Misinformation from Sputum Cultures Without Microscopic Examination

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Only 13 of 38 hospital laboratories surveyed include a Gram stain routinely in microbiological sputum examination. In a prospective three-hospital study, 60% of over 1,200 "sputum" specimens consisted predominantly of saliva, as judged by cell composition. Compared with the results of cultures in which microorganisms presumptively identified on sputum smears were specifically sought ("directed cultures"), cultures of the same specimens processed in the routine manner missed pneumococci 61%, haemophili 23%, and yeasts 44% of the time. The findings were similar in all three hospitals despite differences in administration, staffing, primary culture media, and workload. Unless microscopic examination is routinely included, half of all microbiological information rendered on sputum specimens is meaningless and subject to dangerous misapplication. Furthermore, culture must be guided by microscopic findings, or respiratory pathogens will frequently be missed. Finally, when routine culture and smear disagree, the culture cannot be assumed to be correct. Microscopic examination should be mandatory in sputum microbiology, both for specimen evaluation and as a guide to what to look for in culture.

Microscopic examination of smears is a time-tested, but not particularly honored, part of sputum microbiology. In the belief that culture tells all, the Gram stain has been relegated to the status of an optional accessory. The information it provides is regarded as tentative at best, misleading at worst, and always to be superseded by culture.

Shulman and co-workers (16), analyzing the errors and hazards in the diagnosis of bacterial pneumonias, cited the frequency with which the etiological agent is missed on Gram smears of sputum but did not mention culture as a source of error.

Standard texts in laboratory diagnosis give varying amounts of space to discussion of the Gram stain, but none holds it to be essential. It has been said to be valuable in the diagnosis of staphylococcal and gram-negative pneumonia (4), misleading in pneumococcal pneumonia except in typical cases (1), and even misleading in staphylococcal pneumonia (15). Reference to microscopy for evaluation of specimens is exceptional (7, 13), even though proper collection of specimens is emphasized and the frequent submission of saliva as sputum is appreciated (17).

A number of recent publications (3, 5, 7, 12) have addressed themselves to cellular analysis of sputum without, however, modifying the prevalent views regarding identification of microorganisms. Despite earlier warnings (2, 14, 18), culture remains virtually the sole basis for microbiological diagnosis.

Our experience in sputum microbiology has convinced us that microscopic examination is much too useful to be left to discretion. Overinterpretation may indeed lead to false conclusions. However, without microscopy, culture results are of unknown relevance and, because of the faith placed in them, may be dangerously misleading. In agreement with others, we find that a very substantial proportion of specimens submitted as sputum are so contaminated with saliva that the results of culture (often resistant gram-negative bacilli) cannot possibly be applied to the diagnosis of respiratory infection and that the only way to recognize this fact is to examine every specimen microscopically. In contradiction to widely held beliefs, we find that presumptive identification of respiratory pathogens other than staphylococci and gram-negative bacilli, specifically pneumococci and haemophili, in Gram-stained smears is quite feasible.

Finally, a survey of area hospital laboratories indicates that in two-thirds, the practice is to make Gram-stained smears only on request. Evidently, this simple and inexpensive test is not universally accepted, and repeated emphasis on its value is needed.

(This work was originally presented at the 77th Annual Meeting of the American Society for Microbiology [H. S. Heineman and W. E.
MATERIALS AND METHODS

All sputum specimens were handled in the standard manner, as described in the microbiology procedure manual of Philadelphia General Hospital. Processing began within 30 min of receipt in the laboratory. From the most purulent or mucoid portion of the specimen, a smear was made and plates of 5% sheep blood agar, chocolate agar, and MacConkey agar were inoculated.

The smear was Gram stained and read by one of a number of technicians, the interpretation being recorded on the stat copy, which was immediately separated from the remainder of the report and returned to the patient area. An evaluation of the specimen was entered on the chart copy, specifically, "purulent secretions" (polymorphonuclear cells), "respiratory secretions" (ciliated cells), and/or "oral secretions" (squamous cells). Bacterial morphology was noted only on the stat copy. The technician handling the culture plates on subsequent days was usually not guided by the microscopic findings.

