Increased Sensitivity of Acid-Fast Smears
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Evaluation of the centrifuges used in the mycobacteriology laboratory indicated a failure to develop sufficient relative centrifugal force for optimal concentration of acid-fast bacilli. Retrospective analysis of 14,509 specimens received between 1 July 1973 and 30 June 1975 and sedimented at 1,260 × g relative centrifugal force revealed a positive smear rate of 1.8% and a positive culture rate of 7.1%, with a correlation between positive paired smears and cultures of 25.2%. After increasing the relative centrifugal force from 1,260 × g to 3,800 × g at maximum radius, the positive smear and culture rates were 9.6 and 11.6%, respectively, with a correlation between positive paired smears and cultures of 82.4%. The sensitivity of acid-fast smears is directly related to the relative centrifugal force achieved while concentrating the specimen by centrifugation.

The acid-fast smear has been used as an aid in the diagnosis of mycobacterial disease for many years. The reliability and usefulness of the acid-fast smear in clinical practice has recently been challenged. Boyd and Marr (1) examined 4,570 specimens and reported that only 22% (26/118) of their culture-positive specimens had produced positive acid-fast smears. They concluded that the acid-fast smear was a poor screening technique for a low-incidence population. Mar-raro et al. (4) reported a smear/culture correlation of 24.1% (14/58) among 1,983 specimens. Burdash et al. (2) reported a correlation of 42.7% (97/227) from 6,199 specimens and supported continued use of the acid-fast smear as an aid in the diagnosis of mycobacterial infections. The latter investigators were unable to attribute the discrepancy between their results and those of previous investigators to differences in geographical location or laboratory technique. Pollock and Wieman (5) reported a smear/culture correlation of 49.7% (197/396) from 6,880 specimens. They concluded that the low sensitivity of smears with respect to cultures was related to low numbers of organisms present in specimens from patients in a low-prevalence general hospital population, and they supported continued use of acid-fast smears as a reliable diagnostic tool.

The problem of negative smears from tuberculous patients was brought to our attention when one clinic submitting specimens to our laboratory notified us that they had received negative acid-fast smear reports on sputa they had found to be positive when examined as direct smears. In an attempt to confirm these findings, the sputa from six patients with mycobacterial disease were used to prepare direct smears. These sputa were then concentrated according to our usual procedure except that additional smears were prepared from the supernatant fluid before decantation. All six direct smears were positive for acid-fast bacilli. Four of the supernatant fluids contained acid-fast bacilli, and only one of the concentrated sediments was smear positive for acid-fast bacilli (Table 1). These alarming results prompted us to determine the effect of relative centrifugal force (RCF) on the rates of smears and cultures positive for acid-fast bacilli.

MATERIALS AND METHODS

Specimens. Data from 38,867 specimens received between July 1973 and December 1978 were tabulated. The predominant specimen was sputum, but data from urine, gastric lavage, bronchial washings, and pleural fluids were included in the study. Data from specimens that were contaminated when cultured were excluded from this study and reported unsatisfactory. Specimens were received from new cases, reactivated cases, and patients undergoing drug therapy. The data include all potentially pathogenic mycobacteria isolated, 30% of which were species other than Mycobacterium tuberculosis. Isolates of 211 saprophytic mycobacteria, predominantly Mycobacterium gordonae, were excluded from the data, because all had negative smears and three or fewer colonies on culture.

Digestion and concentration of specimens. Specimens were digested and decontaminated by the N-acetyl-L-cysteine–sodium hydroxide method (3).

Centrifuges. Two IEC/Damon model UV centrifuges were in routine use during the first 24 months. These were replaced with one IEC/Damon model PR-J centrifuge for the next 18 months. A Beckman J-6 centrifuge with windshield was purchased for the mycobacteriology laboratory and was in use in the last 24 months of this study. The centrifuge characteristics during the three periods of the study are presented in Table 2. Falcon 50-ml plastic conical tubes were used during the study.
Microscopy. Smears were prepared from concentrated sediments, heat fixed, and stained using the fluorochrome method (7). Slides were examined using a Leitz Dialux microscope equipped with 100 W halogen illumination and incident fluorescence attachment with BG23 and KP500 filters. The smears were scanned with a 25× objective, and the presence of acid-fast bacilli was confirmed using a 63× objective. The ocular magnification was ×10. The smear was called positive if three or more acid-fast bacilli were seen per smear.

Culture. Lowenstein-Jensen and Middlebrook 7H10 slants were inoculated with concentrated sediments. The cultures were incubated at 36°C in 10% CO2 and observed for growth over a 6-week period.

Paired specimens. To compare our results with those of previous investigators (1, 2, 4, 5), we used the method recently described by Kubic (Bull. Int. Union Against Tuberc., in press) and presented in Table 3.

