ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Comparison of Cerebrospinal and Pleural fluid analysis using manual and automated hematology analyzer at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

PI: Mulualem Aschenik

Advisors:

Aster Tsegaye (PhD)

Rahel Alemu (MSc)

Yared Mamushet (MD)

Henok Kebede (MSc)

Tewodros Tamire (MSc)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Masters Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology).

August, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Mulualem Aschenik entitled with: "**Comparison Cerebrospinal (CSF) and Pleural fluid analysis on Sysmex XT-4000i automated hematology analyzer at Tikur Anbessa specialized hospital, Addis Ababa"** Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_________________ Signature ____________Date _____________

Internal Examiner__________________ Signature ____________Date _____________

Advisor___________________________Signature ____________Date _____________

Advisor__________________________Signature_____________Date______________

Advisor__________________________Signature_____________Date______________

Advisor__________________________Signature_____________Date______________

Advisor__________________________Signature_____________Date______________

____

Chairman of the Department or Graduate Program Coordinator

Acknowledgments

I owe a debt of gratitude to many people whose help has been essential to the completion of my thesis. I would like to acknowledge Addis Ababa University, College of Health Sciences, and Department of Medical Laboratory Sciences for providing this opportunity to develop this thesis and for financing the study. I would like to express my heartfelt gratitude to my advisors Dr. Aster Tsegaye , Rahel Alemu, Dr. Yared Mamushet, Henok Kebede and Tewodros Tamire for their constructive comments and suggestion from the beginning of my study. Finally, I would like to sincerely thank Saifu Hailu for all his kind support and those who helped me directly or indirectly to finalize my thesis work.

Last but not least, I am and will always be grateful to my wife for her love, unwavering support, and steadfast belief in me.

Table of contents

List of Tables

List of Figures

Abbreviations

AF	Ascetic fluid
ASRA	American Society of Regional Anesthesia
AUC	Area Under the curve
BF	Body Fluid
CBC	Complete Blood Count
CI	Confidence Interval
CSF	Cerebrospinal Fluid
CTscan	Computerized Tomography scan
CV	Convecient of Variations
EDTA	Ethylene Diamine Tetra-Acetic acid
LoB	Limit of Boundary
LoD	Limit of Detection
LoQ	limit of Quantification
LP	Lumbar Puncture
MN	Mononuclear Cells
MRI	Magnetic Resonance Image
OM	Optical Microscopy
PF	Pleural Fluid
PMN	Poly Morphonuclear cells
R	Regression
RBC	Red Blood Cells
SF	Synovial Fluid
TASH	Tikur Anbessa Specialized Hospital
TAT	Turn Around Time
TNC	Total Nuclear Cells
US	Ultrasound
WBC	White Blood Cell

Abstract

Background: Analysis of body fluids provides important information for assessing various medical conditions. Bothe manual and automated hematology analyzers are used for cell counting. However, no published study is available to validate the diagnostic performance of the Sysmex 4000-i (Sysmex, Japan) system for the analysis of CSF and Pleural fluids.

Objective: To compare the manual counts of cerebrospinal fluids (CSF) and pleural fluids (PF) with the Sysmex XT-4000*i* automated hematology analyzers.

Methods: In this cross-sectional study, 240 body fluid samples were analyzed using the Sysmex 4000-i system in the body fluid mode and light microscopy at Tikur Anbessa Specialized Hospital. Body fluids included were pleural and cerebrospinal fluid (CSF). The results of the manual and automated counts were compared using the Pearson correlation test.

Results: A comparison between the Sysmex 4000-i system and manual counting demonstrated great correlations with regard to pleural fluid WBC, Polymorph nuclear cells and Mono nuclear cell counts where r =0.82, 0.96, 0.95, respectively in both methods of the counts. Cell count from CSF also showed good correlations for both manual and automated counts of WBC, PMN % and MN% where r=0.83, 0.94, and 0.92, respectively. The Sysmex 4000-i system also demonstrated good performance for differential cell counting (r =0.9028). CSF particularly showed a good correlation.

Conclusions: The use of the Sysmex 4000-i system for cell counting and differential analysis of body fluid samples might be an effective and automated alternative to chamber counting in laboratory routine analysis, thereby enhancing laboratory workflow and clinical effectiveness.

Keywords: CSF, Pleural fluid, Manual count, automated count.

1. Introduction

1.1. Background

Body fluids analysis includes a variety of nucleated cells including nucleated red blood cells, white blood cells, lining cells (such as mesothelial cells, ependymal cells), and other malignant cells that are counted as total nucleated cells(TNC)(1).Cytological fluids gave important clues for the diagnosis and monitoring of various diseases and were also used to treatment decisions for patients. Cytology and biochemical molecules are measured in such fluid. In the cytological study the determination of white blood cells, red blood cells, and their differentiation into polymorphonuclear (PMN) or mononuclear leukocytes(MN)(1,2).

Numerous inflammatory diseases are known by the presence of abnormal values of WBC, in biological fluid rather than the blood. As a result, the total WBC and the differential neutrophils, eosinophils, monocyte, lymphocyte counts have immense diagnostic value and may pave the therapy guide. The clinical values of WBC in different body fluids have been studied in detail, but the result becomes vague. Despite this, the role of WBC is critical for diagnosis; parameters have undoubtedly been considered, and nowadays, it is added in clinical diagnostic guidelines (3).

The total and differential cell counts in body fluid (BF) samples are of huge diagnostic value. They guide treatment decisions and follow-up of several illnesses, including those involving body cavities, such as neoplasm's, haemorrhages, inflammatory processes, and infectious diseases (4-6).

The cerebrospinal fluid (CSF) is a clear fluid that is produced as a result of the ultra-filtrate of plasma. CSF is found in both spinal and intracranial compartments. It is consistently being secreted by the choroid plexus at a persistent rate inside the ventricles of the brain and circulates in the subarachnoid space of the brain and spinal cord through CSF pathways. The total amount of CSF in the adult is approximately 140 mL. CSF is produced at a rate of 0.2–0.7 mL per minute or 500–700 mL per day (7).

Cerebrospinal fluid has the main role in the functioning of the brain, and variation in its content, flow, and pressure can badly affect the normal functions of the brain. Laterally and abnormal brain function can alter CSF content. Therefore, the collection and analysis of CSF provide important information to examine neurological abnormalities (8).

Cellular investigation of cerebrospinal fluids has a crucial role in the diagnosis and follows up of the central nervous system (CNS) disorders. Rising numbers of white blood cells (WBC) in CSF samples (i.e., $>5\times 10^6$/L in adults, $>7\times10^6$/L in children, or $>27\times10^6$/L in neonates) is a foundation for diagnosing encephalitis, meningitis, neurologic disease, and leukemic infiltrations in CSF (9).

Cerebrospinal fluid is collected easily by lumbar puncture (LP). Disturbance in CSF content may be the same in different pathological conditions and result in interpretation difficulties. Bringing together a set of CSF constituents like protein, albumin, immunoglobulin, glucose, lactate, and cellular changes, and additionally, antigen and antibody testing for infectious particles will boom the diagnostic sensitivity and specificity (10).

Pleural fluids are normally found in small amounts between the parietal and visceral membranes of the thoracic cavity surrounding the lungs. The inner surface of the chest wall is lined with parietal membranes, while the visceral membranes line the lungs. Pleural fluid mediates as a lubricant between the lungs and internal chest wall during breathing. Pleural fluids productions take place at capillary hydrostatic and osmotic pressure from parietal vasculature. The production of pleural is maintained by absorption, mainly by lymphatic stomata and venous uptake. Rising in pleural production, as well as lower in lymphatic absorption, resulting in fluid accumulation is known as effusion. Causes of pleural effusion include pressure in the lung circulation like congestive heart failure or pulmonary emboli, alteration in pulmonary vascular permeability that may enhance inflammation or infection, accumulation of pus during infection, movement of non-pleural fluids into the thoracic cavity, haemorrhage, or a variety of disorders, which reduce or hinder the absorption of fluid back to the lymphatic capillary (11).

Many small pleural effusions may be clinically asymptomatic and detected only during radiologic studies such as chest X-ray, CT, MRI, or ultrasound (US).Patients with symptomatic pleural effusions may report shortness of breath, difficult or painful breathing, or general pulmonary symptoms such as cough (11). Sometimes they may also be febrile if an underlying infection is present.

Body fluid cell count routinely is performed by manually manual microscopy examination. The method used for cytological analysis requires manual chamber counting of red blood cells and white blood cells as well as cytocentrifuge and staining preparation reagents for leukocyte differentiation (12). These procedures consume time and it requires around 60 minutes completing the whole procedures. Also, it is a manual technique that exposed a subjective interpretation of cytological findings resulting in significant inter-observer variability. An encouraging choice could be the automated cell analysis of these fluids. Sysmex corporation in 2007 introduces a fully automated hematology analyzer dedicated to body fluid mode (XE-500) was innovated. This helps to overcome the limitations of manual methods and open the door for applying automated analysis of cells in the liquid cavity for diagnostic purposes (13-15).

In summary, in a recent setup, three methodologies were used to generate data for counting and differentiating cells in body fluids. Those methods are manual microscopy, automated flow cytometry, and automated impedance technology. The manual microscope is the gold standard traditionally; but, in the last decade, automated analysis using flow and/ or impedance technology put a hand over in the clinical laboratory. The use of automated cell counters versus conventional microscopy are issues of discussion, even though, each method comes with its limitations. To select between methodologies, many factors such as technical capability, sample volume, type of body fluid, and patient category come into play. Every laboratory should make its own choice based on the advantage and disadvantages of each existing technology (3).

