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 (350cells/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.
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Cerebrospinal fluid

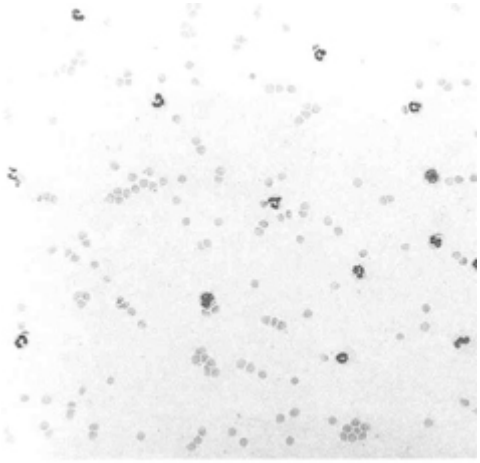
The diagnostic Importance of the cellular examination of cerebrospinal fluid (CSF) has been repeatedly emphasized.

It is well known that the CSF cytomorphology is dependent upon the techniques of cellular collection and pro-cessing. Since Sayk [1] described the so-called sedimentation technique, many cytopreparations, including the standard counting chamber, direct smear, centrifugation, and mem-brane filters have appeared [2]. More recently considerable experience with a new centrifugation technique of cell processing called cytocentrifuge has been acquired.[3-9]. Cells in samples of CSF subjected to clinical centrifuges showed severe distortion, and special filtration or sedi-mentation chambers were therefore designed. [10,11] Meth-ods for using membrane filters have also been recommend- ed.[12,13] However, some laboratories have suggested that the cytocentrifuge technique is superior to other cell-catch procedures. [5] One major consideration that has been stud-ied 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 appara-tus.

Materials and Methods

Human peripheral blood cells were chosen for specimens, because they are composed of single cells with a predicta-ble size in range most cellular samples. Twenty-four sam-ples were obtained from healthy persons and were cen-trifuged 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 Finetechnical, Tokyo, Japan) and type B apparatus (Cytospin® 2, Shandon Souththern 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-Grünwald-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).

Table I. Cellular Recovery Rate with Cytocentrifuge under Different Conditions

					Type A apparatus						Type B apparatus			
Rotation		**Number of**			**Cell counts,**			**Recovery**			**Cell counts,**			**Recovery**
Speed and	**Sample**	**nucleated**			**mean ? SD**			**Rate**			**mean ? SD**			**rate**
Time	**No**	**cells/0.5 ml**	**High**			**Low**		**(%)**	**High**			**Low**		**(%)**
1,000 rpm,														
5 min														
High	1)	3.500	2.025					58,6	1664					47,5
	2)	3.500	2.218	2.079 ± 121				63,4	1443	1.619 ± 158				41,2
	3)	3.500	1.995					57,0	1751					50
Low	4)	350				242		69,1				167		47,7
	5)	350				229	239 ± 9	65,4				148	146 ± 21	42,3
	6)	350				247		70,6				125		35,7
1,000 rpm,														

10 min														
High	1)	3.500	2.244					64,1	1377					39,3
	2)	3.500	2.237	2.191 ± 85				63,9	1814	1.610 ± 22				51,8
	3)	3.500	2.093					59,8	1641					46,9
Low	4)	350				190		54,3				174		49,7
	5)	350				213	208 ± 16	60,9				150	168 ± 16	42,9
	6)	350				220		62,9				180		51,4
2,000 rpm														
5 min														
High	1)	3.500	2.127					60,6	1506					43
	2)	3.500	2.088	2.069 ± 69				59,7	1229	1.388 ± 143				35,1
	3)	3.500	1.992					56,9	1431					40,9
Low	4)	350				215		61,4				109		31,4
	5)	350				220	216 ± 3	62,9				167	128 ± 34	47,1
	6)	350				214		61,1				107		30,6
2,000 rpm,														
10 min														
High	1)	3.500	2.168					61,9	1468					41,9
	2)	3.500	2.140	2.148 ± 17				61,1	1435	1.478 ± 49				41
	3)	3.500	2.138					61,1	1532					40,6
Low	4)	350				245		70,0				135		38,6
	5)	350				262	251 ± 11	74,9				149	142 ± 7	42,6
	6)	350				244		69,7				142		40,6
Mean ± SD				2.122 ± 87*						1.524 ± 166*				
							228 ± 20**						146 ± 24**	
Average (%)								63,0						42,5
*T=10,48606, P < ,01														
**T=8,49715, P < ,01														

