ORIGINAL ARTICLE

ACCURACY OF AUTOMATED CELL ENUMERATION METHOD FOR VARYING CONCENTRATION OF WBCS FOR VARIOUS BODY FLUID SAMPLES

Sana Brohi, Muhammad Shariq Shaikh, Bushra Moiz

Aga Khan University, Karachi Pakistan

ABSTRACT

Background: Body fluids (BF) including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids are now being analyzed by fully automated methods that are replacing the manual methods. The aim of this study is to assess the accuracy of automated instrument at varying concentrations of WBCs of various body fluid samples.

Material and Methods: This cross-sectional study was conducted at the section of Hematology, Department of Pathology and Laboratory Medicine, the Aga Khan University, Karachi, Pakistan from November 2020 to April 2021. Forty body fluid samples with suspicion of infection including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids (CSF) were analyzed to verify accuracy of white blood cell counts on fully automated XN-1000™ hematology analyzer (Sysmex Corporation, Kobe, Japan) against Neubauer chamber method (a manual method of differentiating WBCs via microscopy). The culture results of the body fluid samples were also analyzed. EP Evaluator version 10.3.0.556 (Data Innovations, LLC, VT, US) was used for statistical analysis with other supporting statistics such as Correlation Coefficient (R), Bias, Mean and Standard Deviation were included.

Results: Eleven (n=11) of forty (n=40) body fluid samples showed high WBC count above the normal range. Samples with normal WBC (n=29) were also included in accuracy study to assess instrument performance at varying concentration of analyte. An average Error Index of -0.27 (-0.99 to 0.74) for WBC was obtained. Microbiological cultures grew *Escherichia coli in 1* and *Acinetobacter* species in 1 CSF samples and *Staphylococcus aureus* in 1 pleural sample.

Conclusion: The study verified accuracy of varying concentration of WBC counting by fully automated Sysmex XN-1000 analyzer for various body fluid samples.

Keywords: Body fluids, validation, automated analyzer, performance specifications, accuracy verifications, high white blood count, infectious.

BACKGROUND

Body fluids (BF) including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids are a source of nutrition, waste disposal and necessary movement of enclosed organs. Each of these fluids has its own unique biochemical characteristics.[1] Over decades**,** hemocytometer (counting chamber) has been used for enumerating cells in body fluids. However, with the availability of automated cell counters, conventional hemocytometers are being readily replaced in clinical laboratories.[2] Several analyzers are available nowadays by various manufacturers that claim high accuracy.[3-5]Although manufacturers usually provide performance specifications in their instruction

manuals, it is the responsibility of each lab to verify these in their own setting before initiating patient reporting. Accrediting bodies such as Clinical Laboratory Standard Institute (CLSI), International council for standardization in hematology (ICSH) and college of American pathologists (CAP) recommend each lab to verify performance specification including accuracy, for each quantitative test offered by the laboratory.[1,6,7]

Understanding the difference between validation and verification is essential.[8] Validation is carried out by the manufacturer and provides performance characteristics of the method being used. If validation has not been carried out by the manufacturer, then it must be carried out by the laboratory by following any local guidelines and recommendations for establishing a laboratory-developed test (LDT).[1,8] However, verification is defined as a confirmation of the validation performed by the manufacturer that gives evidence that the analyzer can meet the specific requirements within a given test site.[8] Hence, this verification must be carried out by each laboratory before the analyzer is used for testing.[8]Accuracy study, one of the most important performance specifications verifies closeness of the measured value to the true value of an analyte.[8]

Correspondence: Dr Sana Brohi, Resident, Aga Khan University, Pakistan, Islamabad Pakistan

Email: sanamumtazbrohi@gmail.com

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White cell count is routinely analyzed in various body fluids. Causes of elevated WBC count in body fluids include infection, inflammation, hemorrhage, malignancy, seizures etc.[9]

The aim of our study was to verify accuracy of varying concentration of WBC counting by fully automated Sysmex XN-1000 analyzer against traditionally used manual method in various body fluid samples as automated method decreases the turn-around time of body fluid sample reporting.

MATERIAL AND METHODS

Forty body fluid samples received in Hematology department; Aga Khan University Hospital Karachi from November 2020 till April 2021 were randomly included. Sixteen cerebrospinal, eight peritoneal, seven pleural, six pericardial, three synovial fluids with suspicion of meningitis, peritonitis, pneumonia, pericarditis and synovitis were included in the study. The body fluid samples were collected without anticoagulant in plain glass or plastic tubes, syringes or with anticoagulant like EDTA. These samples are normally discarded after finalizing results and dispatching reports to the patients. Therefore, before discarding we analyzed these samples on fully automated XN-1000^{TM} hematology analyzer (Sysmex Corporation, Kobe, Japan) as well. No additional samples were drawn from the patients. Bile drainage, bronchoalveolar lavage and other fluids likely to be contaminated by external material were excluded. These fluids might be unsuitable for evaluation because of the presence of clots, crystals and high viscosity.