Plates were incubated in 5% carbon dioxide overnight; if growth was too slight for isolation of colonies, incubation was continued for 24 h more. On the basis of gross visual inspection, suspect colonies were subcultured and subjected to standard procedures for bacterial identification. Pneumococci were identified as alpha-hemolytic streptococci inhibited by optochin; haemophili were identified as gram-negative coccobacilli showing characteristic growth on chocolate agar and satellitism around a streak inoculum of Staphylococcus aureus on sheep blood agar.

Parts of study. (i) Evaluation of specimens. The criterion for specimen evaluation was the estimated proportion of saliva and inflammatory secretions (operationally equivalent to sputum), respectively, an attempt was made to compute the proportions of saliva and sputum from the numbers of these cells. Using a calibrated loop (of the type designed for urinary colony counts), loopfuls of normal saliva from three volunteers were carefully placed on slides and allowed to dry without spreading. After staining, several oil immersion fields were scanned, and it was determined that the average field contained 3 to 5 squamous cells. The same procedure was carried out with grossly purulent sputum, yielding 50 to 100 polymorphonuclear cells per average oil immersion field. Since each field represented the dried residue of approximately equal volumes of saliva and sputum, it was determined for the purpose of specimen evaluation that a volume of purulent sputum contains approximately 20 times as many polymorphonuclears as there are squamous cells in an equal volume of saliva. Therefore, a smear showing more than 1 squamous cell per 20 polymorphonuclears, regardless of absolute numbers, was regarded as consisting of more than 50% saliva.

Approximately 500 Gram-stained smears were examined under low-power (×100) magnification, and note was made of the relative proportion of cells. Smears of specimens estimated to contain more than 50% saliva (Fig. 1, left) were not examined further. Smears computed to consist mostly of inflammatory exudate (Fig. 1, right) were examined under oil (×1,000) for microbial morphology.

(ii) Predictability of culture results from smears. The microorganisms were not only described by morphology and staining reaction, but specific identification was attempted for certain known and suspected respiratory pathogens, namely, pneumococci, staphylococci and haemophili (Fig. 2 through 4). The presence of disproportionate numbers of other gram-negative bacilli (Fig. 5) or of yeast cells (Fig. 6) was likewise noted as abnormal. Gram-positive bacilli, long-chain streptococci, and gram-negative cocci, as well as mixtures of many types of bacteria, were considered grounds for predicting "normal flora" in culture.

After the smear interpretations had been recorded, the culture results were retrieved from the laboratory records.

(iii) Validity of culture results. Because of a disappointing lack of correlation between certain apparently diagnostic smear findings and culture results (see Table 1), the possibility was investigated that the smear interpretation was correct and the culture result was incorrect. Consecutive smears were examined, and allowed to dry without spreading. After staining, several oil immersion fields were scanned, and it was determined that the average field contained 3 to 5 squamous cells. The same procedure was carried out with grossly purulent sputum, yielding 50 to 100 polymorphonuclear cells per average oil immersion field. Since each field represented the dried residue of approximately equal volumes of saliva and sputum, it was determined for the purpose of specimen evaluation that a volume of purulent sputum contains approximately 20 times as many polymorphonuclears as there are squamous cells in an equal volume of saliva. Therefore, a smear showing more than 1 squamous cell per 20 polymorphonuclears, regardless of absolute numbers, was regarded as consisting of more than 50% saliva.

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approximately 60 were selected on the basis that they showed either (i) yeast cells or (ii) a combination of numerous polymorphonuclear cells and a predominance of bacteria resembling pneumococci or haemophili, as in Fig. 2, 4, and 6. In other words, they were felt to be virtually diagnostic of infection or significant overgrowth. Cultures were handled in the routine manner, but in addition, unknown to the technicians, duplicate subcultures were performed by one of the authors (W.M.L.), who intercepted the primary culture plates at the time of discarding (generally 1, occasionally 2, days after the initial examination by the technician) and reexamined them, particularly for the organisms thought to be recognized on the smear. The criteria for bacterial identification were the same as used in the routine culture. A comparison was then made of smear interpretation, results of routine culture, and results of culture as directed by microscopic findings ("directed culture").