They are many kinds of commercial hematological analyzers available for the cytometric of biological fluids (16, 17). Unusual modules or applications are needed to count number of cells in the biological fluid. The main advantages of these automated systems over manual systems are lower response times and reduced inter observer variability. Lowering time gaps helps with a correct interpretation of the results since the samples are not stable and can degrade easily. Thus, scientific guidelines always recommend studying such samples in the shortest time possible (18). Minimizing the inter observer variability is also essential, especially for monitoring pathologies, since the variability could result in interpretations of false improvement or worsening. It permits the comparison of results obtained in different centres. Because of these, many laboratories have replaced manual counting with automated systems for the study of biological fluids (18).

Misleading techniques and the incorrect result could lead to over diagnosis of curing inflammatory diseases like meningitis, and peritonitis (19).

1.2. Statement of the problem.

Cytological analysis of body fluids is an important tool for the diagnosis and management of disease. Even though it is not conclusive, leukocyte differential count could suggest the stage of inflammatory response (19). Total and differential WBC counting from body fluid samples have huge diagnostic value. That paved the way for decisions and follow-up of several illnesses, including those involving body cavities, such as neoplasm's, hemorrhages, inflammatory processes, and infectious diseases (4-6).

For diagnosis of these diseases manual microscopy using hemocytometer also known as "counting chamber", is traditionally used to determine the concentration of WBC, RBC, in body fluids. The differentiation of WBC into MNs and PMNs are made from a stained cytocentrifuged slide. Cytocentrifugation is a cell preparation system that uses centrifugal forces to deposit cells onto a slide. Then, the slide is stained and followed by a 100- 200 cell count differentiation (depending on laboratory protocol). Operating parameters (speed, timing, sample volume) may vary from laboratory to laboratory. This can lead to having an impact on quality of slides, and end up with quantitative differences in evaluations study. However, the combined techniques are remaining as gold standard even if they have their limitations (20, 21).

Hemocytometer concentration measurement is prone to subjective skill and understanding. The critical concerns in differential counting method are the preparations of cytospin slides. The hemocytometer apart from being subjective is labor-intensive and has a high inter and intra-assay imprecision. Due to centrifugations, vulnerable cells are lost or gain aberrant looking, while macrophages and mesothelial cell clusters can be mistaken for malignancy. Generally, the result obtained from manual microscopy method viewed skeptically as a result potential sources of errors can lead to misinterpretation of the result (22).There are significant limitations of manual technique, besides with continuous booming of workload and scarcity of skilled personnel (23). These limitations are especially relevant in emergency laboratories. However, the greatest limitation is that the results vary according to the observer, which has been widely demonstrated even among qualified personnel (2).

Many laboratories opted for alternative methods by adopting the use of automated hematology analyzers, and more recently, urine analyzers to perform BF cell counts. Automation of BF cell counts has provided most of the time, improvements in laboratory exactness, precision, effectiveness, and productivity, besides reducing costs and time until result release (24). Since the use of automated cell counters opens the road to immense improvement in BF analysis, and its manipulation increasing, its acceptance is still limited as choice to manually microscopy. The limitations nowadays of BF mode includes lack of precisions in the low ($<20\times10^6$/L) counting range, incapable to identify malignant cells, and interference by non-cellular particles (bacteria, lipids, crystals) leading to specious WBC and RBC result, inability to flag unusual cells (13, 25).

In our country, Tikur Anbesa Specialized Hospital, as a tertiary care referral hospital, receives different kinds of body fluid samples like cerebrospinal fluid (CSF), pleural fluid (PF), synovial (SF), ascetic fluid (AF), and also, other biological fluids of various origins. This study examined or analyzed CSF and PF using both manual and automated platform. The current available automated analyzer Sysmex XT-4000i which has been dedicated for BF mode is compared to manual microscopy.

1.3. Significance of the study.

The significance of this study is to generate data that blood cell count from CSF and pleural fluid using Sysmex 4000i in body fluid mode are comparative to the manual microscopy method. This will have significant importance for diagnosis and provide the clinician reliable result to come up with good diagnosis and treatment for their client. Besides, to the best of the investigators' knowledge there is no study conducted to know either the manual or the automated method is more relevant to diagnose a patient based on the body fluid count in Ethiopia. This study provides data regarding the method which is more reliable to count CSF and PF fluids.

2. Literature review

A study conducted in Vajira University hospital by Sirin and coworkers revealed that Comparison of RBC and WBC counts using sysmex-4000-i of the two methods were analyzed by using Spearman's rank correlation test (*r*s). The acceptable value of 218 of red blood cells count was in the range of more than 1,000 cells/µL (RBC count <1,000 cells/µL with the *p*-value =0.037). The correlation of RBC count were *r*s = 0.857 (CSF), 0.748 (synovial fluid), 0.944 (pleural fluid) and 0.973 (ascitic fluid). The correlation of WBC count of 253 were *r*s = 0.950 (CSF), 0.936 (synovial fluid), 0.956 (pleural fluid) and 0.967 (ascitic fluid). RBC and WBC count by two methods were indicated that the correlation were high and in the same direction in any specimen except for synovial fluid. The correlation of differential polymorphonuclear cell (PMN) (*r*s=0.903) was better than mononuclear cell (MN) (*r*s=0.894). The background count, accuracy, precision, linearity and carryover for RBC and WBC count were found to be in the range. The correlation of counting WBC was better than RBC (1).

RBC and WBC count of body fluid are routinely performed by the microscopic analysis or manual method. It has been the gold standard examination for determination of cell count and differential WBC in the most laboratories (7).

A method comparison studies between Sysmex XN-9000 body fluid mode and conventional optical microscopy was conducted for forty-four samples in Italy involving four sites namely: Clinical Pathology Laboratory, Hospital of Lecco; Transfusion Center, Hospital of Bolzano; Clinical Pathology Laboratory, Hospital of Bolzano; and Section of Clinical Biochemistry, University of Verona. The studies carried out in these different sites showed the mean difference was 1.6×10^6 cells/L for total white blood cells (95% CI: -21.8×10^6 to 25.1×10^6 cells/L), 1.3×10^6 cells/L for polymorph nuclear cells (95% CI: -13.9×10^6 to 16.5×10^6 cells/L), and -0.6×10^6 cells/L for mononuclear cells (95% CI: -21.5×10^6 to 20.3×10^6 cells/L). The imprecision was getting to be less than 5% and carryover was found to be less than 0.01%. The body fluid mode for XN-9000 was identified as very good linearity in the range values comprised between 85×10^6–$3{,}197\times10^6$ cells/L, with correlation coefficients (r) always equal to 1.00 (P<0.001) (26).

Another comparative study was done to evaluate new body fluid module on Sysmex UF1000i (UF1000i-BF) for analysis of white blood cell (WBC) and red blood cell (RBC) in cerebrospinal fluid (CSF) in Italy by using UF1000i-BF and Fuchs–Rosenthal counting chamber in 67 CSF samples. The result indicated that the agreement between UF1000i-BF and manual WBC counts was optimal in all CSF samples (r = 0.99; y = 1.05x + 0.09). A limited overestimation was seen in samples with WBC < 30×10^6/L (r=0.95; y= 1.21x − 0.15). A satisfactory agreement was observed for RBC counts (r=0.98; y=1.15x + 0.55), particularly in samples with RBC 18×10^6/L (R= 0.98; y=1.01x +8.90). Precision between days was good with coefficient of variations (CVs) lower than7.2% for both WBC and RBC. The LoBs were 0.1×10^6 WBC/L and 1.2×10^6 RBC/L, the LoDs were 0.7×10^6 WBC/L and 5.5×10^6 RBC/L, the LoQs were 2.4×10^6WBC/L and 18.0×10^6 RBC/L, respectively. Excellent linearity was observed for both WBC and RBC (r=1.00). Carryover was negligible. The excellent diagnostic agreement was obtained at 4.5×10^6 WBC/L cut-offs (sensitivity, 100%; specificity, 97.4%) (9).

A study was performed to evaluate automated Sysmex XE-2100 (differential) WBC counting in comparison with manual counting that was done in Netherland from patients undergoing diagnostic thoracocentesis on 45 pleural fluid samples. The result revealed that the automated WBC count was highly correlated with that of the microscopic reference method (r2s0.95; WBC-analyzers0.97=WBC reference methodq16; ns45). Good agreement was also observed for the absolute lymphocyte count (r2s0.92; WBC-analyzers0.99=WBC-reference methodq32; ns36), neutrophil count (r2s0.94; WBC-analyzers0.91=WBC-reference methodq6; ns35), and monocyte count (r2s0.73; WBC-analyzers0.83= WBC-reference methodq6; ns38). The functional detection limit for WBCs was calculated at $50=10^6$/L (coefficient of variation 20%) (21).

The study carried out to evaluate the analytical performance of the Sysmex XE-5000 hematology analyzer examined pleural and ascitic fluids in the routine laboratory of a large university hospital in Brazil. A total of 56 samples (35 ascetic and 21 pleural fluids) were analyzed by manual optical microscopy (OM) and XE-500s automated hematology analyzer. The analytical performance contains carryover, linearity, functional sensitivity, and comparison of patient samples. As a result, performance studies showed linearity up to 25,825 WBC-BF/µl (r2 = 0.999), WBC-BF showed carryover of 0.18%, and the lower limit of quantization was set at 22 WBC-BF/µl. Good correlations between the methods were observed just for total cell (TC-BF) and white blood cell (WBC-BF) counts in pleural and ascetic fluids. The high-fluorescence cell count (HF-BF) showed poor correlation but high positive predictive value (PPV) for both fluids (94.74% for pleural and 96.97% for ascitic fluid. XE-5000 provides an accurate and precise count for TC-BF, WBC-BF, and polymorphonuclear cells (PMN-BF) in pleural and ascetic fluids in medical decision levels, but the morphological differentiation should continue to be held by optical microscopy (OM)(27).