The data was analyzed on EP Evaluator software version 10.3.0.556 (Data Innovations, LLC, VT, US). The CLIA limit of 15% for WBC was set as an acceptable Allowable Total Error (TEa). Deming Regression Statistics (slope and intercept) and other supporting statistics including Correlation Coefficient (R), Bias, Mean ± SD (Standard Deviation) were used to measure agreement between 2 methods i.e. Automation versus manual method.

The study was exempted from ethical approval by ethical review committee of Aga Khan University Hospital, Karachi (2020-5531-14621).

RESULTS

A total of 40 patients' body fluid samples were analyzed. Specimens were compared over a range of 0.007 to 25.94 x 10^3/uL for WBC. The difference between the two methods was within allowable error (i.e. 15%) for 40 of 40 specimens (100%). The average Error Index (Y-X)/TEa was -0.27 (-0.99 to 0.74). Correlation coefficient (R) is 0.9998 (-1.0 to 1.0) Mean ± SD by automated method and manual methods were 2.28 ± 4.46 and 2.29 ± 4.47 respectively. Results of key statistics obtained and their acceptable limits are shown in Table-I, which confirms the accuracy of automated method. The study passed the criteria of accuracy. For lymphocytes and polymorphs; refer to Table-II.

Further, 11 out of 40 body fluid samples showed high WBC count (refer to Table-III). High WBC counts in different samples were as follows: 6 out of 16 CSF samples (i.e. WBC: > 0.005 x 10E3/uL), 3 out of 8 peritoneal samples (i.e. WBC: > 0.5 x 10E3/uL), 2 out of 7 pleural samples (i.e. WBC: > 0.5 x 10E3/uL), none of the pericardial and synovial samples showed high WBC counts. Also, the WBC reported by both methods i.e. Automation and manual method showed no significant difference between the two. The microbiological culture of all 40 samples was performed with suspicion of infection by primary physician. Total 3 (2 in CSF and 1 in pleural fluid) out of 40 body fluid samples were culture positive for bacterial growth. The 2 culture positive CSF showed *Escherichia coli* in one and *Acinetobacter species* in other CSF sample, whereas growth of *Staphylococcus aureus* was present in 1 pleural fluid sample.

Table-I: Key statistics of accuracy study for WBC obtained with their acceptable limits.

Statistics	Values obtained	Acceptable limits	Results: Acceptable or Unacceptable
Average error index	-0.27	-0.99 to 0.74	Acceptable
Correlation coefficient (R)	0.9998	-1.0 to 1.0	Acceptable
Slope	0.9998	0.991 to 1.005	Acceptable
Intercept	-7.9	-40.7 to 24.8	Acceptable
Bias	-12.4	< -0.5 %	Acceptable
Mean ± SD (Automated method range)	2.28 ± 4.46	0.007 to 25.94 (10^3/uL)	Acceptable
Mean ± SD(Manual method range)	2.29 ± 4.47	0.008 to 25.939(10^3/uL)	Acceptable

Table-II: Key statistics of accuracy study for lymphocytes & polymorphs obtained with their acceptable limits.

Statistics	Values obtained for Lymphocytes	Values obtained for Polymorphs	Acceptable limits	Results: Acceptable or Unacceptable
Correlation coefficient (R)	0.9368	0.9375	-1.0 to 1.0	Acceptable
Slope	0.861	0.861	Lymphocytes: 0.76 to 0.96 Polymorphs: 0.76 to 0.96	Acceptable
Intercept	11.09	3.09	Lymphocytes: 3.61 to 18.57 Polymorphs: -0.78 to 6.97	Acceptable
Bias	1.31	<-0.99%	Lymphocytes: < 1.85 % Polymorphs: <-3.44	Acceptable
Mean ± SD (Automated method range)	0.0727 ± 0.0228	0.006 ± 0.097		Acceptable
Mean ± SD (Manual method range)	0.0704 ± 0.261	0.005 ± 0.009		Acceptable

Table-III: High WBC cut-off for various body fluid samples.

