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Determination of Leukocyte Counts in Cerebrospinal Fluid With a Disposable Plastic Hemocytometer

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Manual microscopic cell counting in a Fuchs-Rosenthal (FR) chamber has been the gold standard for quantification of leukocytes in cerebrospinal fluid (CSF). However, for accurate determination of the number and differentiation of cells by chamber counting, hemocytometers must be prepared carefully and kept clean. Improper fitting of the chamber and coverslip changes the volume of sample introduced into the chamber well. Moreover, because conventional hemocytometers are used repeatedly and are breakable, there is a risk of exposure to potentially infectious material. To address these issues, disposable plastic hemocytometers have been developed. However, the accuracy, precision, and clinical usefulness of disposable chambers for CSF cells counting have not been determined. In the present study, we evaluated use of a disposable plastic counting chamber (C-Chip DHC-F01) by comparing its performance with that of an FR chamber for counting of CSF specimens and cell suspensions. Within-run precision of C-Chip counting was comparable or superior to that of FR chamber counting, and excellent correlation between cell counts obtained with the C-Chip chamber and FR chamber was observed. However, C-Chip chambers that were kept at 4°C yielded significantly low cell counts. The disposable hemocytometer will reduce the risk of exposure to potentially infectious material. However, use of C-Chip chambers should be avoided in cold environments. J. Clin. Lab. Anal. 21:282–285, 2007.

Key words: CSF; cells counting; hemocytometer

INTRODUCTION

Manual microscopic cell counting in a Fuchs-Rosenthal (FR) chamber has been the gold standard for quantification of leukocytes in cerebrospinal fluid (CSF). Leukocyte differentiation is very important in the clinical differential diagnosis of meningitis: viral meningitis is frequently characterized by a predominance of mononuclear cells, whereas polymorphonuclear cells often predominate in bacterial meningitis (1). However, accurate manual counting requires careful cleaning and drying of counting chambers and proper fitting of the hemocytometer and coverslip. Improper fitting changes the volume of sample introduced into the chamber well by capillary action. Moreover, because conventional hemocytometers are used repeatedly and are breakable, there is a risk of exposure to potentially infectious material. To address these issues, disposable plastic hemocytometers have been developed. However, the accuracy, precision, and clinical usefulness of disposable chambers for CSF cells counting have not been determined. In the present study, we evaluated use of a disposable plastic counting chamber by comparing its performance with that of an FR chamber for counting of CSF specimens and cell suspensions.

Abbreviations: CSF, cerebrospinal fluid; FR, Fuchs-Rosenthal; PBS, phosphate-buffered saline; SD, standard deviation; Sy|x, standard deviation from a regression line to the y axis direction.

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MATERIALS AND METHODS

We evaluated the C-Chip DHC-F01 (SKC Co., Ltd, Seoul, Korea) disposable plastic (polymethylmethacrylate) counting chamber. This hemocytometer has two enclosed chambers and does not require placement of a coverslip on the slide. The grid pattern of the C-Chip DHC-F01 is same as that of the FR chamber. It consists of 16 large squares (1 mm × 1 mm), which are each bounded by three lines on all four sides (total area, 4 mm × 4 mm). The chamber is 0.2 mm deep, providing a total counting area volume of 3.2 μL. Cell counting with the C-Chip was performed according to the manufacturer's instructions.

We studied CSF samples that were collected routinely from 34 patients and submitted to our laboratory (10 patients with brain malignancy, nine with meningitis, four with leukemia, four with malignant lymphoma, and seven with other disorders) and cell suspensions. To prepare cell suspensions, mononuclear cells and granulocytes were isolated from whole blood by gradient density centrifugation with Histopaque-1077 and Histopaque-1119 (Sigma-Aldrich, St. Louis, MO). A total of 3 mL of Histopaque-1077 (density, 1.077) was layered onto an equal volume of Histopaque-1119 (density, 1.119) in a glass tube. Then 6 mL of heparinized whole blood collected from a healthy adult was layered onto the upper gradient. After centrifugation at 700 *g* for 30 min, two distinct opaque layers (mononuclear cells and granulocytes) were separated. Cells from each layer were washed twice with phosphate-buffered saline (PBS) (200 *g*, 10 min) and resuspended in an appropriate volume of PBS. Pappenheim-stained leukocytes were counted with the use of the C-Chip and the FR chamber. We also used glutaraldehyde-fixed erythrocyte suspensions (fixed red blood cells [RBCs]) for samples.

