Clinical and Laboratory Analyses of Cytospin-Prepared Gram Stains for Recovery and Diagnosis of Bacteria from Sterile Body Fluids

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The smear of a clinical specimen provides essential laboratory information that is used to make therapeutic decisions. For this study, smears were made by centrifugation in a Beckman Microfuge 11 (Beckman Instruments, Palo Alto, Calif.) and in parallel by using a Cytospin 2 apparatus (Shandon Inc., Pittsburgh, Pa.). Of 350 consecutive body fluid specimens examined, 50 (14.3%) grew bacteria. Both methods were culture and smear positive for 24 (6.9%) specimens; 18 (5.1%) specimens were cytocentrifuge smear positive, culture positive, and high-speed centrifugation (HSC) negative; 3 (0.8%) were culture negative and positive by both smear methods; and 1 (0.2%) was HSC smear positive, culture positive, and cytocentrifuge negative. Seven (2.0%) specimens were culture positive and negative by both smear methods. Clinically, cytocentrifuge preparations showed greater sensitivity for culture-positive specimens and a closer correlation with the CFU per milliliter than HSC did, resulting in a greater ability to treat patients with specific therapies. In addition, analysts needed to examine only a 6-mm-diameter area on the slide, cells and microbes were somewhat larger and more regular in appearance, and smears stained more uniformly. Because of the increased clinical and laboratory utility of the cytocentrifuge, its use is recommended in clinical microbiology laboratories for all sterile body fluid specimens.

Rapid diagnosis by Gram staining for a suspected infectious organism from a sterile body fluid is a major responsibility of our Clinical Microbiology Laboratory. In addition to suggesting a diagnosis, it guides clinicians in the choice of antibiotics. This is especially critical in patients with life-threatening diseases such as meningitis, peritonitis, and obstetric infections, in which the clinical efficacy is dependent on the therapeutic intervention that is used. Because the concentration of microorganisms in body fluid specimens may be low and/or the volume of specimen submitted for study may be extremely low, a technique for concentrating body fluids is recommended for body fluids that are to be used in smears and cultures (5, 6, 9). In the Hematology and Cytology laboratories, a cytocentrifuge technique is commonly used to prepare body fluid specimen smears when the intent is to observe the morphologies of the cells (2, 4, 8). These methods sometimes demonstrate organisms that were not observed in the smears prepared from the same specimens by the Clinical Microbiology laboratory. Because of this phenomenon, we undertook an investigation into the sensitivity and specificity of smears prepared with the cytocentrifuge compared with those of smears prepared by the high-speed centrifugation (HSC) method currently used in our laboratory. Both methods were evaluated for laboratory and clinical utility.

MATERIALS AND METHODS

Specimens. Three hundred fifty consecutive sterile body fluids from sterile body sites submitted to the Clinical Microbiology Laboratory for evaluation were included in this study. Specimens were processed according to standard laboratory procedures, as follows. For clear or nonviscous cerebrospinal fluid (CSF) or dialysis fluid, 5 to 10 drops (0.25 to 0.50 ml; 5 drops [0.25 ml] was used, unless >5 ml was received by the laboratory) was used. For nonpurulent or noncellular fluid (any body fluid that was not obviously bloody or cloudy), 2 to 3 drops (0.10 to 0.15 ml) was used. For cellular or purulent specimens (gross pus, bloody, or cloudy), 1 to 2 drops (0.05 to 0.10 ml) was used, and 1 to 2 drops of sterile distilled water, which acted as a lysing agent for bloody specimens, was added. All specimens were centrifuged at 2,000 rpm (350 × g) for 8 min. Two smears were prepared for each specimen, one by HSC and the other by cytocentrifugation with the Cytospin 2 apparatus (Shandon Inc., Pittsburgh, Pa.). Prior to use, slides were dipped in alcohol, flamed, and air dried. After preparation of the smears, the specimen was air dried and fixed in absolute methanol for 2 min, and both smears were stained with Gram stain. HSC smears were examined as part of the routine processing of a specimen. In examinations of smears that were prepared by cytocentrifugation, the observer was blinded to the original results of the HSC smear and culture, and the results were correlated at a later date. Both smears were read by the same investigator, and smears prepared by cytocentrifugation were not read simultaneously with the HSC smear. The smears obtained by both procedures were quantified for cells and microorganisms as well as morphology and Gram stain characteristics.

Smear 1: HSC. The first parallel smear was evaluated by HSC, which entailed centrifugation at 11,000 rpm or 10,000 × g for 5 min in a Beckman Microfuge 11 (Beckman Instruments, Palo Alto, Calif.). A measured volume of fluid was not used for HSC preparations. The volume used was dictated by the amount of specimen received. A maximum of 1.0 ml could be added to the tube that fit into the microcentrifuge. This maximum volume was used whenever possible. Sediment was then used for preparation of the smear and inoculation of the appropriate medium.

Smear 2: cytocentrifuge. On the basis of our preliminary observations, the following procedure was used for smears prepared by cytocentrifugation. The volume of specimen

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pipetted into the cytofunnel chamber (Shandon Inc.) was adjusted on the basis of its cellularity and/or viscosity, as described above.