The hematological analyzer (Sysmex XE-500) offers a channel to quantify the total cell count of body fluids. The comparative study was carried out on traditional manual microscopy using the Fuchs-Rosenthal counting chamber in technical sensitivity and specificity, intra assay variability, turnaround time(TAT), and cost for knowing CSF cell counts for both methods. The mean coefficients of variation (CV) for total cell counts in CSF for the Fuchs–Rosenthal chamber and the XE-5000 were 15.2% (range: 2.8–47.5%) and 12.5% (range: 1.9–50.6%).When the Fuchs–Rosenthal chamber was used as 'gold standard', the results revealed a sensitivity of 100% and a specificity of 75% for the XE-5000 to detect a pathological cell count (6 cells/ lL). In another round, the sensitivity and specificity to detect a severely pathological cell count (20 cells/ lL) were 100% for both. There were higher cell counts with the XE-500 when analyzed by Bland and Altman analysis (28).

The automated hematology analyzer Sysmex XN series has been fitted with a body fluid mode to count and differentiate leukocytes in BF. But, its diagnostic accuracy was not reliable for CSF samples with little cell concentration. To minimize this limitation, a current flow cytometry-based technology, termed "high sensitive analysis (hsA) mode," had been developed. Plus, the XN series analyzer has been equipped with the automated digital cell imaging analyzer DI-60 to classify cell morphology including normal leukocytes differential and abnormal malignant cells detection. By applying various BF samples, the performance of the XN has mode and DI-60 was evaluated comparatively to manual microscopy examination. The repeatability of the XN-hsA model showed good results in samples with low cell densities (coefficient of variation; % CV: 7.8% for 6 cells/μL). The linearity of the XNhsA mode was settled up to 938 cells/μL. The cell number gained using the XN-hsA mode correlated highly with the corresponding microscopic examination. There was a good correlation observed between the DI-60 analyses and manual microscopic classification for all leukocyte types, except monocytes (29).

A study was conducted to compare two cells counting method in body fluids by using a Neubauer chamber, and automated cell counting using an XN-350 hematology analyzer in Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym

University College of Medicine, Anyang, Korea. The cell was collected from32 body fluid samples were counted by manual examination and by an automated analyzer. Total cells (TC), white blood cells (WBC), red blood cells (RBC), polymorphonuclear leukocytes (PMN), mononuclear leukocytes (MN), neutrophils, lymphocytes, monocytes, and eosinophils were each counted by both methods. The results were compared using the Pearson correlation test, Bland-Altman regression analysis, and Passing-Bablok regression analysis. The two methods indicate a very strong correlation in TC, WBC, RBC, PMN, and MN counts, strong correlation in % neutrophils, and % lymphocytes, and weak correlation in % monocytes and % eosinophils. The result by Bland-Altman regression analysis, the mean biases for TC, WBC, and RBC were -270, -257.4, and -1,256.09, respectively, and 0.15 for PMN and MN. Study parameters were compared as well: mean biases were -1.31, -2.46, -5.16, and -3.58 for % neutrophils, % monocytes, % lymphocytes, and % eosinophils, respectively. Passing-Bablok regression equations were y=1.039x+20, y=1.037x+19, y=1.259x+0.0, y=0.983x+1.541, and y=0.983x+0.125 for TC, WBC, RBC, PMN, and MN, respectively. The equations were y=0.955x+2.194 for % neutrophils, y=0.965x+1.184 for % monocytes, y=1.003x+0.161 for % lymphocytes, and y=x+0.75 for % eosinophils (30).

An evaluation study was conducted in a clinical chemistry laboratory in Italy by module of BF Mindary BC-6800 (BC-6800-BF) for cytometry analysis of ascitic and pleural fluid from 99 ascetic and 45 pleural samples. The analysis was done by BC-6800-BF and optical microscopy method in which limit of blank(LoB), the limit of detection(LoD),limit of quantification(LoQ), carryover, linearity, and diagnostic agreement between two methods were evaluated. The result showsTC-BF, LoB was 1 9 106 cells/L, LoD was 3 9 106 cells/L, and LoQ was 4 9 106 cells/L. Linearity was excellent (r2 = 0.99) and carryover was negligible. TC-BF performed with the two methods showed Pearson’s correlation of 0.99 (P < 0.0001), Passing–Bablok regression y = 1.04x 1.17, and bias 33.7 cells. In ascitic fluids, polymorphonuclear cells (PMN) showed an area under curve (AUC) of 0.98 (P < 0.0001). Mononuclear cells (MN) and polymorphonuclear cells (PMN) in pleural fluids displayed an AUC of 0.79 (P < 0.0001) and 0.93 (P < 0.0001), respectively (31).

Another comparative study with the same Mindray BC-6800 hematology analyzer and the manual hemocytometry method for determination of total WBC count in peritoneal and pleural fluids was done in Turkey. The study required 109 peritoneal fluid and 34 pleural effusion fluid. The result revealed a statistically significant difference between manual count and hemogram mode ($p < 0.001$for both peritoneal and pleural fluids). Also, there was a statistically significant difference between the hemogram mode and BF mode too for both fluid types ($p < 0.001$). But, there was no statistically significant difference between the manual count and BF mode (for peritoneal fluids p=0.236, for pleural fluids p=0.627). For cell count less than100 cells/mL, there was a statistically significant difference between each of the counting methods (Manual count vshemogram mode $p < 0.001$, manual count vs BF mode p= 0.012, hemogram mode vs BF mode $p < 0.001$) (32).

In most African countries, including Ethiopia, there is a scarcity of published data that compares the automated analyzer and manual body fluid count for diagnosis of different diseases to understand which method was more recommended to use it. This study is designed to address such gaps in the premier teaching and referral tertiary care hospitals of the country.

3. Objectives

3.1. General Objective

- To compare the manual counts of cerebrospinal fluids (CSF) and pleural fluids (PF) against the Sysmex XT-4000*i* automated hematology analyzers at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

3.2. Specific Objective.

- To compare automated and manual body fluids analysis of CSF and PF total white blood cells count and differential count.
- To determine the diagnostic agreement between-4000*i* and manual microscopy method.

4. Materials and Methods

4.1. Study Area

The study was conducted in hematology laboratory at Tikur Anbessa Specialized Hospital (TASH), which was established in 1972. Tikur Anbessa Specialized Hospital is located in the nation's capital Addis Ababa. It is Ethiopia's largest referral hospital in the country. The hospital was given to Addis Ababa University by the Ministry of Health as the main teaching hospital in the School of Medicine. TASH delivers diagnosis services and treatment for approximately 500,000 patients per year in its 20 outpatient specialty clinics, inpatient, and emergency unit. From different departments, CSF and PF fluid is sent to hematology laboratories for different investigations from outpatient clinics and inpatients wards. The result is sent through the digital system to each department or ward.

4.2. Study Design and period

A comparative study was conducted from January 2021 to June 2021.

4.3. Population

4.3 .1.Source Population

All patients attending at TikurAnbessa Specialized Hospital (TASH) during the study period.

4.3.2. Study Population

All patients who are requested for Cerebrospinal and Pleural fluid analysis at the hematology laboratory at TASH and who fulfill the eligibility criteria.

4.4. Eligibility Criteria

4.4.1. Inclusion Criteria

All patients starting from the age of 1 year whose samples were sent to hematology laboratory of Tikur Anbessa Specialized Hospital (TASH) for Cerebrospinal and Pleural fluid analysis were included.

4.4.2. Exclusion Criteria

- Samples with bloody appearance both for CSF and PF.
- Clotted sample.

- Inadequate sample
- Delayed sample

4.5. Sample Size Determination

A minimum of 30 samples from each fluid type with different WBC count levels (Low, Normal, High) will be included as per the CLSI guide for method comparison [1]. All study participants who fulfilled the eligibility criteria during the study period were included until the required sample size is obtained. Hence a total of 240 CSF and PF samples (120 each) were collected.

4.6. Sampling Technique

All of the patients who are requested for Cerebrospinal and Pleural fluid analysis during the study period that fulfill the inclusion and exclusion criteria were included consecutively.

4. 7.Study Variables

4.7.1. Dependent Variable

- WBC-BF, RBC-BF, MN#, MN%, PMN#, PMN%.

4.7.2. Independent Variables

- Age
- Sex
- Patient-specific diagnosis
- Specimen type

4.8. Data collection

Data on socio-demographic variables and associated factors such as age, sex and patient-specific diagnosis from which sample was sent (inpatient or outpatient), was collected from patient clinical records through pre-structured data abstraction format. Cerebrospinal and pleural fluid samples were collected by experienced physicians following standard operating procedures for body fluid analysis collection and immediately sent to the laboratory for analysis

4.8.1. Cerebrospinal and Pleural Fluid Collection

For analysis cerebrospinal and pleural fluid analysis sample collection is very important to gain good diagnostic value. Especially, lumbar puncture (LP) is critical if the patients have coagulopathy or anticoagulant/antiplatelet drugs. LP was performed based on the time of drug action and guidelines for LP by American Society of Regional Anesthesia- ASRA) can be followed (33). A fasting LP was taken for patients suspected of seizure for cerebrospinal fluid analysis. LP can be done either in a lateral or sitting posture, mostly, under sterile conditions, 22-24G spinal needles are inserted subsequently knowing the lumbar L2-3 or L3-4 space and local infiltration by an experienced physician. As soon as the spinal subarachnoid is identified using loss of resistance, adjusting removal of stylet was done to obtain massive drainage of CSF. The color of the CSF is famed and if blood-stained as a result of traumatic puncture, it is curial to wait for blood to be cleared before samples are collected. Samples are collected in three EDTA tubes each of 3-5ml CSF for analysis Sysmex XN-1000 analyzer (Sysmex Corporation, Kobe, Japan)(34)

Also, the pleural fluid samples are collected by thoracocentesis, using a 0.8 mm = 40 mm needle and a 20mL syringe in EDTA anticoagulated tubes by experienced physicians. In situations for which pleural effusion on chest radiography is large, no ultrasound is used. Sometimes, pleural effusion is collected using a pleural catheter (Pleuracath 8 F, Plastimed, St. Leu la Foreˆt, France) for diagnostic and therapeutic value. Sysmex XE-2100 hematology analyzer.

