

A note on erythrocyte counts in cerebrospinal fluid with the automated hematology analyzer, Technicon H*3

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Abstract:

A note on erythrocyte counts in cerebrospinal fluid with the automated hematology analyzer, Technicon H*3
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Background: At present, the counting of erythrocytes in cerebrospinal fluid (CSF) is still performed manually. Currently, specific automated systems for CSF cell counting are not available. In this study, we evaluated the use of automated hematology analyzer, Technicon H*3, in the analysis of cerebrospinal fluid (CSF) in case of the contamination of erythrocytes in the CSF.

Methods: We tested the hematology analyzer, Technicon H*3, which was not designed for CSF analysis. Twenty CSF samples were analyzed. The counts obtained were then compared with the manual microscopic method.

Results: Linearity was tested by normal saline solution (NSS). The results of the comparison of both methods used for the determination of total erythrocytes in the CSF exhibited a good correlation ($y = 0.836X + 1902.49$; $r = 0.849$, $p < 0.001$).

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Conclusions: According to our study, the use of this automated analyzer for cell counting in CSF is probably feasible. The high degree of accuracy and linearity that is offered by the analyzer should prompt us and the manufacturers to remedy the interfering factors as described by improving the algorithms. Once this is done, these analyzers may be very useful for cell counts in CSF.

Key words: cerebrospinal fluid (CSF), hematology analyzer, red blood cell count

บทคัดย่อ:

ในปัจจุบันการนับเซลล์เม็ดเลือดในน้ำไขสันหลัง (CSF) ยังใช้วิธีนับด้วยมือ เนื่องจากยังไม่มีเครื่องนับอัตโนมัติ การศึกษาครั้งนี้เพื่อใช้เครื่องนับเม็ดเลือดอัตโนมัติ Technicon H*3 มาลองนับเม็ดเลือดแดงที่ละลายในน้ำเกลือด้วยจำนวนเซลล์ต่างๆ กัน พบว่าผลที่ได้สอดคล้องกันเป็นเส้นตรง จึงได้นำน้ำไขสันหลัง 20 ราย มานับด้วยมือเทียบกับเครื่อง ผลปรากฏว่าสอดคล้องกัน $r = 0.849$ $p < 0.001$ จึงอาจสรุปได้ว่า มีความเป็นไปได้ที่จะมีเครื่องนับเซลล์เม็ดเลือดใน CSF แบบอัตโนมัติ เพียงผู้ผลิตปรับปรุงเพื่อมิให้มีสิ่งรบกวนในการนับก็จะเป็นประโยชน์อย่างมากในการนับเซลล์ใน CSF

คำสำคัญ: น้ำไขสันหลัง, เครื่องวิเคราะห์ทางโลหิตวิทยา, การนับเม็ดเลือดแดง

Introduction

At present, the counting of erythrocytes in cerebrospinal fluid (CSF) is still performed microscopically, e.g., using counting chamber in most laboratories.¹ The manual microscopic analysis is still accepted as the gold standard in examining cerebrospinal fluid (CSF) cells and particles.¹ Due to the fact that these countings have not been automated, it is imprecise, has wide variability, and is labor-intensive and time-consuming.

With the concept of total laboratory automation, automated analyzers are essential to release laboratory technicians from simple routine works, allowing them to make use their time for more skilled tasks.² Automation seems to be the solution to improving both the accuracy and the productivity of CSF analysis. Because flow cytometry generally allows accurate and precise quantitative analysis of leukocytes and erythrocytes^{3,4}, some reports mentioned the use of flow cytometry analyzer for as an alternative for CSF cell count.⁵⁻⁷

The objective of this study was to examine to what extent the automated method of the Technicon H*3 instrument could be used as an alternative to the visual counting of red blood cells (RBC) in CSF. In this study, flow cytometric data from CSF were compared with Neubauer chamber counting.