The Cytofunnel is a disposable chamber with a cap and a permanently mounted filter card with a maximum volume of 0.5 ml. The filter card absorbs the supernatant, while cells and microorganisms are centrifuged through a hole in the filter paper strip and are deposited on a 6-mm-diameter circular area on the slide. A clip containing the glass slide and cytofunnel is mounted together and placed in the Cytospin 2 rotor head, the appropriate amount of sample is added with a sterile pipette, and the rotor head is sealed. All samples were processed at 2,000 rpm (350 x g) for 8 min. The cytofunnel with the attached filter card is then discarded, and the clip is placed in Amphi (National Laboratories, Montvale, N.J.) and rinsed in water before the next use.

RESULTS

The number and type of clinical samples evaluated are given in Table 1. The number and percentage of positive specimens in relation to the total number of specimens examined for both leukocytes and organisms are noted in Table 1 for each smear method and are compared with the culture results. Cytocentrifuge-prepared smears showed a better correlation with culture and the presence of inflammation than did HSC for all specimen types examined except CSF specimens. Overall, of 350 sterile body fluid specimens submitted, 50 (14%) were positive by culture. Of that total, 42 (12%) were positive by cytocentrifugation, whereas 25 (7.1%) were positive by HSC. Most critically, peritoneal fluid HSC smears, which typically exhibit poor sensitivity compared with culture, were less often positive than the parallel cytocentrifuge smears were. Of all the peritoneal fluid specimens, 6 (1.8%) specimens were positive by HSC, 14 (4%) specimens were positive by cytocentrifugation, and 17 (4.8%) specimens were positive by culture ($P < 0.05$; Wilcoxon signed rank test). On the other hand, the presence of leukocytes as an indicator of inflammation showed that there was no significant difference between their presence or absence in relation to culture-positive specimens when both smear preparations were examined.

Table 2 shows a comparison of HSC and cytocentrifuge smear results compared with those of culture. Again, cytocentrifuge-prepared smears exhibited significantly higher numbers of positive specimens that correlated with culture results than parallel HSC smears did. Only one specimen was HSC and culture positive but cytocentrifuge negative. There were seven culture-positive specimens that were negative by both smear methods, two of which were positive in broth only. There were also three specimens that were positive by both smear methods but that were culture negative.

Tables 3 and 4 show the correlation of smear methods with culture quantification. Smears were quantified as follows: 0, no organisms; 1+, 0 to 5 organisms; 2+, 5 to 10 organisms; 3+, 10 to 25 organisms; 4+, >25 organisms per oil immersion field. Culture quantification was determined as 0 (no growth), 1+ (growth in the first quadrant), 2+ (growth in two quadrants), 3+ (growth in three quadrants), and 4+ (growth in all quadrants). Table 3 provides a comparison of smear

### Table 1. Comparison of HSC and cytocentrifuge smears of sterile body fluids

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Examined</th>
<th>Cells in HSC smear</th>
<th>Cells in cytocentrifuge smear</th>
<th>Organisms in HSC smear</th>
<th>Organisms in cytocentrifuge smear</th>
<th>Total culture positive</th>
<th>No. of positive cultures/no. of HSC smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>123 (38)</td>
<td>120 (34)</td>
<td>121 (35)</td>
<td>3 (0.8)</td>
<td>3 (0.8)</td>
<td>3 (0.8)</td>
<td>30</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>80 (23)</td>
<td>75 (21)</td>
<td>78 (22)</td>
<td>6 (1.8)</td>
<td>14 (4)</td>
<td>17 (4.8)</td>
<td>15/2</td>
</tr>
<tr>
<td>Amniotic</td>
<td>17 (4.3)</td>
<td>15 (4.3)</td>
<td>16 (4.5)</td>
<td>1 (0.2)</td>
<td>2 (0.5)</td>
<td>2 (0.5)</td>
<td>20</td>
</tr>
<tr>
<td>Other</td>
<td>121 (35)</td>
<td>112 (32)</td>
<td>112 (32)</td>
<td>15 (4.3)</td>
<td>23 (6.6)</td>
<td>28 (8)</td>
<td>27/1</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>322 (92)</td>
<td>327 (93)</td>
<td>25 (7.1)</td>
<td>42 (12)</td>
<td>50 (14)</td>
<td>47/3</td>
</tr>
</tbody>
</table>

* Other, ascitic, pleural, bile, abscess, nephrostomy, pericardial, joint, or dialysate fluid.
* Four smears with positive culture showed no leukocytes.
* One smear with positive culture showed no leukocytes.