RESULTS

The initial period of the study was retrospectively analyzed by examining smear and culture reports from 14,509 specimens received between July 1973 and June 1975. A total of 1,030 (7.1%) of the specimens yielded positive cultures, but only 260 (1.8%) of these specimens were smear positive for a smear/culture correlation of 25.2% at an RCF of 1,260 × g. During the interim period, July 1975 through December 1976, 9,727 specimens were examined, resulting in 1,086 (11.2%) positive cultures and 440 (4.5%) positive smears for a smear/culture correlation of 40.5% at an RCF of 3,000 × g. Between January 1977 and December 1978 a total of 14,631 specimens were processed, yielding 1,701 (11.6%) positive cultures and 1,401 (9.6%) positive smears for a smear/culture correlation of 82.4% at an RCF of 3,800 × g.

A summary of our findings compared to those of previous investigations is presented in Table 4.

DISCUSSION

In contrast to hospital laboratories, public health laboratories receive a significant number of specimens from patients on continuing drug therapy. A review of the smear-positive-culture negative reports revealed that most of these false-positive smears were made from repeat specimens from patients on drug therapy. Some patients remained smear positive for several months after cultures were negative. Of the 334 false-positive smear reports during the past 2 years, 318 were from patients with previously diagnosed mycobacterial disease. The remaining 16 reports were on undiagnosed patients, constituting an actual absolute false-positive smear rate of 0.12% (16/12,946). False-positive smears may result from death or inactivation of organisms by antituberculosis drugs or by severe decontamination procedures (6).

The number of positive smears and cultures increased dramatically as the RCF used for processing specimens increased from the initial 1,260 × g to 3,000 × g. The final increase to an RCF

TABLE 1. Recovery of acid-fast bacilli before and after concentration with an RCF of 1,260 × g at Rmax

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>No. of bacilli recovered (per high dry field, ×630)</th>
<th>Before concn</th>
<th>In supernatant fluid</th>
<th>In sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-10</td>
<td>1-10</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1-10</td>
<td>None</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&lt;1</td>
<td>None</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&gt;10</td>
<td>&lt;1</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Characteristics of centrifuges used during study

<table>
<thead>
<tr>
<th>Period</th>
<th>Centrifuge</th>
<th>Head configuration</th>
<th>rpm</th>
<th>Radius (Rmax)</th>
<th>RCF (× g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1973–June 1975</td>
<td>IEC/Damon UV</td>
<td>8 place</td>
<td>2,400×</td>
<td>195</td>
<td>1,260</td>
</tr>
</tbody>
</table>

* Rmax is defined as the radius in millimeters to the bottom of the centrifuge tube.
* Maximum rpm obtainable.
* Maximum rpm limited by centrifuge tubes.
of 3,800 × g further improved smear rates but failed to alter culture recovery rates. With increased RCF, more viable acid-fast bacilli are present in the sediment for inoculation onto culture media, resulting in earlier growth and more rapid identification of organisms.

An increased sensitivity can be misleading, because it may be accompanied by a decreased true-positive and an increased relative false-positive rate (see Table 4). We believe this can be explained by the large number of treated patients who provide specimens that are smear positive but culture negative. To test this hypothesis we deleted the 318 treated patients mentioned above from the data in the "3,800" column of Table 4 and recalculated the percentages; these new results are presented in the column headed "3,800 corrected" in Table 4.

A large population of treated patients often discharge dead or noncultivable acid-fast bacilli, and the number increases as the RCF is increased. This may be compensated for by increasing from 3 to 10 the number of acid-fast bacilli needed to label as positive a smear from treated patients. To take full advantage of this switch from 3 to 10 bacilli for a positive smear, it is necessary for a diagnostic laboratory to maintain a patient file. Workers can then quickly ascertain whether they are dealing with a new or "old" patient; for new ones, three acid-fast bacilli are required for a positive report whereas "old" ones need 10 acid-fast bacilli per smear (Kubica, Bull. Int. Union Against Tuberc., in press).

Because of the low specific gravity of the acid-fast bacillus, a low RCF will have a buoyant rather than a sedimenting effect, and few organisms will be present in centrifuged sediments. An RCF of 3,800 × g at maximum radius (Rmax) greatly improves the sedimenting effect, but fails to remove all smear-detectable acid-fast bacilli from the specimens. An RCF in excess of 4,500 × g is required to effectively clear the supernatant fluid, but such RCF exceeds the stress limits of most centrifuge tubes used in mycobacteriology laboratories.

Although the maximum recommended centrifugation for the Falcon 50-ml plastic conical tube is 3,000 × g, we avoided a problem with the tubes splitting at 3,800 × g by using rubber cushions. The tubes are used one time and then discarded.

The sensitivity of the acid-fast smear is directly related to the RCF applied during concentration of the specimen by centrifugation. We have observed a significant increase in the number of positive smears and cultures since adopting an RCF of 3,800 × g.

**LITERATURE CITED**