Materials and Methods

CSF samples

Twenty CSF samples were examined for erythrocytes in a paired comparison on the Technicon H*3 and in a microscopic counting chamber (Neubauer chamber). Routine CSF samples were brought from the In-Patient ward, King Chulalongkorn Memorial Hospital, Bangkok Thailand and sent to the Clinical Microscopic laboratory, Faculty of Allied Health Sciences, Chulalongkorn University, for further laboratory analysis. After collection, all specimens were immediately sent to the laboratory for analysis. Because only the material that was left over from routine analyses was used, special consent from the patients was not required.

Initial analyses in defined cell suspensions prepared in our laboratory were used to check the linearity of the analyzer as described in a recent previous study.^{7,8} The cell free plasma was prepared by centrifugation at 4,000 rpm for 10 minute. Thereafter, the packed red cells were diluted with normal saline solution (NSS) in order to obtain the number of cells varied from low to high.

Automated analyzer method

The flow cytometry analyzer used in this study was Techniocy H*3 (Bayer Diagnostics). In general, this machine is an automated hematology analyzer for in vitro diagnostic use in clinical laboratories. The Techniocy H*3 system provide the operator with the results of a complete blood count and a five part differentials for each specimen tested.

Manual microscopic method

Manual microscopic examination was performed in Neubauer counting chambers. For each sample, two microscopic chambers were examined at x 400, and the mean number of cells/mm³ was calculated. RBC examination was performed on all CSF samples. Therefore, CSF samples were prepared by cytocentrifugation (Cytospin; Hettich Tuttington, Germany) of 100 µL of CSF, depending on cell content, onto a glass microscope slide at 1,500 rpm for 5 min. After modified Wright staining, the slides were examined by light microscopy under immersion oil at x 1,000 magnification.

Comparative study

For each CSF sample, analysis by both automated analyzer and manual microscopic methods were performed. Comparison of total erythrocyte count was done.

Statistical evaluation

Agreement between automated cell counts and microscopic data was examined by Spearman correlation analysis. $P < 0.05$ was considered statistically significant. All statistical analysis was performed using SPSS for Window Program.

Results

The linearity of erythrocyte counting was carried out in serial dilutions by NSS (Figure 1). The results of the comparison of both methods used for the determination of total erythrocytes in the CSF showed a good correlation ($y = 0.836X + 1902.49$; $r = 0.849$, $p < 0.001$) (Figure 2).

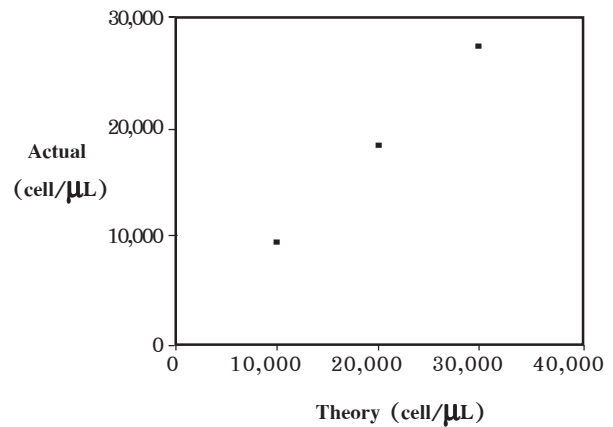


Figure 1 Linearity of erythrocyte counting of cell suspensions on the Techniocy H*3 (Start at theoretical number = 27,000 cells/µL; $r = 0.999$, $P < 0.01$)

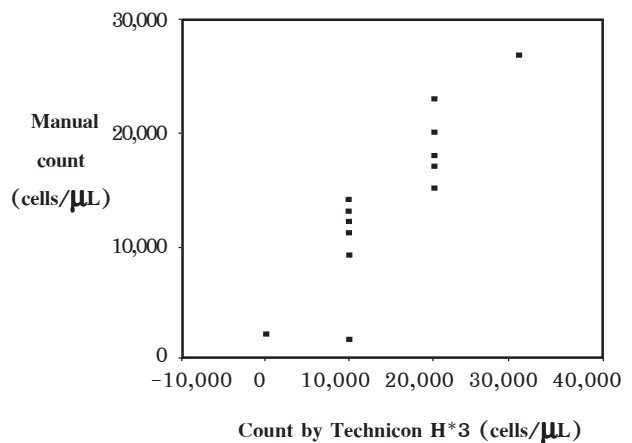


Figure 2 Linearity of erythrocyte counting of CSF suspensions on the Techniocy H*3 vs manual count ($y = 0.836X + 1902.49$; $r = 0.849$, $p < 0.001$)