4.8.2. Cerebrospinal and Pleural Fluid Processing and cell counting

In the beginning, cell counting and leukocyte differentiation were performed by the manual method by expert technologists for both CSF and PF. Then, automatic analysis was done on a Sysmex XT-4000*i*analyzer (Sysmex Corporation, Kobe, Japan) using the body fluid module. Manual WBC counting is performed by microscopy with a Fuchs-Rosenthal hematocytometric chamber after centrifugation of the sample using ordinary centrifugation, and the results are reported for a volume of 1 µl. Cells are counted in a volume of 0.2 mm^3 (one square) when the number is >200, or in 1 mm3 (five squares) when the number is <200. Differential count performed using Giemsa staining for both CSF and PF fluids until 100 cells are counted to gain the related percentage of each cell type.

4.9 Data Analysis and Interpretation

The data was analyzed using SPSS version-23. Linearity was estimated through linear regression analysis using the least-squares method and by the coefficient of determination (r2). Agreement between the counts performed on sysmex-4000*i* (WBC-BF, RBC-BF, MN#, MN%, PMN#, PMN%.) and the manual cell counts using Fuchs-Rosenthal hematocytometric chamber by optical microscope (OM) was analyzed through non-parametric statistical methods: Passing-Bablok regression and Spearman's correlation in which values of $p < 0.05$ were considered statistically significant. In the regression analyses, a linear regression equation is created ($y = b + mx$), in which b is called the intercept and m is the slope. In Bland-Altman analysis, absolute average differences (y-axis) and the arithmetic averages (x-axis) of cell count by OM and those obtained on XT-4000*i* are plotted, with a significant tendency being observed when the average difference is distant from the null value. The 95% confidence intervals (95% CI) were calculated to highlight significant differences between methods. The Spearman's correlation coefficient analysis, the closer the values came to 1 the stronger the positive correlation is between the analyzed variables.

4.10. Quality Assurance

4.10.1. Pre- Analytical Phase

Body fluid samples were collected for diagnostic and therapeutic purposes (or both) by ultrasound-guided procedures by experienced physicians. The collection tubes and the conditions under which the specimen is transported to the laboratory are dictated by the tests ordered. Sample stability was documented to check if there is cellular deteriorations, cell lysis, and bacterial growth. Sample testing was taking place within the time the sample remains stable. Performance specification also checked if it is done for body fluid mode for XT-4000*i* Sysmex like precision, accuracy, analytical sensitivity, and analytical specificity including interfering substance, reference range, patient correlation studies, linearity, and limit of detection, and analytical measuring range.

The background count, accuracy, precision of the Sysmex XT-4000i were studied. The acceptable limits of them were based on the standard limit of ICSH (International Committee for Standardization in Haematology) (19) CLSI (Clinical and Laboratory Standards Institute) (7) and Sysmex Corporation (Kobe, Japan) (18).

Background Count

The acceptable background limits of automatic analyzer were ≤ 0.003 x 106/µL and ≤ 0.001 x 103/µL for RBC and WBC respectively. In the study, the background limits of the Sysmex XT-4000i were in the acceptable limits (18, and 19).

Accuracy

The acceptable accuracy for body fluids cell counts and differential by automatic analyzer are r = .0.80, .0.90, .0.70, .0.70 (RBC, WBC, PMN%, MN %) and the slope are 1 • } 0.30 for RBC and WBC count and 1 • } 0.50 for PMN and MN differential count. In the study, the accuracy of the Sysmex XT-4000i was in the acceptable limits (18, and 19).

Precision

The acceptable precision (reproducibility) of automatic analyzer for body fluids counting are coefficient of variation (CV) ≤ 40 % for RBC count and ≤ 30 % for WBC count. In this study, the precision of the Sysmex XT-4000i were in the acceptable limits (15).

4.10.2. Analytical Phase

Body fluid counting by automated machines needs different handling procedures from peripheral blood samples. Standard operating procedures were used in all steps required for handling the samples, changing modes of the analyzer, and sample running. Pre-treatment of the sample before running if the sample is viscous by hyaluronidase to lower viscosity, background check, and procedures for handling spurious results was checked.

4.10.3. Post - Analytical Phase

The reading from the XT-4000*i* was recorded on the patient request paper as well as the laboratory registration logbook. It is mandatory to check for quality of the result. The result was written with legible handwriting. The leftover sample was disposed following the safety procedures of TASH laboratory.

4.11. Ethical Considerations

The research and ethics committee of the Department of Medical Laboratory Sciences (DRERC) reviewed and approved the study protocol. A formal letter was written to the department of the hematology laboratory of TASH, Addis Ababa, Ethiopia. The data was collected from the patient clinical record using data abstraction format for the study participants. The collected data was secured in a lockable cabinet, confidential identifiers were used and data was analyzed in aggregate to maintain confidentiality and anonymity of information, and finally, the study outcome was delivered to the Hospital. No additional sample was collected for the purpose of this study as samples were referred to the laboratory for CSF and PF analysis as part of their clinical care.

4.12. Dissemination of Result

The result of the study is submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, hematology laboratory, and School of Medicine, Addis Ababa University. The final result will be presented at conferences and published in peer-reviewed journals.

4.13. Operational Definition

Body fluid: A fluid found in various cavities of the body under normal conditions (i.e., cerebrospinal fluid and synovial fluid), and others during pathological conditions (i.e., serous fluids). a plasma ultra-filtrate that is modified by both active and passive means of transport of substances from the systemic circulation into the central nervous system (CNS). Cerebrospinal fluid is a clear fluid that is formed as an ultra-filtrate of plasma. CSF is present in both the intracranial and spinal compartments. The cerebrospinal fluid (CSF) is a dynamic, metabolically active substance that has many important functions.

Pleural Fluid: A fluid that serves as a lubricant between the lungs and inner chest wall during breathing. It is produced under normal conditions by capillary hydrostatic and osmotic pressure from the parietal vasculature.

Lumbar puncture: An invasive technique that accesses the restricted compartment of the subarachnoid space to sample CSF. This procedure involves introducing a needle below the termination of the spinal cord, passing through the Dura matter of the spinal cord, and permitting access to the subarachnoid space.

5. RESULTS

5.1 Demographic and clinical characteristics of patients

A total of 240 samples (120 Pleural fluid and 120 CSF samples) were collected and evaluated in this study. The samples were collected from 123 males and 117 females with a male to female ratio of 1.05:1 (male: female). All the samples were analyzed using the Sysmex 4000-i system and Manual counting, and the evaluations were compared. Table 1 and 2 shows the distribution of samples by sex and age of the subjects. Body fluids were divided into two categories (Pleural and CSF). Both pleural and CSF fluids showed a higher representation of younger individuals. Meanwhile, CSF was represented by a relatively higher proportion of teen age patients in the age ranges (1-10) and (11-20).

Table1. Gender based distribution of body fluids among patients at TASH, Addis Ababa, January to June 2021

Body fluids	Male (%)	Female (%)	Total
CSF	64(53.3)	56(46.7)	120
Pleural fluid	59(49.2)	61(50.8)	120
	Male: female	1.05:1	240

Table 2. Age groups based body fluids distribution among patients at TASH, Addis Ababa, January to June 2021

Age groups (years)	Pleural fluid n(%)	CSF n(%)
1-10	3(2.5)	24(20.0)
11-20	18(15.0)	38(31.7)
21-30	32(26.7)	31(25.8)
31-40	25(20.8)	20(16.7)
41-50	12(10.0)	5(4.2)
51-60	11(9.2)	0(0)
61-70	19(15.8)	2(1.6)
Total	120	120

Table 3 shows the distribution of patient diseases and total body fluids. Diseases were divided into three broad categories: inflammation, malignancy, and “others.” The inflammation category comprised various inflammatory diseases: meningitis, pneumonia, cholangitis, and urinary tract infection, among others. The grand total prevalence of malignancy among the two body fluids were concluded to be 168 (70%). The malignancy category included tumors and hematologic cancers (stomach cancer, hepatocellular carcinoma, lung cancer, leukemia malignancy of unknown origin, etc.). The “other” category included liver cirrhosis and chronic kidney disease, among others. In the total body fluids, the malignancy category formed the largest of the three categories (70% among the two body fluids). CSF and pleural fluid (68.3% and 71.6%, respectively) constituted a large proportion of malignancies among the three categories. The inflammation category was predominantly observed in CSF (23.3%).

Table 3. Distribution of diseases condition among patients at TASH, Addis Ababa, January to June 2021

Samples	**Malignancy (%)**	**Inflammations (%)**	**Others (%)**
CSF(n=120)	82(68.3%)	28(23.3%)	10(8.3%)
Pleural fluid(n=120)	86(71.6%)	18(15%)	16(13.3%)
Total(n=240)	Grand(168)=70%	46(19.2%)	26(10.8%)

5.2 Comparison of automated and manual cell count using CSF and Pleural fluid

Sysmex 4000-i system was compared with the manual counting method for WBC counts and differential counts of the pleural fluids where (n=120) (Table-3). **Fig. 1** shows that the comparison of WBC counts between the Sysmex-4000-i system and chamber method for pleural fluids. In this comparison between the two methods, there was a good correlation, the Passing and Bablok regression slope was 1.17 (95% CI, 1.04 to 1.30) where the correlation coefficients r=0.824, (p<0.001).