Because the thermal expansion coefficients of plastic are larger than those of glass, we examined the effect of chamber temperature. We exposed chambers to different temperatures, 4°C, 22°C, and 37°C, for 30 min each. After each exposure, fixed RBC suspension kept at room temperature (22°C) was loaded immediately into the chambers, and cells were counted. The procedure was repeated 10 times for each chamber.

We analyzed differences in cell counts obtained with the C-Chip and FR chambers by means of Student's *t*-test. Welch's *t*-test was used when variance was unequal. The Dunn test was used for multiple comparisons. Pearson's correlation coefficient was used to examine the relation between cell counts obtained with the C-Chip and FR chambers. A significance level of 0.05 was used for all statistical tests, and all tests were two-tailed. Statistical analyses were performed with SPSS for Windows 11.0J (SPSS, Inc., Chicago, IL) or Stat Flex (Artech, Inc., Osaka, Japan).

RESULTS

Within-run precision of the C-Chip counting and that of FR chamber counting were assessed with the use of three cell suspensions and three fixed RBC suspensions. The results are shown in Table 1. There were no significant differences in mean cell counts obtained by the two chamber countings. Within-run coefficients of variation (CVs) of the C-Chip counting were nearly the same or smaller than those of FR counting. Correlation between leukocyte counts of CSF samples determined by C-Chip chamber counting and by FR chamber counting is shown in Fig. 1. The regression equations for C-Chip (y) and FR chamber counting (x) of polymorphonuclear cells and mononuclear cells were $y = 1.10x - 0.13$ cells/μL ($r = 0.994$, $Sy|x = 0.668$ cells/μL, $n = 34$) and $y = 1.01x + 0.001$ cells/μL ($r = 0.998$, $Sy|x = 1.31$ cells/μL, $n = 34$), respectively. The equation for the line representing total cell counts was $y = 0.99x - 0.19$ cells/μL ($r = 0.999$, $Sy|x = 1.17$ cells/μL, $n = 34$). No major discrepancies were observed.

Cell counts of the fixed RBC suspension (theoretical cell numbers = 68.2 cells/μL) obtained with C-Chip and FR counting chambers that were kept at 4°C, 22°C (room temperature), and 37°C are shown in Fig. 2. Mean cell counts for the C-Chip chambers kept at 4°C, 22°C, and 37°C were 56.6 (standard deviation [SD],

TABLE 1. Precision studies*

Sample[a]	C-Chip chamber counting		Fuchs-Rosenthal chamber counting	
	Mean ± SD (cells/μL)	CV (%)	Mean ± SD (cells/μL)	CV (%)
A				
Granulocyte	10.0 ± 3.02	30.2	9.2 ± 3.61	39.2
Mononuclear cell	109.8 ± 15.32	14.0	112.8 ± 14.91	13.2
Total	119.8 ± 15.85	13.2	122.0 ± 17.41	14.3
B				
Granulocyte	61.7 ± 10.27	16.6	59.1 ± 11.81	20.0
Mononuclear cell	103.5 ± 10.41	10.1	99.5 ± 11.14	11.2
Total	165.2 ± 19.09	11.6	158.6 ± 18.08	11.4
C				
Granulocyte	22.2 ± 5.45	24.5	22.2 ± 6.25	28.2
Mononuclear cell	80.8 ± 10.41	12.9	82.2 ± 11.86	14.4
Total	103.0 ± 14.55	14.1	104.4 ± 16.31	15.6
D	160.8 ± 7.54	4.7	165.6 ± 8.92	5.4
E	72.7 ± 10.73	14.8	75.3 ± 10.70	14.2
F	29.9 ± 4.01	13.4	32.6 ± 5.80	17.8

*n = 10.

[a]A, B, and C are cell suspensions. D, E, and F are glutaraldehyde-fixed erythrocyte suspensions.

