The Bacteriological Examination of Sputum

RICHARD C. TILTON, Ph.D.,* EUFRONIO MADERAZA, M.D.,* PAUL IANNINI, M.D.,† AND RICHARD QUINTILIANI, M.D.†

*University of Connecticut Health Center, Farmington, CT 06032 and
Hartford Hospital, Hartford, CT 06115

ABSTRACT

Pitfalls in the collection, transport, processing, and interpretation of the expectorated sputum culture are discussed. Data are presented which suggest that, under strictly controlled conditions, the culture of sputum may be a valuable adjunct to the diagnosis of bacterial pneumonia.

In 1971 there were 2,068,000 diagnosed cases of bacterial pneumonia resulting in 34,183,000 days of disabled activity and 10 percent of hospital admissions. As expected, since pneumonia is common, the demand for sputum cultures is great; in fact, over 37 million dollars is spent annually on this laboratory test. Yet, for such a frequently performed test, sputum cultures are still plagued by diagnostic uncertainty with scientific obscurity.

When an infectious agent is isolated from a site which is normally devoid of microorganisms such as the cerebral spinal fluid, the blood, or the pleural fluid, its pathogenic role is easily established. Many parts of the body, such as the respiratory, gastrointestinal or genitourinary tracts have, however, a "normal flora." Consequently, recovery of a microorganism from these areas does not necessarily indicate infection. Sputum cultures are one of the most notorious examples where the problem is not the detection of a pathogen but rather the determination as to which one of the many bacteria found is responsible for the patient's infection.

Of the many facets of the bacteriological examination of sputum, three are of particular importance. They include current methodology, difficulty encountered in the use of these methods and possible solutions for better laboratory diagnosis of respiratory infection.

Current Methodology

THE GRAM STAIN

The Gram stain is the most widely used and abused procedure in microbiology but it can be one of the most valuable, if correctly performed and intelligently interpreted. All respiratory secretions should be Gram stained and expeditiously examined. This simple procedure provides (1) an assessment of the quality of the specimen, (2) a hint as to the microbial etiology of the disease and (3) information on the cellular response to infection.

In preparing sputum for staining, the most purulent portion should be applied to
the slide, spread so as to provide for reading and air dried. The Gram stain involves four step wise procedures: (1) primary staining, (2) addition of a mordant, (3) decolorization and (4) counterstaining. The most critical step is decolorization. The time required to decolorize a smear will vary depending upon the ingredients of the decolorizing reagent (ethanol being the slowest and acetone the most rapid) and the thickness of the smear. If this step is not well standardized, then quality control slides containing a mixture of gram positive and gram negative organisms should be included in a staining run at least once a day.

Morphological evaluation of the stained secretions provides valuable information on the source of the specimen. Secretions, allegedly sputum but in fact contaminated by saliva, contain many epithelial cells but few, if any, polymorphonuclear (PMN) leukocytes. The bacterial nature of such a specimen reveals many different morphological types of bacteria suggestive of normal mouth flora. Frequently, gram positive, lancet shaped diplococci resembling \textit{Streptococcus pneumoniae} are superimposed on or attached to epithelial cells. Subsequent culture often reveals these organisms to be the alpha hemolytic streptococci of the oral cavity. In the sputa of patients with bacterial pneumonia, there is usually a proliferation of PMN's and mucous strands apparent on Gram stain. \textit{Streptococcus pneumoniae}, if present, may be observed without resorting to a specific capsular stain. Other bacterial agents of pneumonia, such as the Staphylococci, \textit{Hemophilus influenzae} and \textit{Klebsiella pneumoniae}, may be presumptively identified by Gram stain. An attempt should be made to determine the predominant organism as all of the aforementioned bacteria can be seen in the respiratory secretions, especially those of hospitalized patients who may or may not have a pulmonary disease.

**Cultural Techniques**

The guidelines for the culture of respiratory secretions are very broad. Although the decision as to the choice of media is best left to the microbiologist or the pathologist, culture protocol should include an enrichment medium (e.g. blood agar), a medium which provides for the growth of fastidious organisms requiring hemin and NADH, (e.g. chocolate agar) and a medium which selectively suppresses flora and allows the growth of either gram negative or positive bacteria (e.g. MacConkey's agar, Mannitol Salt agar). Incubation of non-selective media in a CO$_2$-enriched environment is also beneficial.

Sputum should be inspected grossly in the laboratory. The presence of excessive froth indicates a specimen which is primarily saliva. The laboratory must be prepared to reject saliva samples since they will provide meaningless and misleading data. Sputum should be inspected for areas of pus which provide the best material for smear and culture. Unfortunately, the large daily volume of sputum specimens, the frivolous nature of sputum collection and the failure to differentiate between sputum and saliva has led to a deterioration of specimen quality.

The time a specimen is taken should be recorded on the requisition. Respiratory secretions are an effective growth medium for bacteria; if allowed to stand at room temperature for more than three to four hours, there will be bacterial overgrowth and subsequent difficulty in identifying the etiological agent.