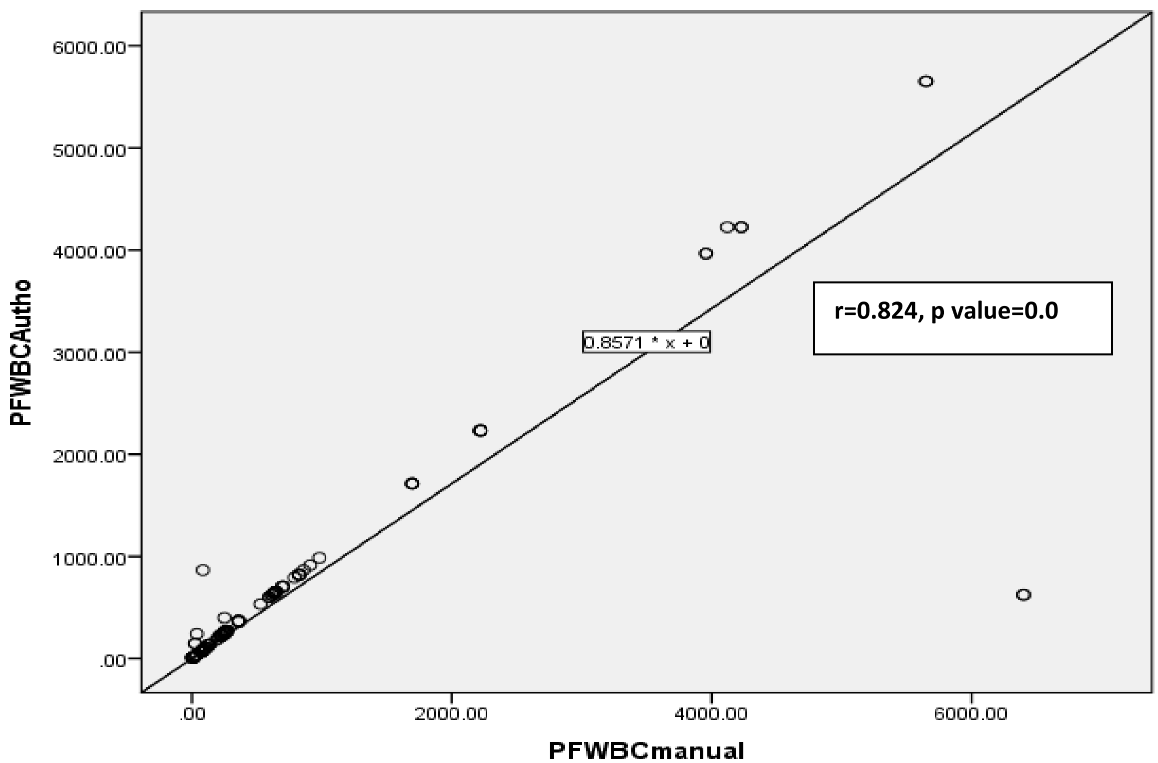

Figure 1. Correlation of WBC count using automated and manual methods of Pleural fluid analysis: X-axis: Manual WBC counts, Y-axis automated WBC counts **Y= 0.8571x+0**

Figure 2 Shows the comparison of PMN cell counts between the sysmex-4000 system and chamber method for pleural fluids. In this comparison, the Passing and Bablok regression slope was 1.07 (95% CI, 0.95 to 1.23). The overall correlation of differential cell counts for PMN cells between the Sysmex-4000-i system and chamber method was statistically significant and very high (r=0.96, P<0.001) (Table-4).

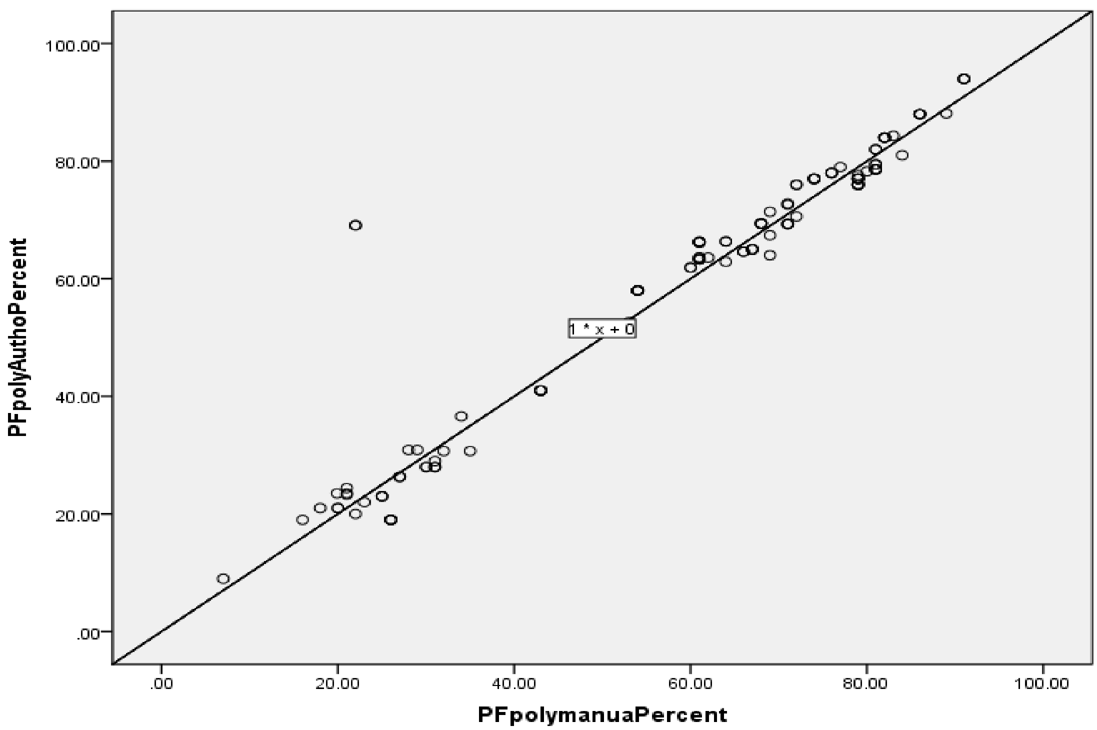

Figure 2. Correlation of PMN count using automated and manual methods of Pleural fluid analysis: Y= 1x+0, r= 0.958, P value=0.0, X-axis: Manual polymorph nuclear cell counts, Y-axis: automated polymorph nuclear cell counts for pleural fluids.

Table 4: Correlation of WBC counts and differential WBC counts between manual and Sysmex XT-4000i Method for CSF and Pleural fluids at 95%CI.

Fluid type	Mean	Min	Max	SD	Pearson correlations(r)	P value
CSF (n=120)						
Manual	60.7	3	621	84.8	0.83	< .001
SX-4000-i	60.5	2	617	86.3	0.83	<.001
Pleural Fluid(n=120)						
Manual	754	2	6400	1319	0.824	< .001
SX-4000-i	673	7.2	5652	1095.3	0.824	<.001
CSF(n=120)						
Manual PMN%	41.0	8	91	25.1	0.94	< .001
SX-4000-I PMN%	41.1	6	94	25.2	0.94	< .001
CSF(n=120)						
Manual MN%	58.2	9	92	25.1	0.92	< .001
SX-4000-I MN%	58.4	6	94	25.3	0.92	< .001
Pleural Fluid(n=120)						
Manual PMN%	58.5	7	91	22.8	0.96	< .001
SX-4000-i PMN%	59.6	9	94	22.9	0.96	< .001
Pleural fluid(n=120)						
Manual MN%	41	9	93	22.6	0.95	<0.0
SX-4000-i MN%	40.8	6	90	22.9	0.95	<0.0

Figure 3 shows that the comparison of pleural fluid mononuclear (MN) cell counts between the Sysmex-4000-i system and chamber method. In this comparison, the Passing and Bablok regression slope was 1.07 (95% CI, 0.95 to 1.23). The overall correlation of differential cell counts for MN cells between the Sysmex 4000-i system and chamber method was statistically significant and very high (r=0.95, P＜0.001).

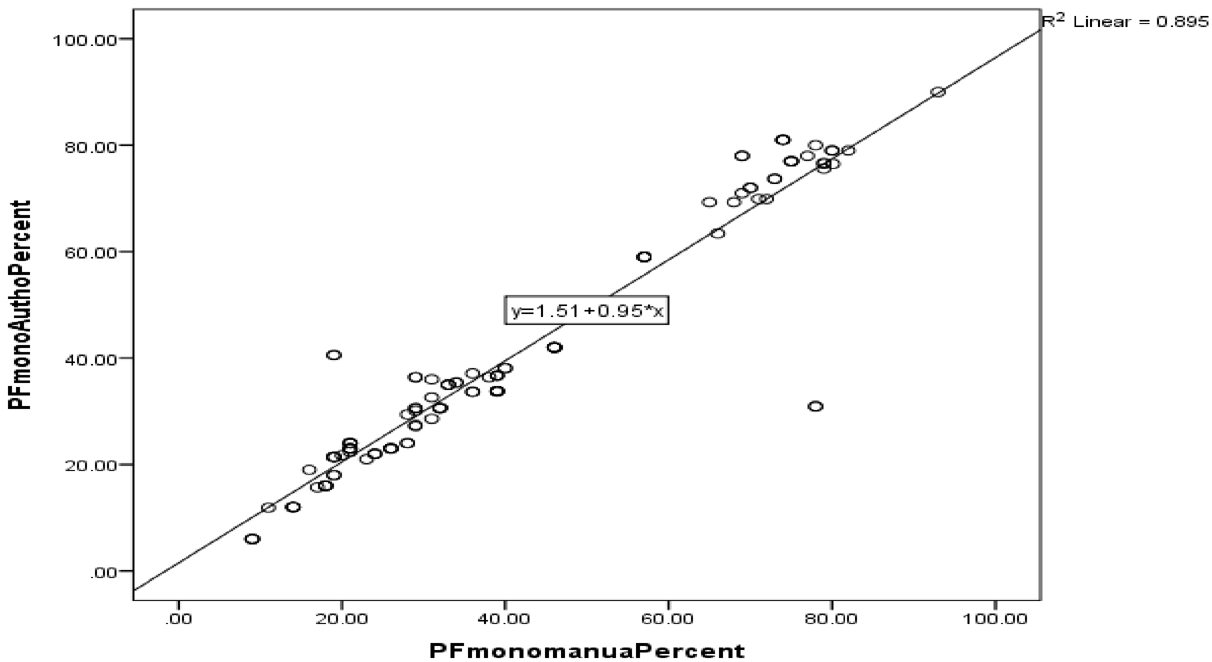

Figure 3. Correlation of mononuclear cells count using automated and manual methods of pleural fluid analysis: X-axis: The manual count of MN% for pleural fluid, Y-axis: The automated count of MN% for pleural fluid. Where r=0.95, R squared=0.90, y=0.95x+1.15

Figure 4, Shows a very good correlation coefficient of 0.83 for WBC counts for CSF, 0.94 for PMN%, and 0.96 for MN% cell counts. CSF showed excellent correlation for differential counts (PMN% and MN %)(r=0.94, and 0.96 respectively where p<0.001).