Body Fluid	Total no: of specimen	Total no: of specimen with high WBC count	Cut-off for reportable high WBC count (10E3/uL)	Total no: of culture positive specimen
CSF	16	6	>0.005	2
Peritoneal	8	3	>0.5	0
Pleural	7	2	>0.5	1
Pericardial	6	0	>0.5	0
Synovial	3	0	>0.2	0

DISCUSSION

Analysis of body fluids provides valuable insight in the diagnosis of many medical conditions such as meningitis, joint pathology, peritonitis and malignancies.[10-12] There are many challenges to manual assessment of body fluids such as skilled handling and subjectivity in interpretation making automated analyzers to be regarded as a fast and accurate tool for assessing body fluids.[13-14] Automated analyzers like Sysmex have body fluid mode which helps in analysis of body fluids with rapid and accurate enumeration of WBCs.[15]

The instrument manufacturers provide specifications such that users' i.e., laboratories have to perform only verifications to justify the manufacturer's intended use.[5] In case manufacturers do not provide specifications then the responsibility lies on laboratories to validate their specifications using international guidelines.[16] In this study, we have verified Sysmex XN -1000's body fluid mode against Manual Neubauer chamber via microscopy for accuracy using patients' samples. Previous study by DD Castellone *et al*. (2010) proved that automated analyzers like ADVIA® 2120/2120i compared well to manual methods of enumeration of RBC and WBC in various body fluids like, pleural and peritoneal.[17]

In our study, accuracy or trueness was verified by method of comparability that was performed using split sample testing, with retained patients' samples. Our results passed the criteria of accuracy verification. Both methods were equally effective, but keeping in view the time consumed by manual method, the automated analyzer proved to decrease the turnaround time in less than 4 hours to more than 4 hours in manual method. Also being a manual mode of counting, the chances of errors such as pipetting errors, faulty sample preparations, volume of sample introduced into the chamber are high with it.

Sirin Lohajaroensub *et al*. (2015) studied 253 body fluid samples and compared the results between automated analyzer Sysmex XT-4000i and manual methods and found correlation of RBC and WBC count was high in ascitic fluid followed by pleural fluid, CSF and synovial fluid.[18]Manual microscopy of body fluid are time-consuming and labor-intensive and subjective while material handling, but fully automated analyzers meet the demand of increase in turnaround time, quality requirements and are objective in material handling.[18]

The Deming regression analysis didn't yield significant constant or proportional bias between the manual and the automated methods. The slope and intercept were computed assuming that the two methods have comparable precision (i.e., the same representative SD). In our study, the Mean ± SD by automated method and manual methods for WBC were 2.28 ± 4.46 and 2.29 ± 4.47 respectively. Method comparison studies usually limit statistical calculation to

correlation coefficient between the two methods and in some instances, correlation does not mean that the two methods agree. Therefore, we also tested our study based on acceptable allowable total error (TEa). Based on biological variations, TEa is a variable that expresses the degree of error in a test result that can be tolerated without negatively impacting patient care. For example, acceptable CLIA values of TEa for red blood cells, white blood cells, and platelets are 6%, 15%, and 25%, respectively. We used same TEa of 15% for WBC analysis. Error Index (EI) is the ratio of the difference between 2 methods to TEa. We obtained an average error index of -0.27 for WBC (Table-I). An index greater than 1.00 or less than -1.00 is unacceptable which means that the difference between methods exceeds TEa. If an excessive number of specimens have an unacceptable error index, the experiment fails. Excessive number of specimens occurs if the EI is unacceptable for at least one specimen if n = <20 or if the EI for more than 5% of the specimens is unacceptable when n = >20. We also found in our study that 3 samples were positive and 37 were negative for any bacterial growth. Since cause of raised TLC can be infectious and noninfectious i.e. reactive, malignancy, autoimmune, drugs etc, therefore, it is better to analyze all body fluid samples by microbiological culture. Also, several factors affect positivity of culture such as antibiotic treatment and bacterial load affect the outcome. Additionally, infectious causes may be other than bacterial or fungal e.g. viral. So infectious etiologies cannot be completely ruled in or out for complete inclusion of infection.

LIMITATION

The limitation of our study is the correlation of culture negativity of majority body fluid samples for infectious cause. Also, there are various other possibilities like fungal or viral cause, bacterial load, antibiotic use or resistance, so the infectious cause cannot be completely ruled out in culture negative cases. Hence, more diagnostic tests such as molecular tests are now available to test for multiple etiologies at one time. Further characterization of fluid is beyond the scope of this laboratory-based paper. It is at physicians' discretion to further characterize body fluids based on their patient's clinical history and other laboratory data while dealing with individual patients.

CONCLUSION

The study has verified the accuracy of WBC counting by Sysmex XN-1000 analyzer in body fluids against manual method in various body fluid samples. Automated method is rapid, accurate and less labor intensive and must be adopted by laboratories for efficient patient management

CONFLICT OF INTEREST

Authors declare no conflict of interest

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None

AUTHOR CONTRIBUTION

Sana Brohi: Conception, analysis, collection and interpretation of data, manuscript writing and revision.

Muhammad Shariq Shaikh: Conception, interpretation of data, manuscript writing, critical revision.

Bushra Moiz: Conception and critical review

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