Evaluation of Cytocentrifuge Apparatus with Special Reference to the Cellular Recovery Rate

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Two types of commercially available cytocentrifuge apparatus (type A and type B apparatus) using disposable funnels were compared for percentage of cell recovery and degree of cell preservation. The cellularity of each cell suspension was determined using a Sysmex micro cell counter for blood analysis, and the cell recovery was obtained by counting cells in the total smeared area on the May-Grünwald Giemsa (MGG)-stained slide. Overall recovery rate by the type A apparatus was between 54.3% and 74.9% with a mean of 63.0%, whereas, the recovery rate for type B apparatus was between 30.6% and 51.8%, with a mean of 42.5%, indicating that the type A apparatus was significantly better. In the type A apparatus, a higher yield of all cells was obtained (69.7-74.9%) in the group of low cell counts (350 cells/a 5 ml), which was run for 10 minutes at 2,000 rpm. On the other hand, in the type B apparatus a higher yield of all cells was obtained (38.6-42.6%) in the group of low cell counts, which was run for 10 minutes at 2,000 rpm. Cellular structure was better preserved on the slides in the type A apparatus. However, the percentage of ghost cells was somewhat higher in the type B apparatus. The cytocentrifugation of the type A apparatus consistently recovered a higher percentage of cells than with the type B apparatus.

Using the type A apparatus, a high rate of cellular recovery, which is extremely important, such as for accurate morphological evaluation of cerebrospinal fluid, can be consistently obtained.


Cerebrospinal fluid
The diagnostic importance of the cellular examination of cerebrospinal fluid (CSF) has been repeatedly emphasized. It is well known that the CSF cytomorphology is dependent upon the techniques of cellular collection and processing. Since Sayk described the so-called sedimentation technique, many cytopreparations, including the standard counting chamber, direct smear, centrifugation, and membrane filters have appeared. More recently considerable experience with a new centrifugation technique of cell processing called cytocentrifuge has been acquired. Cells in samples of CSF subjected to clinical centrifuges showed severe distortion, and special filtration or sedimentation chambers were therefore designed. However, some laboratories have suggested that the cytocentrifuge technique is superior to other cell-catch procedures. One major consideration that has been studied quantitatively by several investigators is the potential for cell loss with various techniques. In clinical practice there is a need for a simple and rapid method, free from pathogen contamination, during the process whereby body fluids can be cytologically examined. Recently, two different types of instruments using a disposable chamber have been developed. It was our purpose in the study reported here to investigate and compare the cellular recovery rate of each cytocentrifuge apparatus.

Materials and Methods

Human peripheral blood cells were chosen for specimens, because they are composed of single cells with a predictable size in range most cellular samples. Twenty-four samples were obtained from healthy persons and were centrifuged at 2,500 rpm for 5 min. The supernatant and buffy coats were then carefully removed by pipette. The cellularity of each cell suspension was determined using a micro cell counter (CC-108, Sysmex Inc, Kobe, Japan) for blood analysis. Each cellular suspension was then centrifuged at 2,000 rpm for 5 min again. The cell count was adjusted using Cellent solution (CE-310, Sysmex Inc., Kobe, Japan).
After gentle mixing, 0.5-ml aliquots of cellular suspension were immediately subjected to cytocentrifugation both with the type A (Cyto-Tek® Cytocentrifuge, Miles Inc, Elkhart, IN Autosmear, Sakura Finetechical, Tokyo, Japan) and type B apparatus (Cytospin® 2, Shandon Southem Ltd., Cheshire, England at different centrifugation speed with two dif-ferent cell counts. All glass slides, which were used in this study, were uncoated. By means of cytocentrifugation, cells suspended in a fluid are protected against a microscopic slide positioned vertically, which closes the outer end of the chamber. Between the outlet of the chamber and the slide, a filter card punched with a 7 square mm (type A apparatus) and a 6 mm circle (type B apparatus) hole absorbs excess fluid, and the cells are sedimented upon the slide within a 49 mm2 and 28 mm2 area respectively. Further technical data on the similar modeis are given elsewhere ~4 The type B apparatus has a permanently attached white filter card, while the filter card of type A was separated from the disposable chamber. Wet slides are drawn out, dried, and stained by a May-Grünewald-Giemsa (MGG) method. The total number of cells on each slide was counted directly in a microscope at 100X magnification using an eyepiece micrometer (OC-M, Olympus, Tokyo, Japan).

All cell counts were performed by one of the authors (U.M.). In order to eliminate any possible bias, the total number of cells sedimented within a certain area was determined by careful microscopic observation. This procedure is time consuming; it takes least 30 min per slide for the total cells to be counted. The means and the standard deviations of the counted cells in each group were calculated (Table I). The significance of the difference between the two pairs of means was estimated with Student’s test. Each preparation was rated as having “good” preservation if more than 95% of cells present were well preserved and showed good chromatin detail; a “poor” preservation was given if only 70-95% of the cells showed apparatuses on a quantitative basis in order to determine good detail and preservation.