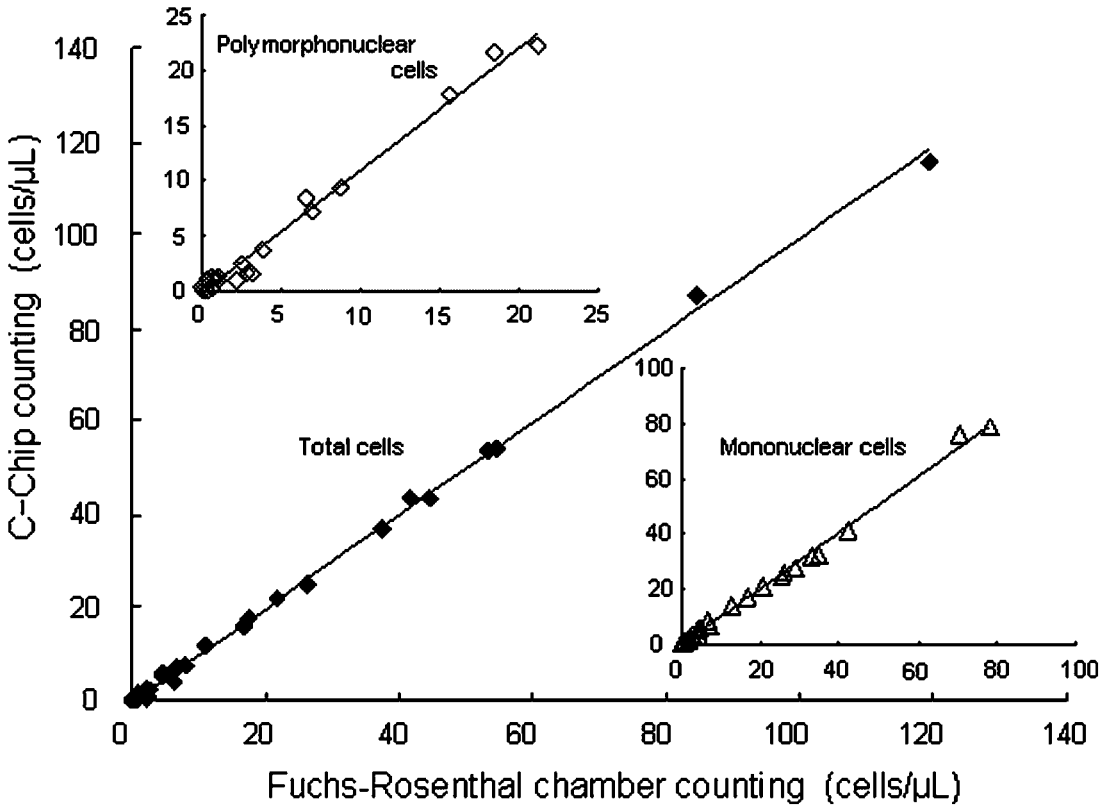

Fig. 1. Correlation between leukocyte counts of CSF samples determined by C-Chip chamber counting and by FR chamber counting. The equation for the line representing the total cell count is y = 0.99x–0.19 cells/μL (r = 0.999, Sy|x = 1.17 cells/μL, n = 34). The upper inset shows correlation for polymorphonuclear cells (y = 1.10x–0.13 cells/μL, r = 0.994, Sy|x = 0.668 cells/μL, n = 34). The lower inset shows correlation for mononuclear cells (y = 1.10x–0.13 cells/μL, r = 0.994, Sy|x = 0.668 cells/μL, n = 34).

4.53) cells/μL, 63.9 (SD, 6.41) cells/μL, and 62.1 (SD, 6.19) cells/μL, respectively. The mean cell count in the chamber kept at 4°C was significantly lower than that in the chambers kept at 22°C ($P < 0.05$). There was no difference between the mean counts obtained in chambers kept at 22°C and 37°C. In contrast, significant differences were not observed between cell counts determined in FR chambers kept at the various temperatures. Mean cell counts in FR chambers kept at 4°C, 22°C, and 37°C were 63.8 (SD, 6.52) cells/μL, 63.6 (SD, 7.13) cells/μL, and 66.9 (SD, 5.94) cells/μL, respectively.