(iv) **Comparison of three hospital laboratories.** Since routine culture at Philadelphia General Hospital frequently failed to yield potential pathogens that could be found by directed search (see Table 2), the possibility of defective laboratory practices at this hospital was investigated by extending the study to two other hospitals, namely, a 500-bed university hospital and a 250-bed community hospital. With the cooperation of the director of the microbiology laboratory and the bacteriology supervisor at each hospital, all Gram-stained smears and primary culture plates were saved. Again, seemingly diagnostic smears were selected for directed culture, which was performed at Philadelphia General Hospital, and the results were correlated with the results of culture at the hospital of origin.

(iv) **Survey of hospital practices.** Because of the important implications of our findings, as discussed below, a questionnaire (Fig. 7) was sent to the labo-
QUESTIONNAIRE ON SPUTUM GRAM STAINS

Table 1. Predictability of routine culture results from Gram stain interpretation

<table>
<thead>
<tr>
<th>Gram stain interpretation (no. of samples)</th>
<th>No. culture positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci (15)</td>
<td>11</td>
</tr>
<tr>
<td>Pneumococci (10)</td>
<td>2</td>
</tr>
<tr>
<td>Haemophilus (4)</td>
<td>2</td>
</tr>
<tr>
<td>Other gram-negative bacilli (31)</td>
<td>22</td>
</tr>
<tr>
<td>Yeasts (5)</td>
<td>2</td>
</tr>
</tbody>
</table>

TABLE 1. Predictability of routine culture results from Gram stain interpretation

RESULTS

Evaluation of specimens. Of 496 consecutive specimens examined under low-power magnification, 198 had a polymorphonuclear-squamous cell ratio of 20:1 or greater, and 298 had a ratio of less than 20:1. Thus, the proportion of specimens labeled "sputum" that consisted of at least half saliva was 60% at Philadelphia General Hospital. The proportions at the other two hospitals were remarkably similar, 232/400 (58%) and 181/304 (60%). In at least half of the unacceptable specimens, squamous cells actually outnumbered polymorphonuclears, indicating the virtual absence of any sputum at all (Fig. 1a).

Predictability of culture results from smears. Sixty-five smears were selected as being virtually diagnostic. The extent to which culture results confirmed this impression is shown in Table 1. Only 39 of 65 predictions (58%) were correct overall, the best performance being recorded for staphylococci (11/15, or 73%) and gram-negative bacilli other than haemophilus (22/31, or 71%). The smear predictions least often confirmed by culture were for pneumococci (2/10, or 20%).

Validity of culture results. Fifty-nine additional smears interpreted as showing pneumococci, haemophilus, or yeasts specifically were selected because of the previous poor showing of the Gram stain in predicting their growth in culture (Table 1). As illustrated in Fig. 2, 4, and 6, there was good reason to consider the organisms as pathogenically significant, based on their preponderance and association with inflammatory cells. Table 2 depicts the results. In 10 of 30 cases (33%), the prediction of pneumococci from the smear was borne out by routine culture. However, directed culture yielded posi-
tive results in 19 more, so that smear interpretation was actually confirmed 29 of 30 times (97%). For haemophili, undirected culture confirmed the smear 13 of 18 times (72%), whereas directed culture confirmed 4 more, for a total of 17 of 18 (94%). In the case of yeasts, all 15 (100%) were confirmed by directed culture, in contrast to only 9 of 15 (60%) undirected cultures.

Comparison of three hospital laboratories. Table 3 compares the sensitivity of routine cultures at the three hospitals. Also given are data regarding directorship, supervision, staffing, workload, and procedure. Despite the differences, all three commonly reported cultures negative for pathogens that were identifiable on smears.