Results
The comparison of cell recovery rate by two different cytocentrifuge apparatuses (type A and type B) with two different cell counts, i.e., high cell counts (3,500 cells/0.5 ml) and low cell counts (350 cells/0.5 ml) together with rotation speed and time is shown in Table I. As can be seen, the overall average recovery rate for the type A apparatus was 63% whereas the average recovery rate for the type B apparatus was 42.5%. These rates were also compared in association with the number of cells used in the preparation, length of time of centrifugation, and rotation speed. In all comparison studies, recovery of cells is better with the type A apparatus than the type B apparatus (Fig. 1). The specimens showed significantly many more cells with the type A apparatus than the type B apparatus in both low cell count (P < .01) and high cell count (P < .01) groups. Recovery rate for individual variations was relatively small (Table I).

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Table I. Cellular Recovery Rate with Cytocentrifuge under Different Conditions

<table>
<thead>
<tr>
<th>Rotation Number</th>
<th>Type A apparatus</th>
<th>Type B apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed and Sample</td>
<td>Cell counts, Mean ? SD</td>
<td>Recovery</td>
</tr>
<tr>
<td>Time No cells/0.5 ml</td>
<td>Low (%)</td>
<td>High</td>
</tr>
<tr>
<td>1,000 rpm, 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High 1) 3.500</td>
<td>2.025</td>
<td>68.6</td>
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<tr>
<td>2) 3.500</td>
<td>2.079</td>
<td>63.4</td>
</tr>
<tr>
<td>3) 3.500</td>
<td>1.996</td>
<td>57.0</td>
</tr>
<tr>
<td>Low 4) 350</td>
<td>242</td>
<td>69.1</td>
</tr>
<tr>
<td>5) 350</td>
<td>239 9</td>
<td>85.4</td>
</tr>
<tr>
<td>6) 350</td>
<td>247</td>
<td>70.8</td>
</tr>
<tr>
<td>1,000 rpm, 5 min</td>
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</tbody>
</table>
### Discussion

Cellular samples of small volume and unknown cell content must be prepared with special care in order to prevent loss of cell and cellular morphology. As far as we know, there are two types of cytocentrifuge apparatus using disposable chambers that are commercially available in Japan. In this study, we compared these cytocentrifuges apparatuses on a quantitative basis in order to determine which cytocentrifuge technique using a disposable chamber would be most suitable for use with cytological materials of small volume.

In the type A apparatus, a high recovery rate was found at high rotation speed and longer length of time of centrifugation. The best cellular recovery rate was between 69.9% and 74.9% with a mean of 71.5% in the group of low cell counts in the type A apparatus, which was run for 10 min at 2,000 rpm. On the other hand, these rates in the type B apparatus were between 42.9% and 51.4% with a mean of 45.0% in the group with low cell counts which was run for 10 min at 1,000 rpm. In the type A apparatus, the sample with a low cell count is higher than those of high cell count groups except at a
rotation speed of 1,000 rpm for 10 min. In no instance was the sample cytocentrifuged by the type B apparatus considered inferior to the sample cytocentrifuged by the type A apparatus. Overall, 21 (88%) of the 24 samples from the type A apparatus showed “good” preservation (Fig. 1) and the remaining 3 (12%) samples showed “poor” preservation. However, 18 of the 24 samples (75%) from the type B apparatus showed “good” preservation and the remaining 6 (25%) samples showed “poor” preservation. We felt that the morphologic details were consistently better in the type A apparatus than in the type B apparatus. The latter sometimes revealed various degrees of nuclear degeneration, i.e., ghosts or sometimes revealed various degrees of nuclear degeneration, i.e., ghosts of autolytic cells. The results of this comparison show that cytocentrifuge of type A apparatus produces a greater total cell yield than does the type B apparatus. We achieved an average 60.7% recovery rate with the type A apparatus in the high cell count group and an average 43.3% with the type B apparatus (Table I). In the group with low cell count, there was a 65.3% recovery rate with type A apparatus and 41.7% with type B apparatus. These results indicated the potential for cell loss with type B apparatus as compared to type A apparatus.

Barrett and King 15 reported a higher recovery rate and better cellular morphologic detail with a Millipore technique, compared to the cytocentrifuge technique. They achieved an 81% recovery rate with Millipore filter, 64% with a Gelman filter, 59% with a Nuclepore filter, and 11% with a cytocentrifuge technique. Recovery of cells with the filter technique is undoubtedly greater, but most cytologists are concerned with obtaining distortion-free cells that can be easily observed by the cytologist or cytopathologist for rapid diagnostic reporting. Barrett and King15 also indicated that the cytocentrifuge technique presented unpredictable recovery and preservation of cells. In our experience, three slides from each group run simultaneously with the same technique may produce similar results; however, the deviation of recovery rate was smaller in type A apparatus than in the type B apparatus (Table I). Since the task of counting all the cells on the smeared area using 100 X magnification is extremely time consuming, we only ran 24 cellular samples on each apparatus for this comparison.

Many investigators have searched for a satisfactory method for concentrating CSF.5, 12, 13, 16-18 The major advantages of the cytocentrifuge technique are the ease and rapidity of processing specimens, the excellent morphologic presentation, and the possibility of utilizing a variety of special staining procedures including those of cytochemistry, immunoperoxidase, and DNA in situ hybridization that cannot usually be utilized with other methods such as the membrane filter technique. We believe that excellent recovery and preservation of cells can be obtained using the type A apparatus, which could contribute to an improved diagnosis of the cytological specimens especially for the CSF samples.

References