DISCUSSION

Quantification of leukocytes in CSF by automated cytometric analysis has been reported as an alternative to manual microscopic analysis (2–9). However, automated methods cannot be used to replace manual microscopic analysis for routine CSF analysis until several problems are resolved: sample volume requirement, cell recognition, and differentiation (4). Microscopic chamber counting requires practical experience, is labor intensive and time consuming, and is subject to interobserver variability (10). However, it requires only 50 μL of CSF sample per analysis, and it provides not only cell counts and differentials but also information about cytologic atypia including nuclear atypia. We believe that manual microscopic analysis is mandatory for CSF samples from patients with leukemia or cerebral tumor. Microscopic chamber counting will remain the standard method for quantification of leukocytes in CSF.

For accurate determination of the number and differentiation of cells by chamber counting, hemocytometers must be prepared carefully and kept clean. Proper fitting of the chamber and coverslip is required. In this respect, the disposable hemocytometer is advantageous because it does not require placement of a coverslip on the slide and it is for single use only.

Within-run precision of C-Chip counting was comparable or superior to that of FR chamber counting. Absence of a difference between mean cell counts obtained by the two different counting chambers indicates that the grid pattern and charged sample volume for the counting area of the C-Chip chamber are exactly same as those for the FR counting chamber. Furthermore, because the C-Chip chamber is thinner

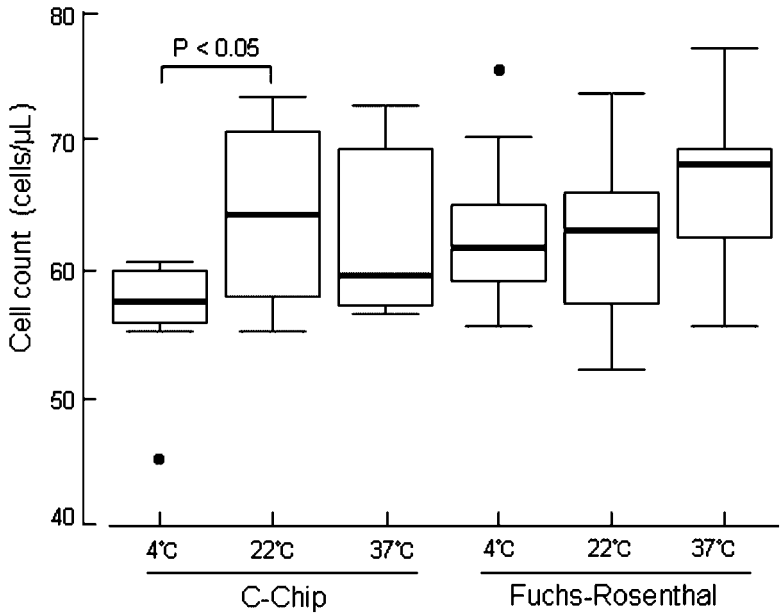

Fig. 2. Effects of chamber temperature. A fixed erythrocyte suspension was analyzed 10 times under several conditions. The horizontal bars indicate median values. The vertical bars indicate the range, and the horizontal boundaries of the boxes represent the first and third quartiles. Mean cell counts in the C-Chip chambers kept at 4°C, 22°C, and 37°C were 56.6 (SD, 4.53) cells/μL, 63.9 (SD, 6.41) cells/μL, and 62.1 (SD, 6.19) cells/μL, respectively. Mean cell counts in FR chambers kept at 4°C, 22°C, and 37°C were 63.8 (SD, 6.52) cells/μL, 63.6 (SD, 7.13) cells/μL, and 66.9 (SD, 5.94) cells/μL, respectively. ● indicates outliers. *P* values were obtained by the Dunn test.

than the FR counting chamber, shapes of nuclei were more distinct. Excellent correlation between cell counts obtained with the C-Chip chamber and the FR chamber reflects the exact grid pattern and depth of the C-Chip chamber. However, C-Chip chambers that were kept at 4°C yielded significantly low cell counts. This could be due to a temperature-induced reduction in volumetric capacity of the counting area. Temperature effects were not observed in the FR counting chambers. Therefore, use of C-Chip chambers should be avoided in cold environments.

In conclusion, precision of CSF cell analysis by means of C-Chip counting was similar to or better than that by means of FR chamber counting. The disposable hemocytometer will reduce the risk of exposure to potentially infectious material. However, use of the C-Chip chamber under cold conditions may yield low cell counts compared with use at room temperature.

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