Survey of hospital practices. Completed questionnaires were received from 37 hospital laboratories, processing an aggregate of 5,300 sputum specimens per month. The average number per month for all hospitals, including Philadelphia General, was 145, with a range of 20 to 470. An analysis of the replies is presented in Table 4. A number of findings deserve note.

First, only one in three laboratories routinely makes smears from sputum. Second, one in four does not describe cellular composition. Third, only one in four reports out an evaluation of the specimen. Fourth, only one in three uses microscopic findings to guide culture. Fifth, not one rejects a specimen because microscopic examination indicates that it is unsatisfactory.

When grouped according to workload, 7 of the 13 laboratories routinely making smears are among the 11 processing more than 200 specimens per month. Also, specimen evaluation is offered by 45, 28, and 15% of laboratories with monthly workloads of more than 200, 100 to 200, and fewer than 100 specimens, respectively. Thus, it appears that more use is made of microscopic examination in laboratories with larger workloads. However, one laboratory processing 300 sputum specimens per month reported that smears were made only on request, the cells were not described, no evaluation was offered, and culture was performed independent of microscopic findings.

DISCUSSION

Our findings support the viewpoint that any attempt to diagnose respiratory infection by sputum culture without microscopic examination invites confusion and misinformation. Unfortunately, the limitations of the Gram stain seem to have received much more emphasis than its virtues. This bias is reflected both in the spotty use of the Gram stain and in the noninterpretative manner in which findings are usually reported.

As a means of judging the quality of a specimen, microscopic examination is unequalled. In contrast to gross inspection, it permits unequivocal identification of inflammatory and salivary cellular constituents. This application of sputum

Table 2. Sensitivity of routine culture and culture directed by Gram stain interpretation

<table>
<thead>
<tr>
<th>Gram stain interpretation (no. of samples)</th>
<th>No. culture positive</th>
<th>Routined</th>
<th>Directed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumococci (30)</td>
<td>10</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Haemophili (18)</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Yeasts (15)</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

*a On 4 of 59 Gram stains, both pneumococci and haemophili were recognized. They are tabulated separately.

*b Culture independent of Gram stain interpretation.

*c Attention directed to organism identified on Gram stain.

Table 3. Sputum microbiology in three hospital laboratories

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Description of laboratory</th>
<th>Sensitivity of routine culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Technical personnel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of sputum specimens/mo</td>
</tr>
<tr>
<td>Municipal</td>
<td>Director</td>
<td>Supervisor</td>
</tr>
<tr>
<td>University</td>
<td>Internist (M.D.)</td>
<td>B.S. (biology)</td>
</tr>
<tr>
<td>Community</td>
<td>Microbiologist (Ph.D.)</td>
<td>M.S. (microbiology)</td>
</tr>
<tr>
<td></td>
<td>Pathologist (M.D.)</td>
<td>MT (ASCP)</td>
</tr>
</tbody>
</table>

*a Numerator, number of routine cultures positive; denominator, number of directed cultures positive.

*b SBA, Sheep blood agar; CA, chocolate agar; MC, MacConkey agar; HBA (A/A), horse blood agar in duplicate, aerobic and anaerobic; EMB, eosin-methylene blue agar.

c Minimum training for at least half of bench technicians.

*d MLT (ASCP), certified Medical Laboratory Technician (American Society of Clinical Pathologists).

*e MT (ASCP), certified Medical Technologist (American Society of Clinical Pathologists).
microscopy has been recognized for a considerable time (3, 5, 7, 12, 13), and it is remarkable that the majority of laboratories continue to report "sputum culture" results without qualification on specimens easily recognizable as consisting wholly or partly of saliva.