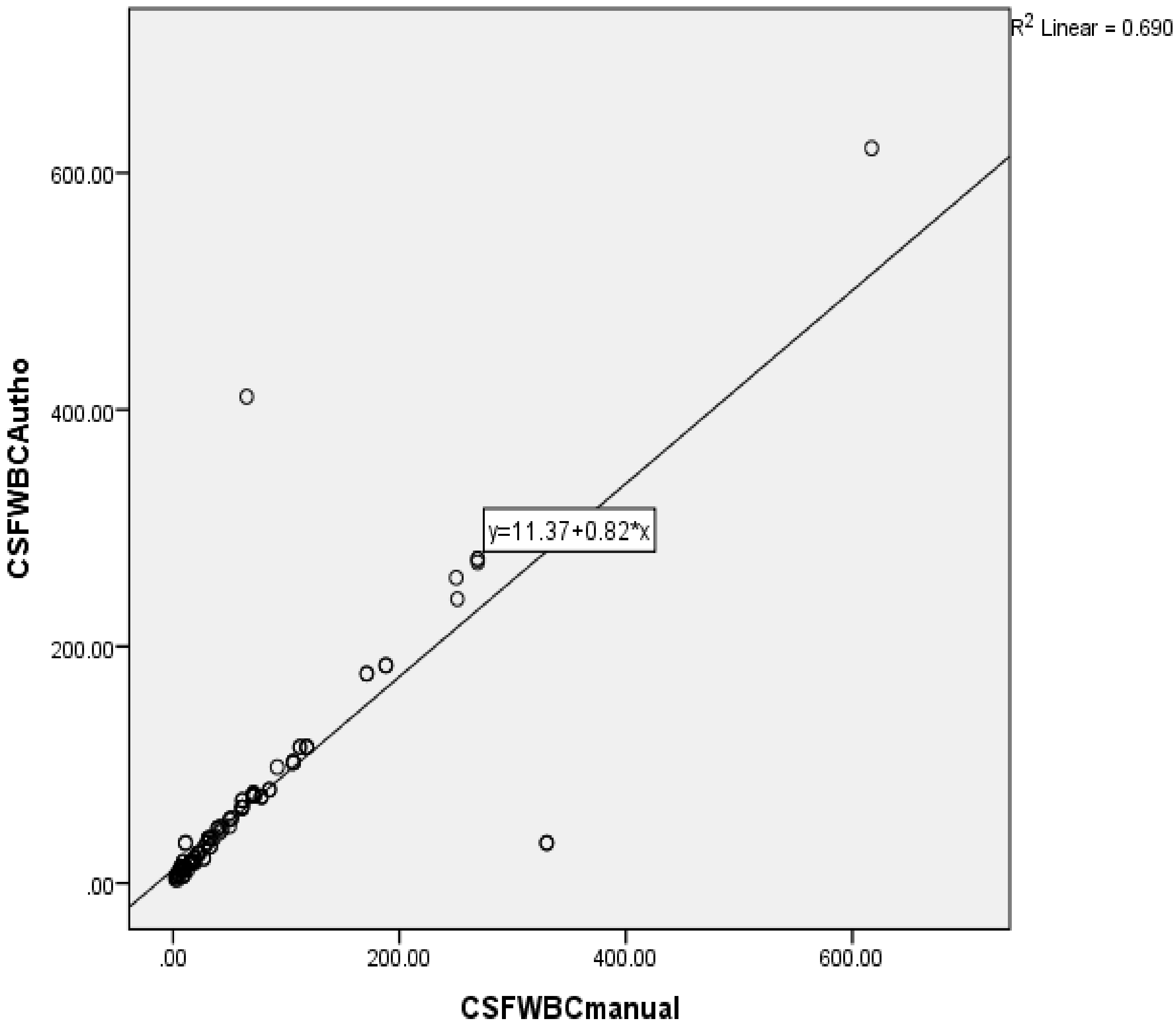

Figure.4. Correlation of WBC count using automated and manual methods of CSF analysis: X-axis the manual count of WBC for CSF count, Y-axis the automated count for CSF count, where r=0.83, R squared=0.7, p value=0.0

Figure 5 shows the comparison of PMN cell counts between the Sysmex-4000-i system and chamber method. In this comparison, the Passing and Bablok regression slope was 0.95 (95% CI, 2.17 to 3.12), the correlation between the manual and the automated count was very strong and it was statistically significant where (P<0.001).

The slope and the intercept of the manual and automated PMN% cells were 0.95 and 2.17 respectively. It indicates that there is a strong correlation between the two methods where

Y=0.95x+2.17

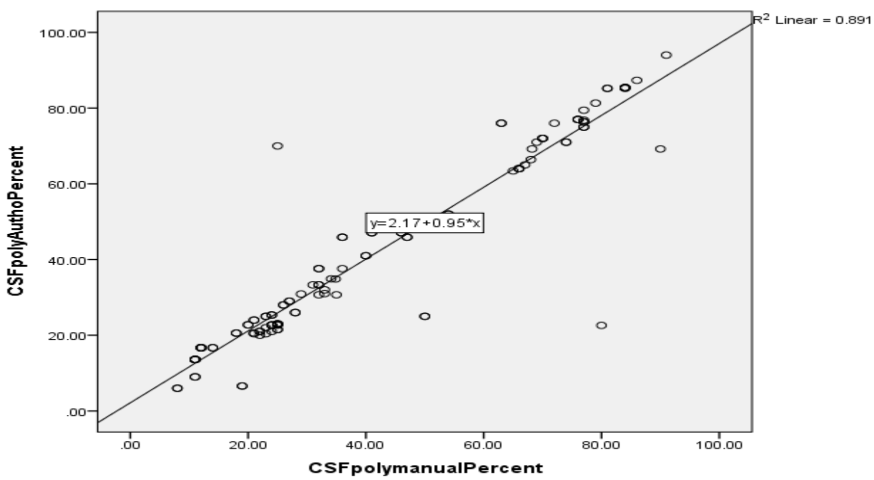

Figure.5 Correlation of PMN count using automated and manual methods of CSF analysis X-axis the manual count for PMN% of the CSF and Y-axis the automated PMN% count for CSF, r=0.94, R squared =0.89

6. DISCUSSION

This study aimed at comparing WBC, polymorphonuclear and mononuclear cells count using Sysmex 4000 I system and manual count using Fuchs-Rosenthal hematocytometric chamber from CSF and pleural fluid samples sent to hematology laboratory of TASH. From the sampled pleural fluid the highest number of patients seen was in the age group (21-30) i. e n=40. In contrast, younger individuals (younger than 20 years of age) accounted for 50% of the CSF fluid samples. From the history of the clients that was collected, many younger individuals and children were evaluated for meningitis and hematologic malignancies to rule out meningeal inflammation and metastasis of hematologic malignancies (lymphoma and leukemia), respectively.

The results obtained from the sysmex 4000-i system with those obtained by manual methods were compared. The results showed good correlations for WBC counting between these two methods. Moreover, the Sysmex 4000-i system demonstrated good performance for differential cell counting of body fluids. The evaluation of the sysmex 4000-i system was carried out according to the ICSH guidelines (7, 12), and the performance of the sysmex 4000-i system was consistent with the declarations of the manufacturer.

In previous studies, WBC counting and analysis of MN and PMN cell distributions from body fluid samples have been performed using microscopic quantification in chambers. However, this method is time-consuming and labor-intensive. Therefore, automated body fluid analysis methods have been developed, particularly for hematology parameters (4-8, 10, 13-15). Most recently, the sysmex 4000-i system has been used to evaluate CSF samples (10). Previous reports have suggested good correlations between automated WBC counts and traditional microscopic analysis, consistent with the current findings (4-7,9, 10).

The study also examined the correlations of each fluid type. The finding indicated that both body fluids, that is, pleural and CSF showed good correlation coefficient. Pleural fluid relatively showed a high correlation coefficient in the differential count. Overall, the two body fluids showed good correlation, and CSF was the most relevant and clinically useful.

Although the Sysmex 4000-i system in body fluid mode is capable of accurate WBC counts, it is critical to manually inspect samples with high WBC counts to identify pathological cells because the sysmex 4000-i system cannot differentiate malignant cells from normal cells. Therefore, the manual method is still needed to differentiate and detect malignant cells and to determine specific cell types in body fluids. Moreover, although the overall agreement between the Sysmex 4000-i system and chamber method was statistically significant, there were some samples with high standard deviations, particularly for PMN and MN cell differentiation. These findings may be explained by the presence of debris or fragments in the analysed samples, which may be interpreted by the instrument as PMN cells.

Although the analyser cannot replace microscopic analysis, microscopy should be performed for samples with high proportions of abnormal cells and samples with very low or high WBC counts. Nonetheless, automation can reduce the routine workload of technicians and enable rapid analysis in laboratories with the help of non-skilled technicians.

The current findings suggest that the sysmex 4000-i system might be useful for the initial screening of body fluids in urgent situations, when the availability of experts and trained personnel cannot be ensured.