Discussion

With the increasing request for CSF analysis in cases of suspected meningitis, laboratory technicians frequently must squeeze microscopic chamber counting into tight laboratory schedules with increasing workloads, additionally needing to consider several other factors (e.g., number of samples and

quality of the cells). Furthermore, a large number of erythrocytes existed in CSF may contribute to a laborious microscopic chamber counting and the subjectivity of individual laboratory workers adds to the unreliability of the results frequently observed. However, until now, microscopic counting is still in use even though it is time-consuming, and may constitute a problem in emergency diagnostics. Although analyses performed with the modified sedimentation chamber technique according to Sayk or with centrifugation have contributed to the optimization of CSF cytology^{9,10} they do not, however, solve the primary problem of the accurate determination of the number of cells. Fully automated analyzers, which meet time and quality requirements are the objective in this item.

On the basis of these requirements, in this study, we evaluated the use of an automatic flow cytometer (Technicon H*3) in the routine analysis of CSF. Finally, we found that the machine could count cells in NSS and exhibited a good linearity. Generally, a good agreement was obtained between automated counts by the Technicon H*3 and the counting chamber. The examinations of the accuracy and linearity of the two analyzers showed that it was not irrelevant to test them for CSF analyses although they had not been designed specifically for that purpose.

Conclusion

According to our study, the use of this automated analyzer for cell counting in CSF is probably feasible. The material to be analyzed is handled by the fully automated analyzers, meeting time and quality requirements, and in an objective manner. The high degree of accuracy and linearity that is offered by the analyzer should prompt us and the manufacturers to remedy the interfering factors as described by improving the algorithms these analyzers have to offer. Once this is done, these analyzers may be very useful for cell counts in CSF.

References

1. Gray LD, Fedorko DP. Laboratory diagnosis of bacterial meningitis. *Clin Microbiol Rev* 1992; 5: 130-145.
2. Tadano J. The new generation of laboratory automation systems. *Rinsho Byori* 2000; Suppl 114: 26-33
3. Cohen CY, Sahar E. Rapid flow cytometric bacterial detection and determination of susceptibility to amikacin in body fluids and exudates. *J Clin Microbiol* 1989; 27: 1250-1256.
4. Alvarez-Barrientos A, Arroyo J, Canton R, Nombela C, Sanchez-Perez M. Applications of flow cytometry to clinical microbiology. *Clin Microbiol Rev* 2000; 13: 167-195.
5. Aune MW, Sandberg S. Automated counting of white and red blood cells in the cerebrospinal fluid. *Clin Lab Haematol* 2000; 22: 203-210.
6. Van Acker JT, Delanghe JR, Langlois MR, Taes YE, De Buyzere ML, Vertraete AG. Automated flowcytometric analysis of cerebrospinal fluid. *Clin Chem* 2001; 47: 556-560.
7. Ziebig R, Lun A, Sinha P. Leukocyte counts in cerebrospinal fluid with the automated hematology analyzer CellDyn 3500 and the urine flow cytometer UF-100. *Clin Chem* 2000; 46: 242-247.
8. Soogarun S, Sirimongkolsakul S, Wiwanitkit V, Siritan-tikorn A, Pimsane K, Srivijam R. Leukocyte counts in cerebrospinal fluid with the automated hematology analyzer, Technicon H*3. *Clin Lab* 2002; 48: 623-629.
9. Busch B. Sayk's sedimentation-chamber method in the cytodiagnosis of body cavity effusions. *Z Med Labortech* 1976; 17: 149-150.
10. Den Hartog Jager WA. Cytopathology of the cerebrospinal fluid examined with the sedimentation technique after Sayk. *J Neurol Sci* 1969; 9: 155-177.