Less well appreciated is the feasibility of identifying certain microorganisms on a Gram-stained smear. Quite reasonably, it is considered dangerous to attempt a definitive diagnosis in this manner, and discrepancies between impressions gained from smear and from culture are usually settled in favor of the latter. This rule, however, needs to be reexamined. Before anaerobic culture achieved widespread clinical application, the smear was often the only clue to the true nature of so-called sterile abscesses (8). One may safely assume that not only anaerobic, but also aerobic and facultative, organisms sometimes escape detection, especially in mixed culture. Unfortunately, no expectorated specimen fails to yield at least some bacteria under all conditions of incubation, so that the stimulus to reconcile microscopic and cultural findings has not been as strong as in the case of sterile specimens. Consequently, it is usually assumed that microscopic findings not borne out in culture have simply been misinterpreted.

However, the fallibility of cultures, as routinely done, should not be underestimated. An extreme example is seen in Fig. 8, thanks to the unmistakable microscopic appearance of the yeast. Less direct, but no less convincing, evidence was presented many years ago by students of pneumococcal infection, who frequently failed to recover the pathogen from the sputum of patients with bacteremic pneumonia (2, 14). On this basis, Barrett-Connor suggested that Gram stain examination was superior to culture, with which we would agree. Others also have reported discrepancies between Gram stain and culture findings both for pneumococci and for haemophilus (10). In all these studies, culture was performed in a routine manner, in which colonial appearance was used to draw attention to the possible presence of pathogens. Negative culture reports were apparently accepted as such and were explained by inadequacy of the specimen, uneven distribution of microorganisms, delay in laboratory processing, or competition from other microorganisms. To our knowledge, however, the question whether sputum cultures that appear negative really are negative has not been systematically studied. Our approach was to search specifically for whatever appeared unmistakable on the smear. With each specimen acting as its own control, we found that as many as half of perfectly typical smears (Fig. 2, 4, and 6) were confirmed in culture only when attention was directed to the suspected organism. The selection of typical smears is emphasized to point out that the search was not directed to a
rare or occasional organism but to the predominant flora, so that what was being missed might well have been clinically significant.

The examination of Gram-stained smears thus contributed materially to diagnosis, not as a source of tentative impression but as a means of improving the performance of culture.

In advocating mandatory microscopic examination of all sputum specimens, we would expect it to serve useful functions in specimen evaluation, culture direction, and clinical interpretation.

Specimen evaluation. Physicians themselves often do not collect sputum from their patients and may have no idea how adequate a specimen is sent for culture. Therefore, they must be told whether information derived therefrom is likely to be relevant to the infection. At the very least, this means appending to the culture report a statement that, rather than merely listing the microscopic findings, clearly states whether the specimen consists predominantly of inflammatory exudate or saliva. Better still, specimens judged to consist mainly of saliva should not be cultured at all, as suggested by Bartlett (3), since the results are meaningless or, worse, misleading. Although Bartlett has also suggested holding an unsatisfactory specimen in case a better one cannot be obtained, we would go further, taking the stand that an unsatisfactory specimen has no diagnostic value, even if it is the only one available, and discarding it without further processing. This approach has ample precedent. Many laboratories will not fully process contaminated specimens, whether contamination is defined as submission in a non-sterile container or (for urine) an implausible mixture of three or more bacterial species. There are as yet no universally accepted criteria for sputum contamination. Our formula differs from those of Bartlett (3) and Murray and Washington (12), and the best one possibly still remains to be developed. Irrespective of detail, the screening procedure is a quickly performed low-power microscopic examination, which will lead to considerable saving of laboratory time and expenses as well as elimination of useless information.