7. Conclusion and Recommendations

The results of the current study provides evidence that the use of the Sysmex 4000-i system for cell counting and differential cell counting in body fluid samples can provide an effective and automated alternative to chamber counting in routine analysis in the laboratory. This in turn enhances the laboratory workflow. In contrast, to many other analyzers, this system also enables differential leukocyte counting. Accordingly, this system may have high diagnostic potential, particularly in the emergency department. Thus, it is recommended that TASH can use both automated using the Sysmex 4000i and manual methods as needed to count cells for the evaluated sample types.

In conclusion, the result of our study provides evidence that the use of the sysmex 4000-isystem for cell counting and differential cell counting in body fluid samples can provide an effective and automated alternative to chamber counting in routine analysis in the laboratory, thereby enhancing the laboratory workflow.

The authors declare that there is no conflict of interest.

8. Limitation of the study

Some limitations of the study are identified to be considered when interpreting the results. First, only we have used relatively a smaller number of samples are used. Furthermore, the distribution of samples was not even or heterogeneous. Therefore, additional studies using a larger number of samples from various sample types or sources are needed to confirm and expand upon the current findings.

9. References

1. Lohajaroensub S, Sakoonwatanyoo P, Sakunthaworn S, Pichanun D. Comparison of Body Fluid Cell Counting between Automated Hematology Analyzer Sysmex XT-4000i and Manual Microscopic Method. Vajira Medical Journal 2015;59(1).
2. Wanjian G, Liang G, Mingde J, Qing Y, Chunbing Z. Operating scheme and experience of executing internal comparisons of blood cell analysis in a clinical laboratory. Clinical laboratory. 2014;60(10):1627-34.
3. Fleming CKA. From Manual Microscopy to Automated Cell Counters for First Line Screening of Body Fluids:"But not without a special body fluid mode". 2016. Rotterdam, The NetherlandS. Available at https://epubs.ogc.nl/?epub=c.fleming.
4. Hussong JW, Kjeldsberg CR. Kjeldsberg's body fluid analysis: ASCP; 2015. Available at https://store.ascp.org
5. Comar SR, Schulz T, de Araújo Machado N, da Silva França F, Haas P. Análise citológica do líquido peritoneal. Estudos de Biologia. 2011;32:76-81.
6. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rded. Wayne, PA: Clinical and Laboratory Standards Institute; 2008; CLSI document CD28-A3.
7. Wright BL, Lai JT, Sinclair AJ. Cerebrospinal fluid and lumbar puncture: a practical review. Journal of Neurology. 2012;259(8):1530-45.
8. Buoro S, Apassiti Esposito S, Alessio M, Crippa A, Ottomano C, Lippi G. Automated cerebrospinal fluid cell counts using the New Body Fluid Mode of Sysmex UF- 1000i. Journal of Clinical Laboratory Analysis. 2016;30(5):381-91.
9. Brainin M, Barnes M, Baron JC, Gilhus N, Hughes R, Selmaj K, et al. Guidance for the preparation of neurological management guidelines by EFNS scientific task forces–revised recommendations 2004. European Journal of Neurology. 2004;11(9):577-81.
10. Porcel JM, Light RW. Diagnostic approach to pleural effusion in adults. American Family Physician. 2006;73(7):1211-20.
11. Bottini P, Pompeo D, Souza M, Garlipp C. Comparison between automated and microscopic analysis in body fluids cytology. International Journal of Laboratory Hematology. 2015;2(37):e16-e8.

12. de Jonge R, Brouwer R, de Graaf MT, Luitwieler RL, Fleming C, de Frankrijker-Merkestijn M, et al. Evaluation of the new body fluid mode on the Sysmex XE-5000 for counting leukocytes and erythrocytes in cerebrospinal fluid and other body fluids. Clinical Chemistry and Laboratory Medicine (CCLM). 2010;48(5):665-75.
13. Paris A, Nhan T, Cornet E, Perol JP, Malet M, Troussard X. Performance evaluation of the body fluid mode on the platform Sysmex XE- 5000 series automated hematology analyzer. International Journal of Laboratory Hematology. 2010;32(5):539-47.
14. Williams JE, SH JW. Gaining efficiency in the laboratory-automated body fluid cell counts: Evaluation of the body fluid application on the sysmex XE-5000 hematology analyzer. Labmedicine. 2011;42(7):395.
15. Harris N, Kunicka J, Kratz A. The ADVIA 2120 hematology system: flow cytometry-based analysis of blood and body fluids in the routine hematology laboratory. Laboratory Hematology. 2005;11(1):47-61.
16. Brown W, Keeney M, Chin-Yee I, Johnson K, Lantis K, Finn W, et al. Validation of body fluid analysis on the Coulter LH 750. Laboratory hematology: official publication of the International Society for Laboratory Hematology. 2003;9(3):155-9.
17. Sysmex America, Inc. Sysmex Announces FDA Clearance of Body Fluids Application on Its Automated Hematology Analyzer XE-2000.PR Newswire. 2004 Mar 26.
18. International Federation of Clinical Chemistry (IFCC) and International Committee for Standardization in Hematology (ICSH). Approved recommendation (1986).On the theory of reference values. J Clin Chem Clin Biochem 1987; 25: 337-42.
19. Dux R, Kindler-Röhrborn A, Annas M, Faustmann P, Lennartz K, Zimmermann C. A standardized protocol for flow cytometric analysis of cells isolated from cerebrospinal fluid. Journal of the Neurological Sciences. 1994;121(1):74-8.
20. de Jonge R, Brouwer R, van Rijn M, van Acker BA, Otten HJ, Lindemans J. Automated analysis of pleural fluid total and differential leukocyte counts with the Sysmex XE-2100. Clinical Chemistry and Laboratory Medicine (CCLM). 2006;44(11):1367-71.
21. DeNicola DB. Advances in hematology analyzers. Topics in Companion Animal Medicine. 2011;26(2):52-61.
22. Bar-Meir S, Lerner E, Conn HO. Analysis of ascitic fluid in cirrhosis. Digestive Diseases and Sciences. 1979;24(2):136-44.

23. Sandhaus LM. Body fluid cell counts by automated methods. Clinics in Laboratory Medicine. 2015;35(1):93-103.
24. Perné A, Hainfellner JA, Womastek I, Haushofer A, Szekeres T, Schwarzinger I. Performance evaluation of the Sysmex XE-5000 hematology analyzer for white blood cell analysis in cerebrospinal fluid. Archives of Pathology & Laboratory Medicine. 2012;136(2):1948.
25. Roccaforte V, Daves M, Proserpio V, Sciarini F, Sangiorgio R, Costanzo A, et al. Evaluation of body fluid mode of Sysmex XN-9000 for white blood cell counts in cerebrospinal fluid. J Lab Precis Med. 2018;3:22.
26. Cognialli R, Comar S, Souza A, Singer G. Avaliação da análise dos líquidos pleural e ascítico no analisador hematológico Sysmex XE-5000. Jornal Brasileiro de Patologia e Medicina Laboratorial. 2017;53(3):150-8.
27. Zimmermann M, Ruprecht K, Kainzinger F, Heppner F, Weimann A. Automated vs. manual cerebrospinal fluid cell counts: a work and cost analysis comparing the Sysmex XE- 5000 and the Fuchs–Rosenthal manual counting chamber. International Journal of Laboratory Hematology. 2011;33(6):629-37.
28. Takemura H, Ai T, Kimura K, Nagasaka K, Takahashi T, Tsuchiya K, et al. Evaluation of cell count and classification capabilities in body fluids using a fully automated Sysmex XN equipped with high-sensitive Analysis (hsA) mode and DI-60 hematology analyzer system. PLoS One. 2018;13(4):e0195923.
29. Lee J, Jeon K, Lee J, Kim M, Kim H-S, Kang HJ, et al. Comparison of Body Fluid Differential Counts Using a Manual Counting Method or an Automated Hematology Analyzer. Journal of Laboratory Medicine and Quality Assurance. 2020;42(1):26-32.
30. Buoro S, Mecca T, Azzarà G, Seghezzi M, Candiago E, Gianatti A, et al. Mindray BC-6800 body fluid mode, performance of nucleated cells, and differential count in ascitic and pleural fluids. International Journal of Laboratory Hematology. 2016;38(1):90-101.
31. SAVCI Ü, Şahin M, Barış E. A Comparison Of Manual Hemocytometry And Different Modes Of Mindray BC-6800 Hematology Analyzer For Cell Count In Peritoneal And Pleural Fluids. Dicle Tıp Dergisi. 2020;47(2):278-85.
32. Horlocker TT, Wedel DJ, Benzon H, Brown DL, Enneking FK, Heit JA, et al. Regional anesthesia in the anticoagulated patient: defining the risks (the second ASRA Consensus

Conference on Neuraxial Anesthesia and Anticoagulation). Regional Anesthesia and Pain Medicine. 2003;28(3):172-97.

33. Fleming C, Russcher H, Brouwer R, Lindemans J, de Jonge R. Evaluation of Sysmex XN-1000 high-sensitive analysis (hsA) research mode for counting and differentiating cells in cerebrospinal fluid. American Journal of Clinical Pathology. 2016;145(3):299-307.

10. Annexes

Annex I. Data collected from sample appearance during the sample came to the hematology laboratory

1. Type of sample

   □ CSF □ PF

2. The appearance of the sample or color of the sample

   □ Clean □ clear to turbid □turbid □ yellow □ bloody

Annex II. Data extraction format from the patient clinical record

1. Name of patient____
2. Card #or ICARE#_________________________
3. Age______
4. Sex

   □ Male □ Female

5. Patients clinical diagnosis specify____
6. The patient has a treatment history before

   □ Yes □ No

7. If the answer is yes for question 6 specify the type of treatment he/she took.

   ____

   ____

Annex III: English Version of Information Sheet

Introduction

The study is carried out at TikurAnbessa Specialized Hospital, Haematology laboratory Addis Ababa, Ethiopia entitled Comparison of Cerebrospinal (CSF) and Pleural fluid analysis using manual and automated hematology analyzer from leftover the sample in hematology laboratory.