### Table 2. Comparison of HSC and cytocentrifuge smears with culture results

<table>
<thead>
<tr>
<th>Result</th>
<th>No. (%)</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture, HSC, and cyta</td>
<td>24 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Culture and cyto, HSC negative</td>
<td>18 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Culture and HSC positive, cyto negative</td>
<td>1 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Culture positive, HSC and cyto negative</td>
<td>7 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Culture negative, HSC and cyto positive</td>
<td>3 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Culture and HSC negative, cyto positive</td>
<td>1 (0.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Cyto, cytocentrifugation.
quantification for each method compared with culture quantification. The positive or negative smear results for these 34 specimens were in uniform agreement regardless of the culture result. For instance, 24 specimens were positive by culture and both smear methods, 3 specimens were culture negative but positive by both smear methods, and 7 specimens were culture positive but negative by both smear methods. Of 34 cytocentrifuged smears, 15 (44%) were in agreement with culture quantification, whereas the same was true for only 5 of 34 (15%) HSC smears. There were 7 cytocentrifuged smears with a higher quantified value than culture, and there were 12 smears with a lower quantified value (35%). Similarly, there were 7 HSC smears with a higher quantified value than that obtained by culture, but there were 22 (65%) HSC smears with a lower quantified value. Cytocentrifuge quantification correlated better with culture quantification, with all growth amounts being >1+. This was most obvious in the 16 cultures that were 4+, in which 4 cultures were 4+ with the HSC smear and 15 cultures were 4+ with the cytocentrifuge smear. Table 4 shows that there was a good correlation between culture and cytocentrifuge quantification for the 18 specimens that were culture and cytocentrifuge positive but HSC negative. However, the cytocentrifuge smear quantification range showed greater variability with these cultures. Finally, Table 5 shows the average range of the cell length and organism measurements determined by two separate examiners, with 10 of each morphologic type of organism examined.

Other parameters that we examined included the use of albumin as an adherence factor. Approximately 100 smears were examined in parallel, and no difference in the rate of positive smears was found. In contrast, it appeared that the albumin created a background precipitation that made examination of the smear more difficult. The addition of an additional agent into a sterile body fluid also adds a potential source of contamination. We also examined minimal concentrations of organisms that could be detected by using the two centrifugation methods by examining cultures of Escherichia coli, Staphylococcus aureus, Neisseria spp., and Haemophilus spp. grown in Trypticase soy broth (Difco Co., Detroit, Mich.) at concentrations ranging from 10³ to 10⁶ organisms per ml. Few organisms were noted on the cytocentrifuge preparations with 10⁶ organisms per ml, and no organisms were seen on the HSC preparations with 10⁵ organisms per ml. At a concentration of 10⁴ organisms per ml, all cytocentrifuge preparations were positive, yet only about 50% of the HSC preparations were positive.

**DISCUSSION**

The presence of microorganisms in normally sterile body fluid specimens may be representative of life-threatening infections. In the clinical microbiology laboratory, the routine protocol for the preparation of a sterile body fluid includes centrifugation of the fluid at 1,000 × g for 15 min or 10,000 × g for 15 min specifically if we are attempting to identify Haemophilus influenzae (5). The most recent edition of the *Manual of Clinical Microbiology* (5th edition) (6) recommends the use of centrifugation at 1,500 × g for 15 min for all specimens. Two main problems exist with the identification of organisms by these protocols. First, small volumes of specimens, such as CSF and joint fluid, sometimes do not allow the concentration of organisms after centrifugation. Second, very large volumes of specimens, such as peritoneal fluid, dialysate, or ascitic fluids, need special concentrating techniques. Previously published information on the sensitivity of the HSC and cytocentrifuge methods claim that they are consistently sensitive at 10⁵ organisms per ml and that both methods are 100% sensitive at 10⁹ organisms per ml (9). We found that the sensitivity was less than this. Therefore, a more sensitive means for detecting those organisms that are present at a low level or that are less visible after Gram staining has been recognized.

For example, acridine orange, Wayson, and other staining procedures have shown enhanced sensitivity for observing organisms (1, 3, 7). However, these stains do not increase the number of organisms on the slide. Because of enhanced morphologic characteristics, cytocentrifugation has been used very successfully in our Cytology Laboratory for the examination of body fluids. We also found that we can routinely use cytocentrifugation with the Cytospin 2 apparatus reliably and easily for the preparation of sterile body fluids for the direct detection of organisms by Gram staining. The smear quantification by cytocentrifugation was better correlated with culture than it was by standard HSC. Cytocentrifugation allowed the use of a smaller specimen volume and a single staining procedure.

The utility of cytocentrifugation in the laboratory was also favorable. Although we did not time the viewing of smears, the distinct impression was that the time required to thoroughly read the smear was significantly reduced. This has also been noted by other investigators for the detection of *Pneumocystis carinii* from cytocentrifuge smears of bronchoalveolar lavage and sputum specimens (3). The reasons included the small defined area in which the specimen was deposited, the uniformity of staining and the spreading of the leukocytes, and the enhanced resolution of the organisms. It
was also helpful for lysing heavily bloody specimens. The addition of the instrument and smear preparation was well accepted by the medical technologists and was preferred over the HSC method. Because of the clinical and laboratory advantages seen with the use of cytocentrifugation, we currently use the Cytospin 2 apparatus for examination of all sterile body fluids in our Clinical Microbiology Laboratory.

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REFERENCES