The results of culture must be evaluated in light of both the clinical presentation and known microbial ecological concepts. For example, more than a billion aerobic and anaerobic organisms are present as normal flora in a milliliter of oral secretions. These organisms can readily contaminate expectorated sputum. The normal anaerobic flora of the oral cavity will grow if
sputum is cultured anaerobically. Thus, anaerobic incubation of sputum specimens is not indicated except for those secretions that are obtained by methods, such as transtracheal aspiration, which bypass the normal oral flora.

Encountered Problems

Several studies have shown lack of correlation between potential pathogens isolated from sputum and the clinical diagnosis even when appropriate technique is employed. Bartlett and Melnick reported that the incidence of identification in Gram stains or isolation in culture of *Streptococcus pneumoniae* was not significantly different in infected and uninfected patients. A subsequent study by Hoeprich revealed that 34 percent of patients with no respiratory disease harbored *Streptococcus pneumoniae* in the sputum. Such results increase the concern as to what relevance, if any, have cultures of expectorated sputum. The advisability of isolation, speciation, and antimicrobial susceptibility on multiple potential pathogens in a sputum is questionable. Reports have documented the alteration in the normal upper respiratory flora of acutely ill patients as a function of length of hospital stay and use of inhalation therapy equipment. The colonizing flora becomes predominately gram negative rods such as *E. coli*, *Pseudomonas*, and *Enterobacter*. Both laboratorian and clinician must recognize the distinctions between colonization and infection. In our laboratory, the presence of three or more individual species of gram negative rods in a sputum specimen are suggestive of colonization, specimen deterioration or contamination by normal flora. These organisms are not followed through unless subsequent consultation with the physician reveals an overriding reason to do so.

The quintessence of the problem appears to be that sputum is contaminated with normal flora and the etiological agent is masked, that sputum is not always sputum but often saliva and that the visualization and/or isolation of a pathogen from sputum does not always indicate infection.

Laboratory Diagnosis of Respiratory Infection

Recognizing the problems that exist, there have been many attempts to overcome them. Sputum has been washed to remove adhering saliva. This procedure was repeated in our laboratory on 50 sputum specimens. There was no significant difference in the Gram stain or cultural results of washed and unwashed sputum. This washing procedure, if done in the open laboratory, produces potentially harmful aerosols, especially from patients suspected of mycobacterial disease. Other studies have proposed that sputum be liquified with a mucolytic agent, diluted in buffer and a quantitative bacterial culture performed. No increase in the numbers of positive cultures or in the numbers of pathogens detected was observed in our laboratory with the use of the quantitative method. Furthermore, there was no reduction in the isolation of mixed flora. These data corroborate the conclusions of Bartlett and Melnick and Hahn and Beaty.

An obvious approach to the problem of sputum specimen quality is to bypass the normal mouth flora by procuring respiratory secretions by means other than expectoration. Such procedures include endotracheal aspiration, trans-tracheal aspiration (TTA), bronchial washing, bronchial brushing, and percutaneous lung puncture and aspiration.

Kalinske et al and Hahn and Beaty studied 102 and 61 patients, respectively, and concluded that TTA was a safe procedure with virtually no complications. Spencer and Beaty, however, reported cardiac arrest in three patients subjected to TTA. Distinct differences have been demonstrated in the microbial flora of sputum versus secretions collected by these bypass procedures.
Kalinske et al. compared cultural results of sputum and transtracheal aspirates in 102 patients. In 30 percent of the patients, gram negative rods were isolated more frequently from the sputum specimen than from the TTA. “False positive” sputums were seen less frequently with *Staphylococcus aureus* (10 percent) and *Streptococcus pneumoniae* (8 percent). A similar but smaller study carried out by the authors (RQ, PI) revealed “false positive” sputum results attributable to gram negative rods (10 percent), *Candida albicans* (10 percent), and *Staphylococcus aureus* (6 percent).

In yet another series by the authors, 22 patients with acute bacterial pneumonia, as indicated by fever, leukocytosis and pulmonary infiltrate, underwent either TTA or percutaneous lung aspiration, expectorated sputum collection and blood cultures. Several samples were obtained from each patient and the sample which appeared most purulent and free of saliva was selected for culture. TTA or percutaneous lung aspiration was performed immediately following these collections, and blood cultures were obtained. All samples were immediately taken to the microbiology laboratory by the investigator (PI) and handled in a similar manner.

A comparison was made in these patients between the cultural results of expectorated sputum, TTA or lung biopsy, and blood culture. There was an 80 percent agreement between sputum culture and TTA or lung biopsy culture. Forty-one percent of the patients (nine patients) had positive blood cultures, and in all nine cases the same organism was found in the sputum, the TTA, and the blood. In no case did TTA yield the offending organism when the expectorated sputum did not, that is, no “false negative” sputum cultures.

The results of our studies suggest that expectorated sputum can be a valuable adjunct to diagnosis if collected, transported, gram-stained and processed under strictly controlled conditions. Although such experience is minimal, it is possible that controlled sputum culture can provide data as reliable as the more invasive technique of trans-tracheal aspiration or percutaneous lung biopsy. If the laboratory and the clinician are unwilling to adhere to these guidelines, then they should be prepared to accept the inevitable consequences, namely sputum culture and Gram stain reports that are unreliable and misleading. In fact, it could be argued that unless strict adherence to an appropriate technique is maintained, it would be preferable to abandon sputum cultures.

References