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 and 81% recovery rate with Millipore filter, 64% with a Gelman filter, 59% with a Nuclepore filter, and l1 % 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 King[15] 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 l00 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

1. Sayk J. Ergebnisse neuer liquorcytologischer Untersuchungen mit dem Sedimentkammerverfahren. Arzt Wochenschr1954; 9:1042-1046.

2. Bigner SH, Johnston WW. The cytopathology of cerebrospinal fluid:
I. Nonneoplastic conditions, lymphoma and leukemia. Acta Cytol (Baltimore) 1981;25:335 353.

3. Hansen HH. Bender RA, Shelton BJ. The Cyto-centrifuge and cerebrospinal fluid cytology. Acta Cytol (Baltimore) 1974;18:259--262

4. Komp VM. Cytocentrifugation in the management of central nervous system leukemia. J Pediatr 1972;81:992-994.

5. Drewinko B, Sullivan MP, Martin T. Use of the cytocentrifuge in the diagnosis of meningeal leukemia. Cancer 1973;31:1331-1336.

6. Bennett JM, Ruberg J, Dixon S. A new semiautomated method for the concentration of cerebrospinal fluid for cytological examination.

7. Choi H-SH, Anderson PJ Diagnostic cytology of cerebrospinal fluid by the cytocentrifuge method. Am J Clin Pathol 1979;72:931943.

8. Watson P. A slide centrifuge: an apparatus for concentrating cells in suspension onto a microscope slide. J Lab Clin Med 1966;68: 494501.

9. Boon ME, Smid L, van Olphen K, Krebber R An improved cytocentrifuge technique for cerebrospinal fluid cytology Clin Neurol Neurosurg 1980;82:203-207.

10. Kömel HM. A method for concentrating cerebrospinal fluid cells. Acta Cytol (Baltimore) 1977;21:154 157.

11. El-Batata M. Cytology of cerebrospinal fluid in the diagnosis of malignancy. J Neurosurg 1968;28:317-326.

12. Gondos B, King EB Cerebrospinal fluid cytology diagnostic accuracy and comparison of different techniques Acta Cytol (Baltimore) 1976,20:542-547

13. Reynaud AJ, King EB. A new filter for diagnostic cytology. Acta Cytol (Baltimore) 1967;11:289-294.

14. Koss LG. Diagnostic cytology and its histopathologic bases. Philadelphia: Lippincott, 1979:1202-1206.

15. Barrett DL, King EB. Comparison of cellular recovery rates and morphologic detail obtained using membrane filter and cytocentrifuge techniques. Aeta Cytol (Baltimore) 1976,20:17-180.

16. Krentz MJ, Dyken PR. Cerebrospinal fluid cytomorphology: sedimentation vs filtration Areh Neurol 1972,26:253-257.

17. Dyken PR, Shirley S, Trefz J, Gammal TE. Comparison of cytocen-trifugation and sedimentation techniques for CSF cytomorphology. Acta Cytol (Baltimore) 1980;24:167-170.

18. Kobayashi TK, Yamaki T, Yoshino E, Higuchi T, Kamachi M. Immunocytochemical demonstration of carcinoembryonic antigenin cerebrospinal fluid cytology with carcinomatous meningitis from rectal cancer. Acta Cytol (Baltimore) 1984;28:430