Culture direction. Competent technologists are expected to examine culture plates and, knowing the source of the specimen, to isolate, quantitate, and identify possible pathogens. Our data, from three independent laboratories, indicate that everyday practice falls far short of this goal. Furthermore, we wonder whether the "nonvalue of sputum culture" described by Barrett-Connor (2) and similar experiences of others (10, 14) do not illustrate the shortcomings of the usual routine as much as the limited potential of culture. Not surprisingly, we confirmed that a careful search uncovers a good deal more than a routine one. And one way to insure a careful search, without relying on the ever-fading effects of education, admonition, and so on, is to know that a presumptive identification has already been made and awaits confirmation. This is the meaning of directed culture, and it requires interpretation of the smear in terms not of shapes and colors but of specific identification. Granting that such identification is not definitive until confirmed in culture, we would emphasize that it is intended primarily for inter-
nal use by the laboratory. With adequate qualification, however, this information can be extremely helpful to the clinician. For example, a predominance of gram-negative bacilli (Fig. 5), although not permitting even a tentative specific diagnosis, does rule out gram-positive coccal infection. For staphylococci, the microscopic appearance is virtually diagnostic (Fig. 3); their presence on a smear should be reported immediately and by name (not as "gram-positive cocci in pairs and clusters") because of the crucial therapeutic implications. Conversely, since staphylococcal infections are characterized by dense bacterial populations (19), failure to recognize them with ease in the smear should cast some doubt on the significance of a positive culture.

Identification of pneumococci is also feasible, provided certain cautions are observed. The poor performance attributed to Gram-stained smears is probably due to at least two correctable factors. First, as discussed above, the error is frequently in the culture. Second, there is unnecessary confusion between pneumococci and viridans streptococci. A common mistake is to attach significance to the occasional gram-positive diplococci in a mixed bacterial population, which, more often than not, are constituents of normal flora. A typical picture, on the other hand, is unlikely to mislead the microbiologist (1) and should be reported unhesitatingly as suggestive of pneumococcal infection. Figure 9 illustrates the different contexts in which gram-positive diplococci are frequently seen.

The presence of pneumococci, if strongly suspected, can in fact be proved by the capsular swelling reaction with polyvalent serum (11), which can be performed on the sputum specimen itself. To do so requires, of course, that the Gram-stained smear be read immediately, while the specimen is still available and reasonably fresh.

Clinical interpretation. The microbiologist alone cannot decide what kind of respiratory infection a patient has, regardless of his findings. However, he makes a twofold contribution in identifying plausible pathogens and finding them in sufficient numbers to distinguish pathological replication from a benign carrier state. All organisms comprising the subject of this study are frequently found in small numbers in the upper airways or mouth. If it takes a concerted (or directed) search to find them in a sputum specimen, does this not mean that their numbers are too few to be significant? The answer to this question is in the method of determining numbers. Conventionally, such determination is based on inspection of primary culture plates with what amounts to a rough colony count, in other words, a count of organisms viable at the time of plating. For various reasons, however, both technical and physiological, many of the bacterial cells present in the specimen
and easily recognized by direct visualization may fail to reproduce in culture. Therefore, when microscopic examination shows a predominance of organisms so as to make a diagnosis virtually certain (a decision that can usually be made in less than 10 s) and the culture does not confirm the expected proportions, the correctness of the culture may well be called into question. Specifically, if the sputum of a patient with a typical clinical picture appears on smear as in Fig. 2 and yields few or no pneumococci in culture, a diagnosis of pneumococcal pneumonia would nonetheless appear justified (2).

In certain clinical situations, even cultures ideally performed yield results that are inherently uninformative, misleading, or likely to be questioned by the clinician. One example is aspiration lung abscess, culture of whose contents usually reveals normal oral flora. Furthermore, antibiotic therapy, prolonged hospitalization (9), or chronic alcoholism (6) may lead to upper respiratory colonization with gram-negative bacilli. If these are detected in culture, there is a danger of relating the pathology to these quasipathogens and misdiagnosing the true bacterial etiology. Only microscopic examination, as shown in Fig. 10, can tell the true story. Conversely, the clinician may disregard a true pathogen because it is rarely associated with respiratory infection. In one such example, coagulase-negative staphylococci were repeatedly isolated from a patient with chronic bronchitis. Only after inspection of the sputum smear (Fig. 11) could the possibility of laboratory contamination or undetected anaerobes be dismissed.

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LITERATURE CITED


