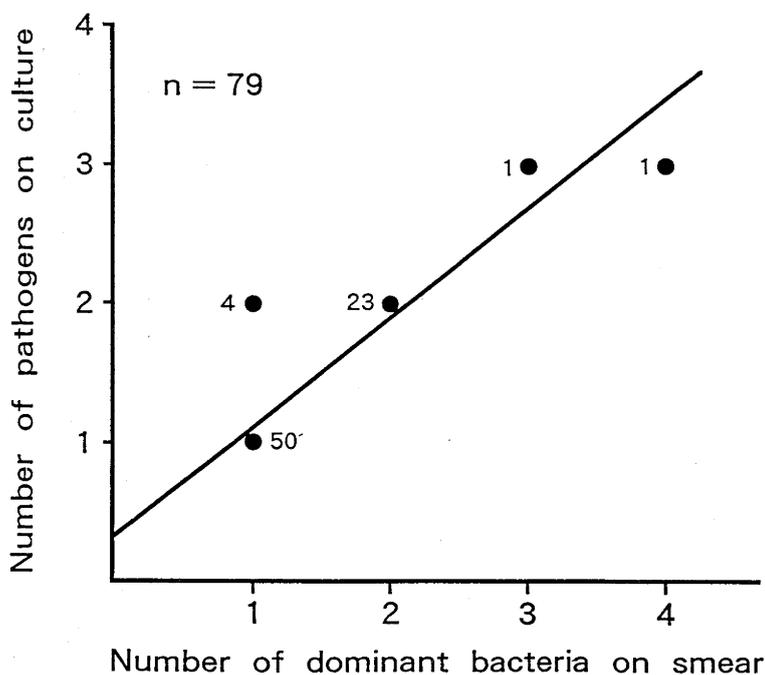
Sputum quality	Number of specimens		
	Prospective	Retrospective	Total(%)
Poor	23	33	56 ( 34.8)
Borderline	1	2	3 ( 1.9)
Good	51	51	102 ( 63.3)
Total	75	86	161 (100 )



**Fig. 1.** Comparison of the frequency of isolated specific organisms from good or poor quality sputum specimens as defined in the prospective and the retrospective study.

Good quality sputum; ▨ Poor quality sputum; □

PA: *Pseudomonas aeruginosa*, HI: *Haemophilus influenzae*,  
 SP: *Streptococcus pneumoniae*, BC: *Branhamella catarrhalis*,  
 SA: *Staphylococcus aureus*.



**Fig. 2.** Correlation between numbers of dominant bacteria on Gram-stained smears and numbers of pathogens subsequently isolated from 79 sputum specimens (28 prospectively and 51 retrospectively). Moderate or numerous bacteria observed on Gram-stained smears, as defined described above, were evaluated to be dominant.

of bacteria within the specimen (Bartlett, 1981). This makes correct recognition of true pathogens difficult. The present study demonstrated that the pathogenic bacteria were more frequently originated from good quality sputum. Some authorities suggested that a cost-effective sputum screening system should reject at least 22% of the specimens received (Heineman and Radano, 1979). On the other hand, 34.8% of specimens examined in this study qualified for rejection. This study further demonstrates that a morphological and semiquantitative assessment of bacteria on Gram-stained smears has a definite role in predicting presence of significant pathogens. Therefore, Gram-stained sputum smears can greatly aid clinicians to make presumptive etiological diagnosis, and permit the laboratory to enhance the isolation of pathogenic bacteria by selective culture methods. A recent report similarly indicated that laboratory-based interpretation of microbiologic results could improve physicians to make decisions (Mizrachi and Valensteine, 1987). It should, however, be noted that isolation from culture of two or more morphologically similar bacterial species could not be distinguished by a Gram-stained sputum smear.

In conclusion, this study shows that Gram-stained smears and quantitative sputum culture together have a significant role in enhancing the diagnostic value of expectorated sputum specimens. The criteria used in this study would appear to be more adaptable for routine laboratory use.

## ACKNOWLEDGMENT

We wish to acknowledge the generous contribution of doctors at Tagami Hospital, Nomozaki Municipal Hospital. We also wish to thank Mrs. Yoko Takayama for her contribution in data analysis. This study was carried out during the first author's participation in JICA (Japan International Cooperation Agency) training course.

## REFERENCES

- 1) Bartlett, J. G. (1981): Bacterial Diagnosis of Pulmonary Infections. Diagnostic techniques in pulmonary disease part II Vol 16 Marccel Dekker Inc. New York, 707-745.
- 2) Heineman, H. S. & Radano, R. R. (1979): Acceptability and Cost Savings of Selective Sputum Microbiology in a Community Teaching Hospital. *J. Clin. Microbiol.*, 10 (4), 567-573.
- 3) Letino, J. R. & Lucks, D. A. (1987): Nonvalue of sputum culture in the management of lower respiratory tract infections. *J. Clin. Microbiol.*, 25 (5), 758-762.
- 4) Martin, R. S., Sumarah, R. K. & Robert, E. M. (1978): Assessment of expectorated sputum for bacteriological analysis based on polymorphs and squamous epithelial cells: six-month study. *J. Clin. Microbiol.*, 8 (6), 63.
- 5) Matsumoto, K., Uzuka, Y., Nagatake, T., Noguchi, Y. & Suzuki, H. (1978): A quantitative sputum culture method: The significance of the quantity  $>10^7$ /ml of isolated organisms. *Jap. J. Thorac. Dis.*, 16 (2), 77-89.
- 6) Matsumoto, K. (1988): Changes of infections and chemotherapy. *Jap. J. Bacteriol.*, 43 (4), 809-820.
- 7) Mizrachi, H. H. & Valenstein, P. N. (1987): Randomized trial interpreting sputum quality in a clinical laboratory. *J. Clin. Microbiol.*, 25 (12), 2327-2329.
- 8) Sonnenwirth, A. C. (1980): Stains and staining procedures. P. 1384. IN A. C. Sonnenwirth & L. Jarett (ed). *Gradwohl's Clinical Laboratory Methods and Diagnosis*. 18th Ed. Vol 2. C. V. Mosby Company. St. Louis.
- 9) Wong, L. K., Barr, A. L. & Horgan, S. M. (1982): Comparison of six different criteria for judging the acceptability of sputum specimens. *J. Clin. Microbiol.*, 16, 627-631.