Objective

The main purpose of this study is the comparison of cerebrospinal (CSF) and Pleural fluid analysis using manual and automated hematology analyzer at TASH, Addis Ababa, Ethiopia

Expected Outcomes and/or Benefits

At the end of this study, we will compare cerebrospinal and pleural fluids analysis using manual and automated hematology analyzer. Also, we will determine the diagnostic agreement between manual and automated body fluid analyzers. They will suggest the best method for analysis of body fluid samples for diagnosis patient's disease more accurately.

Thank you so much in advance.

Part II: English Version of Informed Consent Form

Each information from patients' records would be completely confidential to the research and the data are stored with their name and only used for this study. None of this would affect the care they receive from Haematology laboratory TASH but will help in future planning for the hospital. No identifying names or characteristics will go into my report. Additionally, the analysis of CSF and PF are done from a leftover sample from hematology laboratory therefore no consent for that the patients need to sign.

Principal Investigator: MulualaemAschenick

Addis Ababa University, CHS, School of public health, Department of medical laboratory science

Email: ashenekmulualem@gmail.com

Annex IV: የአማርኛ መጠይቅ ቅፅ

መግቢያ

ጥናቱ በጥቁር አንበሳ ፔሻላይዝድ ሆስፒታል ሄማቶሎጂ ላቦራቶሪ ክፍል ወስጥ ከተለያዩ ዲፓርትመንት ከመጡ የመቅኔ ናሙና ላይ የሚደረግ ይሆናል፡፡ ከላቦራቶሪ ምርመራ ተደርጎላቸዉከተረፈ ናሙና የሚሰራ ይሆናል፡፡

የጥናቱአላማ -

የዚህ ጥናት ዋና አላማው Comparison of Cerebrospinal (CSF) and Pleural fluid analysis using manual and automated haematology analyzer at TASH ሲሆን ዋናዉ ግቡ ተመራጭየ ሆነ ወን ሜትድ ማዎቅ ነ ዉ፡፡

ከጥናቱ የሚጠበቁ ወጤቶች/ጥቅሞች

ይሄ Comparison of Cerebrospinal (CSF) and Pleural fluid analysis using manual and automated haematology analyzer የትኛዉሜትድ ይበልጥ ተመራጭመሆኑን ለማሳየት፡፡

በተጨማሪም ከጥናቱ በሚገኙ ግኝቶች ይበልጥ ህክምና ወጤትን በተወሰነ መልኩ ለማሻሻል እንዲቻል ሚድረግ፤

Annex V: STANDARD OPERATING PROCEDURE FOR BODY FLUID

1. **Test name** WBC or leukocyte count

2. Test method

- **Manual**

3. Introduction

Part of complete blood cell count which shows the number of white blood cell found in micro liter (cubic millimeter) of whole blood. In any given day the WBC count may vary by much as 2000/uliter the WBC count may rise or drop significantly in certain disease but it diagnostically useful only when interpreted in the light of the WBC differential and of the patent current clinical states.

4. Purpose

- To detect infection or inflammation
- To determine the need for further test such as the WBC differential or bone marrow biopsy
- To monitor response to chemotherapy or radiation therapy

5. Equipment

- Citrated or vacutainer test tube
- Syringe with needle
- Tourniquet
- Alcohol swab
- Test tube rack
- Marker
- Neubaure counting chamber
- A=automated hematology analyzer

6. Reagent

- 3.8% trisodium citrate solution
- WBC dilution fluid

7. Reagent preparation

Glacial acetic acid --- 3ml

Distilled water -- 97ml

Mix and ready to use

8. Procedure for manual count

1. Prepare 1:20 dilution of blood to dilution fluid
2. Mix and stand for 2min.
3. Under the 10x objective count the cell in four large corner squares

Use automated hematology analyzer

9. Calculation

- Total count = actual count x 50

10. Normal value

4,000-11,000/mm^3

11. Implication

Elevated WBC count usually signals infection such as

- Abscess
- Meningitis
- Appendicitis
- Tonsillitis
- Leukemia

Tissue necrosis

- Burn
- Mi
- Gangrene

Low WBC count (leucopenia) indicate

- Bon marrow depression resulted from
  - Viral infection

- Treatment of anti neoplastic
- Ingestion of mercury
- Exposure to benzene or arsenicals

Infection

- Influenza
- Typhoid fever
- Measles
- Hepatitis
- Mononucleosis
- Rubella

12. Interfering

- Hemolysis
- Exercise, stress raises WBC count

Drugs

- Antineoplastic drug
- Metrendazol
- Flu cytosine
- Thyroid hormone antagonists
- Indomethacin

Annex VI: STANDARD OPERATING PROCEDURE FOR BODY FLUID CELL COUNT WITH DIFFERENTIAL USING SYSMEX XT-4000*i* HEMATOLOGY ANALYZER

Purpose

The intended purpose of this sop is to explain how to process, examine and report results for body fluid specimens for the characterization of inflammatory, infectious, neoplastic, and immune alterations

Scope

This procedure is intended for use in haematology laboratory when requested by clinicians

Responsibility

- Hematology department personal are required to be knowledgeable of this procedure.
- New employees are trained and assessed for competence before they can handle patient sample

Principle

A combination of fluorescent flow cytometry with a semiconductor laser and impedance technique to measure the following cellular parameters: size, volume, granularity, surface area, and fluorescent signal.

Reagents and supplies

- Cell clean
- Cell pack
- Stromatolyzer-4DL
- Stromatolyzer-4DS,
- Stromatolyzer-FB
- Sulfolyzer
- Control
- Calibrator
- A4 size paper

Equipment

- Sysmex XT-4000i hematology analyzer
- Printer

Sample and container type

Body fluids collected in EDTA tube. Body fluids include CSF, synovial fliud, peritoneal fluid and ascetic fluid.

Environmental and Safety control

- Universal precautions must be used when handling, processing and disposing of patient samples.
- Do not expose the XT-4000*i* to large temperature variations and direct sunlight.
- Avoid shocks and vibrations.
- Switch the power supply to the XT-4000*i* OFF before connecting any additional devices (host computer, printer).

Calibration

The Sysmex haematology calibrators XN CAL, XN CAL PF and SCS-1000 are designed for the calibration of Sysmex hematology systems. The assigned values of XN CAL, XN CAL PF and SCS-1000 are traceable to internationally recognized reference methods for WBC, RBC, HGB, HCT and PLT, according to the recommendations of International Council for Standardization in Haematology (ICSH) and Clinical and Laboratory Standards Institute (CLSI).

The Sysmex XT-4000*i* needs to be calibrated:

- Before initial operation (carried at by the Sysmex service representative!);
- when quality controls show deviations in the same direction which are determined repeatedly;
- When a major component, such as the sample rotor valve, has been replaced.
-

Quality Control

Body fluid controls are not available. Whole blood control materials are recommended. *e*-CHECK Level 1, *e*-CHECK Level 2 and *e*-CHECK Level 3 are used as control material. This is equivalent to the Low, Normal and High level. A quality control should be performed:

- Before any start of operation - prior to analyzing samples
- at least every 8 hours during operation
- after replenishment of components
- after maintenance

- If there is any doubt about the accuracy of the analysis values.

Procedure

1. Turn on the IPU switch and log on screen will appear on the computer. Enter the user name and password.
2. Turn on the main unit on the machine. Self-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear.
3. Double click on the Manual icon on the tool bar and the sample dialogue box will be displayed.
4. Input the sample ID number.
5. Click the body fluid mode for analysis.
6. When all settings are completed click **ok.**
7. When ok is clicked, background check is run. If background check is equal or lower than the given background count, perform the test.
8. Mix the sample tube gently.
9. Hold the opened sample tube under the sample probe and press the start switch but When the READY LED turns off (and two short beeps sound), remove the sample tube.
10. Automatic print of the result will be printed out when analysis finished.

Quality control procedure

1. Bring all the 3 control materials at room temperature.
2. Turn on the IPU switch and log on screen will appear on the computer. Enter the user name and password.
3. Turn on the main unit on the machine. Self-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear.
4. Click the Controller button on the Menu screen.
5. Double-click the QC Analysis icon on the Controller Menu and select QC File dialog box.
6. Select a QC file and click OK.
7. Gently invert eight times the control tubes
8. Hold the opened control tube under the sample probe and press the start switch button.

9. Accept the control result if are within the range of the target limit or repeat the analysis if control results are out of the target limit.
10. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

Calculations

Not applicable

Performance Characteristics

Method was verified for intended use.

Uncertainty measurement

Interferences/Limitations

Fat globules, crystals and high viscous body fluids may cause erroneous or misleading results.

Critical values

Not applicable

Result reporting

Results are reported from automated printing and through computer

Result interpretation

Trauma and hemorrhage may result in increased red and white cells; red cells predominate. White blood cells are increased in inflammatory and infectious processes:
Neutrophils predominate in bacterial infections and Lymphocytes predominate in viral infections.

Biological Reference Interval

Source	**Erythrocyte count** (cells/μl)	**WBC count** (cells/μl)	**PMN**	**MN**
Pleural fluid	<10,000	<1,000	<10%	<25%
Synovial fluid	0	<150	<25%	<80%
Ascetic fluid	<500	<500		-
Peritoneal fluid	<10,000	<200	<25%	-
Pericardial fluid	<10,000	<500	<25%	-
CSF	0-20	0-20	<2%	<90%

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. Candidate: Mulualem Aschenik (BSc.)

Signature:___________________

Date of submission:___________________

This thesis submitted with our approval as an advisor.

Advisor: **Aster Tsegaye** (MSc, Ph.D.)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

RahelAlemu (MSc)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

Yared Mamushet (MD, Neurologist, TASH)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

Tewodros Tamire (MSc, DPHM, TASH)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia

Henok Kebede (MSc, Hematology, TASH)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia