ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Comparison of Microhematocrit Methods (Commercial versus Window Clay Sealant) and Automated Hematology Analyzer (Sysmex XT-4000i) for Determination of Hematocrit and Mean Cell Volume (MCV) at Tikur Anbessa Specialized hospital, Addis Ababa, Ethiopia

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A Research Thesis to be Submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology).

October, 2019

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by **Haymanot Birhanu**, entitled:

Comparison of Microhematocrit Methods (Commercial versus Window Clay Sealant) and Automated Hematology Analyzer (Sysmex XT-4000i) for Determination of Hematocrit and Mean Cell Volume (MCV) 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.

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Acknowledgment

First and foremost I would like to thank my almighty God for helping me in all challenges. I am indebted to thank Addis Ababa University and Tikur Anbessa Specialized Hospital for giving this chance. I would like to thank my advisors Dr. Aster Tsegaye, Mikias Negash and collaborators Elias Bisrat and Henok Kebede for their constructive comments and guidance throughout my study. I would also want to acknowledge all my families and friends for their unreserved support.

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List of Abbreviations

µL	microliter
AAU	Addis Ababa University
CBC	Complete Blood Cell Count
dL	Deciliter
DREC	Department Research and Ethics Committee
EDTA	Ethylene Diamine Tetra Acetic Acid
HCT	Hematocrit
HGB	Hemoglobin
ICSH	International Council for Standardization in Hematology
L	Liter
LoA	Limit of Agreement
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Corpuscular Volume
PCV	Packed Cell Volume
PI	Principal Investigator
RBC	Red Blood Cell
SD	Standard Deviation
SOP	Standard Operating Procedure
TASH	Tikur Anbesa Specialized Hospital
WBC	White Blood Cell

Abstract

Background: Due to several sources of imprecision and inaccuracy of HCT and RBC Indices measurement in manual and automated methods difference in values may impact the clinical decision. Therefore, it would be essential to compare the three methods.

Objective: To compare of Microhematocrit (Manual) Methods and Automated Hematology Analyzer (Sysmex XT-4000i) for determination of Hematocrit and Mean Cell Volume at Tikur Anbessa Specialized Hospital, Addis Ababa.

Method: A hospital based comparative cross-sectional study was conducted in Tikur Anbessa Specialized Hospital (TASH) from January to March 2019 on 240 left over blood samples for Complete Blood Count (CBC) analysis. Hematocrit and MCV were determined Microhematocrit (Manual) using commercial and window clay sealant and Sysmex XT-4000i automated methods. The data obtained from the three methods were entered and analyzed using Statistical Package for SPSS version 23 software for windows. Standard deviation and mean were determined and Independent Student t-test was used to compare mean difference between the three methods on p-value of 0.05. Additionally, precision, correlation coefficient and Bland Altman plot were used to assess correlation and agreement between the tests. Also figures and tables were used for the description of the data.

Result: The mean ±SD for three methods as follow as: HCT values were 40.9±14.91, 40.1±14.84 and 40.3±14.98 for automated, manual methods using commercial sealant and window clay respectively, MCV values were 85.7±9.18, 83.7±10.6 and 83.9±10.12 for automated, manual methods using commercial sealant and window clay respectively. There was no statistically significant difference between the automated and both manual methods ($p>0.05$) for the mean values of HCT. There was statistically significant difference in the mean values of MCV between automated and both manual methods ($p<0.05$), but no statistically significant difference between manual methods. HCT values of automated and manual methods using commercial sealant (positively correlated ($r=0.984$) and limit of agreement range (95%)) and window clay sealant (positively correlated ($r=0.992$) and limit of agreement range (96.25%)) and the manual methods each other had also positive correlation ($r=0.988$) and limit of agreement range of (95.75%).

Conclusion: since there is good performance agreement for HCT, the results of automated method could be checked by both manual methods. Also the two manual methods interchangeably could be used for hematocrit determination in the case of inadequate sample.

Key words: Hematocrit, Microhematocrit, automated HCT

1. INTRODUCTION

1.1 Background

In the advancement of the health care system, laboratory analysis plays a significant role in generating accurate and reliable clinical information for patient diagnosis and treatment by implementing a quality assurance program and committed to it (1). This advancement of laboratory diagnosis for hematological analysis greatly helped by use of automated hematology analyzer that uses different working principles (impedance or optical light scattering) (2) that able to reduce heavy workload and perform in very short period of time with high analytical performance for cell counting and flagging for abnormal findings (3).

Though that is the fact for many automated hematological analyzers to bring substantial input for the diagnostic technology, they could also produce inaccurate and unreliable laboratory results. So they should be compared and evaluated with currently used specific instrument against the manufacturer's specifications claim to verify (4) and this can be achieved by using statistical analysis consists of paired measurements by the two methods and testing for the agreement of the two analytical methods (5,6).

Hematocrit (used by analyzer to compute value) also known as packed cell volume (PCV) (referred to centrifuged blood in capillary tube), which indicate the percentage (%) of compacted red blood cells after being centrifuged or proportion of whole blood occupied by red cells, expressed as a ratio (litre/litre) is used to screen anemia or polycythemia when it is not possible to measure hemoglobin accurately and mains electricity is available to operate a microhematocrit centrifuge. Also qualitatively looking at the color of plasma (straw-normal, colorless- severe iron deficiency anemia, abnormal yellow- bilirubin/hemolytic anemia, pink/red- hemolyzed samples or hemoglobinemia) on the capillary tube (7, 8).

In Ethiopia microhematocrit method is used for determining hematocrit in most of the parts where automated hematology analyzers are unreachable and when insufficient samples are collected (9). Microhematocrit is a technique that uses a small volume of blood is taken by capillarity into an ungraduated capillary tube (usually 75 mm long with an internal diameter of 1.2 mm), leaving about 15 mm unfilled. The end of the tube distant from the column of blood is sealed by modelling clay or a similar product. It is then centrifuged at a specified revolution time (for example 1200rpm), specially designed centrifuge, to separate the column of blood into red cells, buffy coat and plasma. The PCV is read visually on a scale, the buffy coat of white cells and platelets being excluded from the measurement

(8). In TASH, a capillary tube sealed with non-toxic, sticky and dry resistant tube sealant (for example Sigillum MODULOHM I/S) and centrifuged by using KHT- 400 Microhematocrit Centrifuge of 12000 rpm with timer activated operation and an automated balancing system feature is used for hematocrit determination (10).

Sysmex XT-4000i can analyze and output the results for 39 parameters. WBCs and reticulocytes are analyzed by the optical detector block based on the fluorescence flow cytometry method and using semiconductor laser. RBCs and platelet count are analyzed by the RBC detector using the hydro dynamic focusing method. Hemoglobin (HGB) is analyzed by the SLS hemoglobin determination method. Analysis data is displayed on the information processing unit (11).

The RBC indices, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) first introduced in the late 1930s by Wintrobe and could be obtained by calculation from RBC count, HGB concentration and HCT. They are important in the morphologic classification of anemias, defining normocytic, microcytic, and macrocytic anemias (12).

2.1 Statement of the Problem

In the routine laboratory tests, incorrectly reported HCT result may bias clinical decision in follow up of patients, blood transfusion decision, and in diagnosis of hematologic diseases such as severe anemia and polycythemia. Despite the significance, it get much less attentions in research from the standpoint of its reliability than have the measurements of hemoglobin or red cell counts (13).

Microhematocrit method is considered as a gold standard method for hematocrit determination but it also associates with some inaccuracy and imprecisions (14). Spun hematocrit is 1% to 3% higher than the hematocrit from automated instrument due to plasma trapping in the erythrocytes due to change in size or shape, otherwise may reach high up to 6% in conditions like macrocytosis, spherocytosis or polycythemia. Additionally, microhematocrit method could also by affected by operational factors like insufficient centrifugation, delay in reading, excess anticoagulant, clotted sample, not clean capillary tube, inappropriate sealant (e.g. heat) and not performing daily or monthly preventive maintenance (7). Also repetitive measurement could increase the risk of having transmittable diseases, therefore, there is a need of non-invasive method of the hematocrit measurement, because recently used methods are based on the in vitro measurement (15). The type of anticoagulant, i.e. being heparinized or non-heparinized does not show significant difference in hematocrit values (16).

To ensure the accuracy of the hemoglobin and hematocrit a quick mathematical check, the rule of three done for normocytic, normochromic RBC only. If the calculated hematocrit does not agree within +/- 3% of the measured hematocrit, a measurement error or instrument malfunction could have occurred, or the patient could have a pathology that requires investigation. The manually calculated MCHC is lower than that obtained from the automated instrument. When the hematocrit is corrected for trapped plasma, the erythrocyte indices calculated from the centrifuged hematocrit agree with those obtained from automated instruments (12).

The calibration of usually all automated hematology analyzers can be traced in some way back to hematocrit which includes establishing reference range of HCT and red cell indices, for assigning expected or target values of calibrators and controls by avoiding possible errors like trapped plasma, contamination of the red cell layer (WBC or Platelet), indistinct margin from white cell layer, non-flat tube seals, red cell dehydration and oxygenation state (17).

TASH has 5 automated hematology analyzers. Most of these analyzers have never been evaluated for their agreements with manual method like microhematocrit method. Among these, Sysmex XT-4000*i* which this study aimed to study the agreement with microhematocrit method to determine hematocrit

and MCV values. Therefore, this study could be an input in reducing ambiguity occurs during determining the correct hematocrit values.

Generally, method comparison can play an important role in improving the quality of laboratory services and guiding right clinical decisions. Despite these, there is no much tradition of evaluating, verifying and validating new methods, a gap which this study tries to address.

1.3 Significance of the Study

This study finding will help to increase the quality of HCT determination by reducing ambiguity during hematocrit determination and to get correct hematocrit values. It also helps to improve the clinical management of severely anemic patients requiring blood transfusion and polycythemic patients that require phlebotomy and medical treatment. Moreover, it will be helpful for other researchers who will be interested in doing similar work. Finally, this study also intended to remind the use of the manual methods to check the automated hematology analyzers for the proper function and performance.

2. LITERATURE REVIEW

2. Literature Review

A study was conducted in New York, USA in 2016 by Avecilla ST et al. to compare manual hematocrit determinations and automated methods for hematopoietic progenitor cell apheresis products. Significant ($p<0.001$) differences were observed where the manual Hct value was markedly lower than automated HCT values (18).

A study also conducted by Prihirunkit K et al in 2008 on blood samples from dogs and cats to compare the manual and automated for both Hct and Hgb; there were significant differences between manual and automated Hct for both the dog ($P<0.05$) and cat ($P<0.01$) samples, as well as between the manual cyanmethemoglobin and automated cyanide-free methods for both species (dog $P<0.01$, cat $P<0.05$). However, strong correlations using Pearson's correlation coefficient, R, between the two methods were observed (R for Hct of dog = 0.96, cat = 0.98 and R for Hb of dog = 0.96, cat = 0.87). The results indicated that the Hct and Hb values from the automated method could not be used to substitute for those of the manual method, though the values of the two methods were accurate and precise (19).

A comparative study was done by Charuruks N et al in 1993 to compare the manual and automated methods for determining hematocrit (Hct) and mean corpuscular volume (MCV) were done by using three groups of blood specimens: microcyte (MCV < 80 fl), normocyte (MCV 80-96 fl), and macrocyte (MCV> 96fl), with each group containing 100 specimens. The average values of Hct by the automated method were 30.2 ± 43, 38.1 ± 3.1 and 33.4 ± 3.9% and by the manual method 34.7 ± 5.0, 39.6 ± 3.7, and 36.6 ± 4.4% in all three groups respectively. The average values of MCV by the automated method were 69.8 ± 4.8, 883 ± 3.2, and 105.4 ± 4.6fl, and by the manual method 78.9 ± 5.6, 91.1 ± 3.7, and 112.2 ± 5.5fl in all three groups respectively. This study showed that a significant difference between automated values and manual values ($p<0.05$) and Hct and MCV by the automated method was more reliable than by the manual method (20).

In Iraqi, Karem KK et al. conducted a comparative cross sectional study from 28 December 2015 to 28 January 2016 to assess the analytical performance between manual procedure and automated methods for hematocrit and white blood cells determination for EDTA blood samples. The two methods were highly correlated (R=9.75) and were significantly different ($p<0.001$). This study concluded that hematocrit value obtained from hematology analyzer (Sysmex XP 300TM) is different from that of manual, but it is directly proportional in most cases (21).

A comparative cross sectional study was carried out between September and November 2014 to determine the hematocrit values obtained using automated haematology analyzer and the microhematocrit (manual) methods by Ode SA et al in Nigeria. The result showed a strong positive

correlation (r=0.946) between the automated hematocrit and microhematocrit values. The hematocrit values obtained by the automated haematology analyzer were significantly higher than the hematocrit values obtained by microhematocrit method (p=0.0051). The strong positive correlation probably implies that results obtained from both methods are comparable and reliable (22).

Another cross sectional study was conducted in 2016 in Nigeria by Babadoko AA et al to compare the results of some hematological parameters obtained by automated counts with that obtained by manual methods using the same sample at the same time. The mean hematocrit values were 37.5 ± 7.2% by manual method and 37.2 ± 7.3% by automated method. There was no significant statistical difference between the two methods (p>0.05). The Pearson correlation test showed a positive significant ($P < 0.05$) correlation between both methods. They concluded automated analyzers can be used in all laboratories to provide quick and accurate results for patient care (23).

A study was done by Ike SO et al in 2010 to determine the correlation between hematological parameters by Sysmex KX-21N automated hematology analyzer with the manual methods on 60 randomly selected apparently healthy and with certain blood disorders. They found the mean (S.E) values of hemoglobin, packed cell volume, platelet and total white cell counts demonstrated statistically significant difference ($p < 0.001$) and correlated positively when both methods were compared (24).

A study conducted in University of Khartoum on 100 normal Hgb and Hct patient samples from April to May 2003 to compare manual, semi-manual and automated method found significant variation between manual and automated methods for estimating Hct, but there is no significant variation between two methods used for estimating Hb (25).

A comparative cross sectional study was conducted from 28 April to 28 June, 2014 in Yirgalem hospital, Ethiopia to assess the analytical performance between microhematocrit and automated methods for hematocrit determination by Gebretsadkan G et al. The result of this study showed that the correlation coefficient (R=0.95) indicated the strong correlation between manual and automated methods to determine the hematocrit. The manual HCT and automated HCT were significantly different (P<0.002) at 95% confidence interval. The result indicated higher coefficient of variation (CV) in manual method than automated HCT results, which implicated the precision is good for automated method (mindray 3000 plus) and not good for manual method (26).

Generally, the literatures, Clinical and Laboratory Standards Institute (CLSI) and International Council for Standardization in Hematology (ICSH) all agreed and recommended evaluation or comparing the performance of the new method with the current or Gold standard or reference method before replacing it. Concerning the comparison of manual and automated methods were attempted for the hematocrit in Ethiopia from Hawassa University some years ago, but not in Tikur Anbessa Specialized Hospital.

Therefore, this study was done for the first time in this university hospital which also includes MCV beside hematocrit.

3. OBJECTIVES

3.1 General Objective

To compare microhematocrit methods (commercial versus window clay sealant) and automated hematology analyzer (Sysmex XT-4000i) for determination of hematocrit and Mean Cell Volume (MCV) at Tikur Anbessa Specialized hospital, Addis Ababa, Ethiopia from January 1 to March, 2019.

3.2 Specific Objective

- To compare HCT and MCV values between microhematocrit methods that uses window clay and commercial sealant to seal the capillary tube.
- To compare automated HCT and MCV values against microhematocrit method that uses window clay to seal capillary tube.
- To compare automated HCT and MCV values against microhematocrit method that uses commercial sealant to seal capillary tube.
- To study the agreement and correlation of automated and both manual methods HCT and MCV values.
- To compare microhematocrit method that uses commercial sealant to seal capillary tube HCT and MCV values against microhematocrit method that uses window clay sealant to seal capillary tube.
- To study the agreement and correlation of manual methods HCT and MCV values each other.

4. HYPOTHESIS

Ho: There is agreement between results from automated method, microhematocrit (manual) method using commercial sealant and window clay of hematocrit and MCV determination.

5. MATERIALS & METHODS

5.1 Study Area

This study will be conducted at Tikur Anbessa Specialized Hospital (TASH) which is located in Lideta sub city in Addis Ababa. TASH was established in 1973 G.C to serve the needs of the whole country as specialized referral hospital and also as the main teaching hospital of the School of Medicine under Addis Ababa University with bed capacity of more than 700. The faculty is the oldest and the largest among the health training institutions in the country, staffed with the most senior specialists. The Hospital has 201 doctors, 627 nurses, 55 laboratory technologists and over 115 other health professionals dedicated to providing the health care services. The hospital also has over 1300 permanent and contract administrative staffs to support the hospital activities. TASH provides diagnosis and treatment for approximately 370,000-400,000 patients per year (27).

TASH laboratory has different departments and the hematology department is one of them. This department is equipped with different hematology analyzers and equipment used to perform different hematological tests. The hematology laboratory receives in the day time on average 350-400 samples with different tests requested.

5.2 Study Design and Period

Comparative Cross-sectional study was conducted from January to March 2019.

5.3 Population

5.3.1 Source Population

All patients who came for CBC analysis were the source population.

5.3.2 Study Population

Patients who were requested for HCT and MCV as part of CBC analysis and meet the sample acceptance and rejection criteria according to standard operating procedure (SOP) in TASH laboratory.

5.4 Inclusion and Exclusion Criteria

All blood samples came to TASH hematology laboratory for CBC analysis were analyzed based on sample acceptance and rejection criteria according to standard operating procedure (SOP) in TASH laboratory. Based on the SOP; mislabeled, clotted, hemolyzed, over filled, inadequate and old (> 6 hours) samples were rejected.

5.5 Study variables

5.5.1 Dependent Variable

Precision of the measurements and Agreement between the methods for HCT and MCV.

5.5.2 Independent Variables

RBC, HCT and MCV parameters, method types

5.6 Sample Size Calculation and Sampling Method

5.6.1 Sample Size Determination

For the method comparison studies, as stated in Clinical and Laboratory Standard Institute (CLSI) 2013 guideline a minimum of 40 specimens are recommended (28). Using convenient sampling techniques, a total of 240 (for Low, Normal and High values) specimens were analyzed.

5.6.2 Sampling Method

Convenient Non Probability sampling method was used.

5.7 Measurement and Data collection

5. 7.1 Data collection procedure

Whole Blood Samples were collected from patients of any age and gender (Low values from adult Female and children, High values from the adult Male and Normal values from both adult Male and Female HCT values based on the reference range given by Sysmex Corporation for XT-4000i) with any diagnosis at TASH laboratory reception for CBC analysis by phlebotomists and immediately transported to the hematology laboratory section and the leftover samples from the analysis were used and plain (no anticoagulant) capillary tubes were used for manual determination of HCT. Data of age, sex and clinical diagnosis of patients were taken from the request forms.

5.7.2 Laboratory analysis

Automated HCT and MCV Determination

CBC is performed using sysmex 4000i Hematology analyzer whereas manual HCT is performed by microhematocrit technique. The standard operating procedures are found in Annex I and Annex II.

The sysmex 4000i can analyze and output the results for 39 parameters. WBCs and reticulocytes are analyzed by the optical detector block based on the fluorescence flow cytometry method and using semiconductor laser. RBCs and platelet count are analyzed by the RBC detector using the hydro dynamic focusing method. Hemoglobin

(HGB) is analyzed by the SLS hemoglobin determination method. Analysis data is displayed on the information processing unit.

Manual HCT measurement by KHT-400

Anticoagulated whole blood is centrifuged and the volume occupied by the erythrocytes is expressed as a percentage of the total volume. In briefly, from the well mixed EDTA venous blood, a plain capillary tube (75mm in length and having internal diameter of about 1 mm) having filled 75%, then the unfilled side sealed by window clay or commercial sealant. After centrifugation for 5 min in 12000 rpm using KHT-400 microhematocrit centrifuge, PCV measured using Hematocrit reader, and expressed as a percentage.

Calculation of MCV:

- Calculated from RBCs and HCT as follows :

Mean Cell Volume (MCV) = HCT %× 10/ RBCs ($\times 10^{12}$/ L) = fL

MCH and MCHC values were not calculated due to lack of manual methods for the determination of HGB. Also the automated RBC values were used for the manual calculation of MCV due to the frequent encountering of imprecision by the hemocytometer method.

5.8 Data Quality Assurance

Pre-analytical

Patient identification and labeling was made with great care and samples were properly collected and transported without delay. Clotted samples, wrong anticoagulant samples (e.g. CBC in citrate tube) and other that were mentioned in the sample rejection criteria list were also controlled in this stage of quality assurance.

Analytical

Three levels of commercially prepared hematology cell controls (Normal, Low and High) for the Sysmex 4000i analyzer were run. Analysis was performed by following standard operating procedure (SOP) after running and passing of these levels of controls. For the microhematocrit method duplicate run were done as a quality control and +-1.5% values are valid.

Post-analytical

For avoiding any clerical error, printout results that were generated by the analyzers were used.

No results from the screen of the analyzers were recorded by hand. Then the results were confidentially documented and recorded using SPSS version 23.

5.9 Operational Definitions

Anemia- a medical condition of having a lower than normal number of red blood cells or quantity of hemoglobin (< 11g/dL).

Bland Altman plot: a difference plot which put the difference on the dependent axis scattered around the bias which is y- axis and the average of the two methods on the independent axis which is x- axis.

Correlation coefficient - A statistic that indicates the degree, to which two measurements are related, expressed as a value from -1.0 to +1.0, with +1.0 indicating that results are in total agreement, and -1.0 indicating that results are opposite. A 0.0 value indicates that the two measurements are unrelated.

Limit of agreement: It represents the range of values in which agreement between methods will lie for approximately 95% of the sample.

MCH- gives the amount of hemoglobin in pictograms (pg) in an average red cell.

MCHC-gives the concentration of hemoglobin in g/L in 1 litre of packed red cells.

MCV- is a measure of the average volume of red blood cells.

Precision- is how close the measured values are to each other.

Polycythemia- is a disease state in which the hematocrit (the volume percentage of red blood cells in the blood) is elevated

RBC Indices- are absolute values calculated from RBC, HGB and HCT.

5.9 Data Analysis and Interpretation

The data obtained from the tests was entered and analyzed by using MS-Excel 2016 and statistical package for social science (SPSS version 23) computer software for windows and interpreted accordingly. Standard deviation, coefficient of variation and correlation coefficient were used to compare continuous variables. Pearson correlation coefficient was used to determine correlation between manual and automated methods of HCT and MCV values. Bland Altman plot was used to assess agreement between the tests. In the Bland Altman method, the differences in manual versus automated HCT and MCV measurement was plotted against mean of the two methods. Agreement considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases. Precision was determined using coefficient of variation. P-Values < 0.05 considered statistically significant. Additionally, figures and tables were used for the description of the data.

5.10 Ethical Considerations

The study was conducted after getting ethical clearance from the Department Research and Ethics Committee (DREC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. An official support letter of request was written to Tikur Anbessa Specialized Hospital to obtain approval and carry out the study. The leftover specimen from CBC analysis was used only for the intended purposes. All information collected in this study were code in 4 digit numbers. The key to this code numbers kept in a locked file and is accessible to the authorized staffs only.

5.11 Dissemination of the Result

The finalized paper of this study will be presented and submitted to Addis Ababa University, College of Health Sciences, Department of Laboratory science. A copy of this material will be given to TASH laboratory and will be used to improve the laboratory's practice by developing guideline. The finding will also be communicated to the hospital and respective stakeholders. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant workshop, seminars and scientific conferences.

6. WORK FLOW

6.1 Work Flow

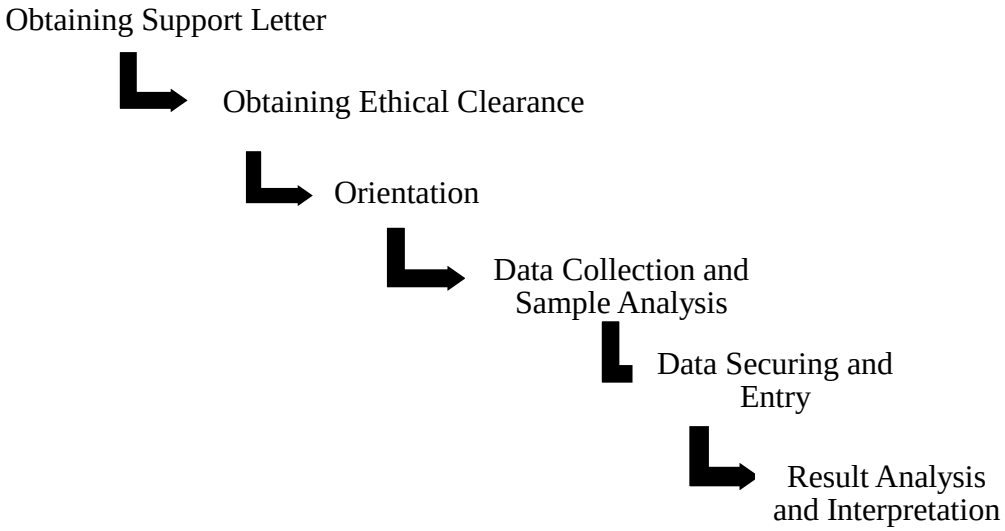


Figure 1 Work Flow

7. RESULT

7.1 Socio-demographic Findings

A total of 240 patients' blood samples including 134 (55.8%) females and 106 (44.2%) males were analyzed. The majority of the participant age was between 21-41 years, 93 (38.8%) (Table1). The mean ±SD as follow as: HCT values were 40.9±14.91, 40.1±14.84 and 40.3±14.98 for automated, manual methods using commercial sealant and window clay respectively, MCV values were 85.7±9.18, 83.7±10.6 and 83.9±10.12 for automated, manual methods using commercial sealant and window clay respectively.

Table 1 Socio-demographic and Clinical characteristics of the study population

Variables	Frequency (240)	Percent (100%)
Sex		
Male	106	44.2%
Female	134	55.8%
Age (years)		
0-20	48	20.0%
21-41	93	38.8%
42-62	63	26.3%
63-82	15	6.3%
Missing	21	8.8%
Clinical Diagnosis*		
Hematological Disorders(Anemia, AML, ALL, CML, CLL, MDS)	51	21.3%
Malignancy(Breast, Cervical, Esophageal, Colon and other)	48	20.0%
Cardiac Cases	70	29.2%
Other Clinical Conditions	71	29.6%

*AML=Acute Myeloid Leukemia, ALL=Acute Lymphocytic Leukemia, CML=Chronic Myelocytic Leukemia, CLL=Chronic Lymphocytic Leukemia, MDS=Myelodysplastic Syndrome, ANC= Anti natal care

7.2 Comparison of the Mean of Different Categories

The mean of HCT and MCV between the three methods to the sex of the studied patients did not show significant difference (P>0.05).

The blood samples were obtained for this study were grouped as 51(21.3%) hematological disorders (anemia, AML, ALL, CML, CLL, and MDS), 48(20%) malignancy (breast, cervical, esophageal, colon and other), 70(29.2%) Cardiac cases and 71(29.6%) other clinical conditions (RVI, accident, ANC, labor and other). The mean HCT and MCV by the three methods did not show significant difference by clinical conditions of patients (P>0.05), but did show slight difference between automated and manual method using window clay for malignancy for MCV (Table 2a, b and c).

Table 2 Clinical Conditions for Automated Vs Manual method using commercial sealant

Parameters*	N	Mean	SD	Correlation	P-value of Paired differences
HemDis Hematocrit by Automated	51	26.0	9.65		
HemDis Hematocrit by Manual Commercial Sealant	51	25.2	9.54	0.984	0.663
HemDis MCV by Automated	51	86.8	11.90		
HemDis MCV calculated from MCSHCT and ARBC	51	83.9	12.86	0.890	0.219
Malig Hematocrit by Automated	48	33.3	8.51	0.985	0.585
Malig Hematocrit by Manual Commercial Sealant	48	32.4	8.46		
Malig MCV by Automated	48	82.4	7.39	0.823	0.080
Malig MCV calculated from MCSHCT and ARBC	48	79.5	8.42		
Cardiac cases Hematocrit by Automated	70	58.0	4.91	0.652	0.200
Cardiac cases Hematocrit by Manual Commercial Sealant	70	56.9	5.38		
Cardiac cases MCV by Automated	70	86.1	8.74	0.747	0.394
Cardiac cases MCV calculated from MCSHCT and ARBC	70	84.8	9.69		
Other CLI Hematocrit by Automated	71	39.8	11.16	0.990	0.887
Other CLI Hematocrit by Manual using Commercial Sealant	71	39.5	11.15		
Other CLI MCV by Automated	71	86.9	8.03	0.899	0.625
Other CLI MCV calculated from MCSHCT and ARBC	71	86.2	9.16		

Table 3 Comparison of Clinical Conditions for Automated Vs Manual method using window clay

Parameters*	N	Mean	SD	Correlation	P-value of Paired differences
HemDis Hematocrit by Automated	51	26.0	9.65	0.989	0.688
HemDis Hematocrit by Manual using Window Clay	51	25.2	9.54		
HemDis MCV by Automated	51	86.8	11.90	0.911	0.219
HemDis MCV calculated from MWCHCT and ARBC	51	83.9	12.86		
Malig Hematocrit by Automated	48	33.3	8.51	0.985	0.537
Malig Hematocrit by Manual Commercial Sealant	48	32.4	8.46		
Malig MCV by Automated	48	82.4	7.39	0.822	0.045
Malig MCV calculated from MWCHCT and ARBC	48	79.5	8.42		
Cardiac cases Hematocrit by Automated	70	58.0	4.91	0.844	0.200
Cardiac cases Hematocrit by Manual using Window Clay	70	56.9	5.38		
Cardiac cases MCV by Automated	70	86.1	8.74	0.815	0.203
Cardiac cases MCV calculated from MWCSHCT and ARBC	70	84.0	10.83		
Other CLI Hematocrit by Automated	71	39.8	11.16	0.989	0.888
Other CLI Hematocrit by Manual Window Clay	71	39.5	11.15		
Other CLI MCV by Automated	71	86.9	8.03	0.894	0.612
Other CLI MCV calculated from MWCHCT and ARBC	71	86.2	9.16		

HemDis=Hematological disorders Malig=Malignancy CLI= other clinical indications

MCSHCT= HCT by Manual method using Commercial Sealant clay

MWCHCT= HCT by Manual method using Window Clay

ARBC= RBC by Automated method

Table 4 Clinical Conditions for the two Manual methods

Parameters*	N	Mean	SD	Correlation	P-value of Paired differences
HemDis Hematocrit by Manual using Commercial Sealant	51	25.2	9.54	0.996	0.975
HemDis Hematocrit by Manual using Window Clay	51	25.3	9.70		
HemDis MCV calculated from MCSHCT and ARBC	51	83.9	12.86	0.952	0.958
HemDis MCV calculated from MWCHCT and ARBC	51	83.8	12.50		
Malig Hematocrit by Manual using Commercial Sealant	48	32.4	8.46	0.995	0.943
Malig Hematocrit by Manual using Window Clay	48	32.3	8.50		
Malig MCV calculated from MCSHCT and ARBC	48	79.5	8.42	0.961	0.817
Malig MCV calculated from MWCHCT and ARBC	48	79.1	8.31		
Cardiac cases Hematocrit by Manual using Commercial Sealant	70	56.9	5.38	0.726	0.512
Cardiac cases Hematocrit by Manual using Window Clay	70	57.5	4.32		
Cardiac cases MCV calculated from MCSHCT and ARBC	70	84.0	10.83	0.863	0.649
Cardiac cases MCV calculated from MWCSHCT and ARBC	70	84.8	9.69		
Other CLI Hematocrit by Manual using Commercial Sealant	71	39.5	11.15	0.995	0.996
Other CLI Hematocrit by Manual Window Clay	71	39.5	11.28		
Other CLI MCV calculated from MCSHCT and ARBC	71	86.2	9.16	0.977	0.992
Other CLI MCV calculated from MWCHCT and ARBC	71	86.2	8.80		

HemDis=Hematological disorders Malig=Malignancy CLI= other clinical indications

MCSHCT= HCT by Manual method using Commercial Sealant clay

MWCHCT= HCT by Manual method using Window Clay

ARBC= RBC by Automated method

The Independent sample t test for 240 samples between the automated and both manual methods didn't show statistically significant difference ($p>0.05$) for the hematocrit, but did show statistically significant difference ($p<0.05$) for the MCV (Table-3), though were positively correlated when tested by a correlation test.

Table 5 Comparison of the three methods mean and standard deviation

Parameters*	N	Mean	SD	Correlation	P-value of Paired differences
Hematocrit by Automated	240	40.9	14.91	0.984	0.570
Hematocrit by Manual Commercial Sealant	240	40.1	14.84		
MCV by Automated	240	85.7	9.18	0.844	0.027
MCV calculated from MCSHCT and ARBC	240	83.7	10.60		
Hematocrit by Automated	240	40.9	14.91	0.992	0.646
Hematocrit by Manual using Window clay	240	40.3	14.98		
MCV by Automated	240	85.7	9.18	0.870	0.033
MCV calculated from MWCHCT and ARBC	240	83.9	10.12		
Hematocrit by Manual using Commercial Sealant	240	40.1	14.84	0.989	0.915
Hematocrit by Manual using Window clay	240	40.3	14.98		
MCV calculated from MCSHCT and ARBC	240	83.7	10.60	0.934	0.900
MCV calculated from MWCHCT and ARBC	240	83.9	10.12		

MCSHCT= HCT by Manual method using Commercial Sealant clay

MWCHCT= HCT by Manual method using Window Clay

ARBC= RBC by Automated method

7.3 Precision (Reproducibility) Study

Table 5 to 6 summarizes the calculated coefficient of variation values of Sysmex XT-4000i for the directly measured parameters. As shown in the Table 5 Sysmex XT-4000i, the precision (reproducibility) values of the directly measured parameters lie within the specification of the manufacturer and comply with predetermined precision values (coefficient of variation). Measurement values for both HCT and MCV by automated are more precise compared to manual methods. Also the measurements for all values found between ±3% for the automated and both manual methods.

Table 6 within sample precision of Sysmex XT-4000i hematology analyzer for HCT and MCV

Parameters Level	Mean	SD	CV (actual)	Manufacture's Specification
LOW HCT	28.6	0.14	0.50	<=1.5%
NORMAL HCT	38.3	0.18	0.48	
HIGH HCT	51.7	0.17	0.33	
LOW MCV	76.4	0.44	0.58	<=1.5%
NORMAL MCV	81.3	0.45	0.55	
HIGH MCV	84.8	0.38	0.45	

Table 7 within sample precision of KXT-400 microhematocrit using commercial sealant

Parameters Level	Mean	SD	CV (actual)	*Manufacture's Specification
LOW HCT	28.3	0.67	2.38	NA
NORMAL HCT	38.8	0.79	2.03	
HIGH HCT	52.2	0.42	0.81	
LOW MCV	75.6	1.99	2.64	NA
NORMAL MCV	82.3	1.86	2.26	
HIGH MCV	85.8	1.07	1.24	

Table 8 within sample precision of KXT-400 microhematocrit using window clay

Parameters Level	Mean	SD	CV (actual)	*Manufacture's Specification
LOW HCT	28.2	0.63	2.24	NA
NORMAL HCT	38.9	0.74	1.90	
HIGH HCT	52.0	0.67	1.28	
LOW MCV	75.3	1.83	2.43	NA
NORMAL MCV	82.0	1.11	1.35	
HIGH MCV	85.9	0.82	0.95	

*NA = Not Available

7.4 Agreement Study

Agreement test of HCT

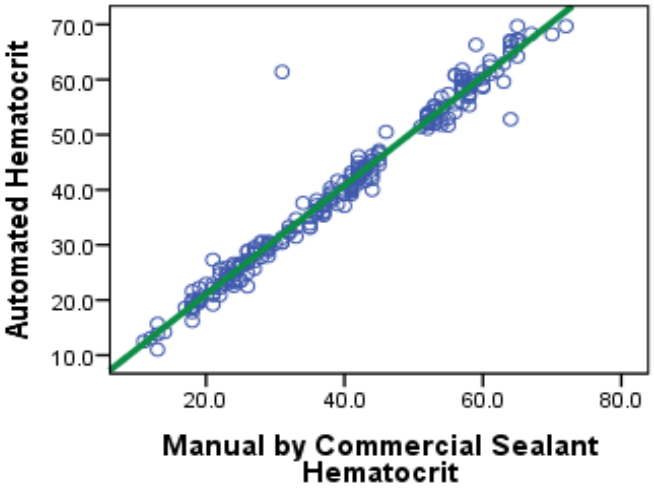
The agreement of Sysmex XT-4000i and Manual method using commercial sealant is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 99.17% of cases and from the linear regression graph, we can see an excellent correlation of r=0.984 with the equation of y= 1.23+0.99x, figure 2 (a).

The agreement of Sysmex XT-4000i and Manual method using window clay is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 97.92% of cases and from the linear regression graph, we can see an excellent correlation of r=0.992 with the equation of y= 1.15+0.99x, figure 2 (b).

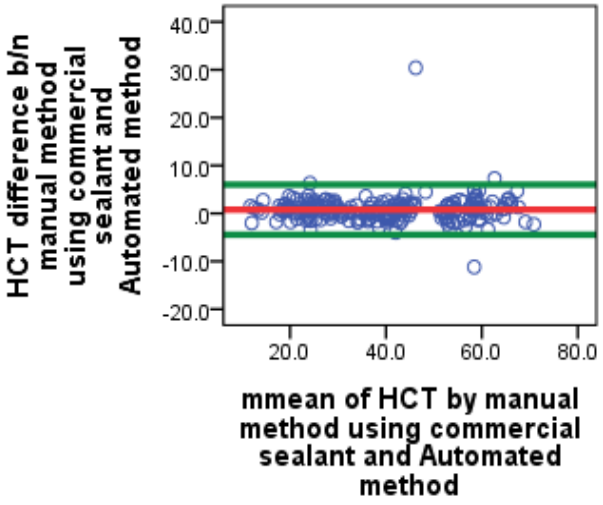
The agreement of Manual method using commercial sealant and Manual method using window clay is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 98.75% of cases and from the linear regression graph, we can see an excellent correlation of r=0.988 with the equation of y= 0.138+1.01x, figure 2 (c).

Figure 2a, b and c: Correlation coefficient and Bland Altman Plot for automated and manual methods for HCT

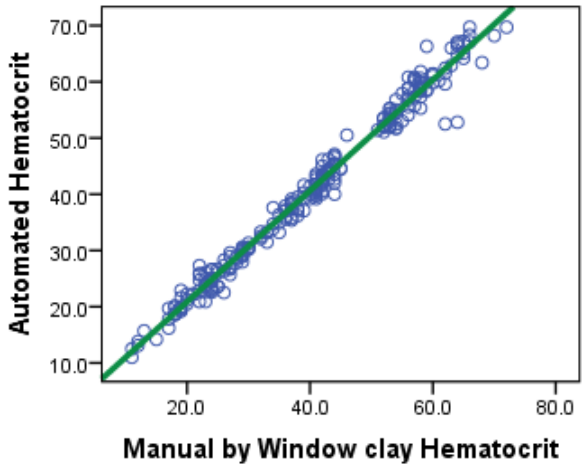
a) r=0.984, y= 1.23+0.99x



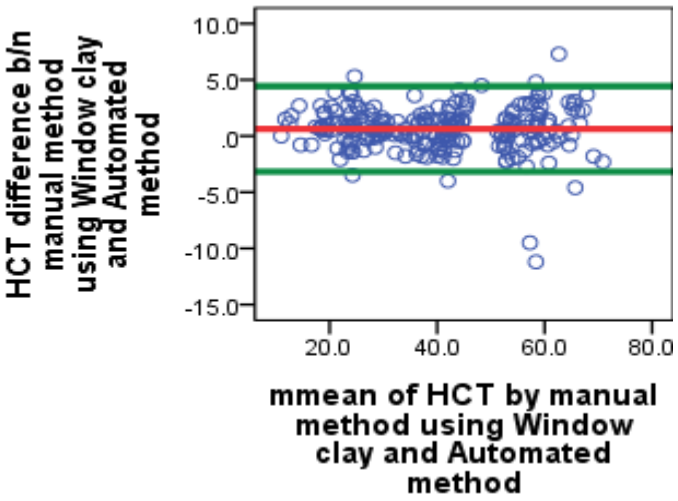
Mean=0.77, SD=2.67, LoA(-4.46,6.01)



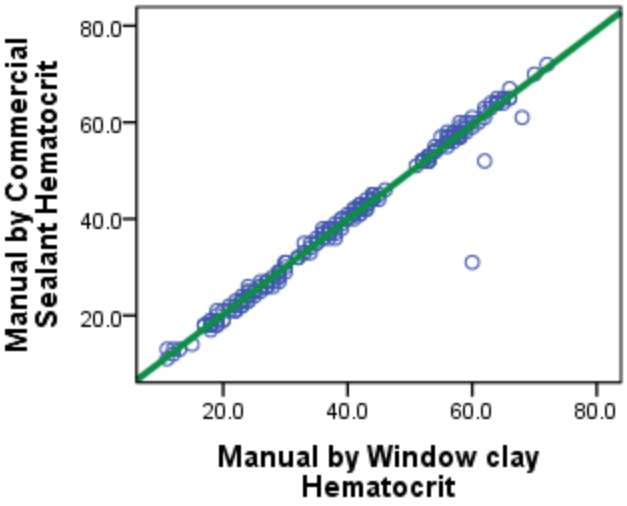
b) r=0.992, y=1.15+0.99x



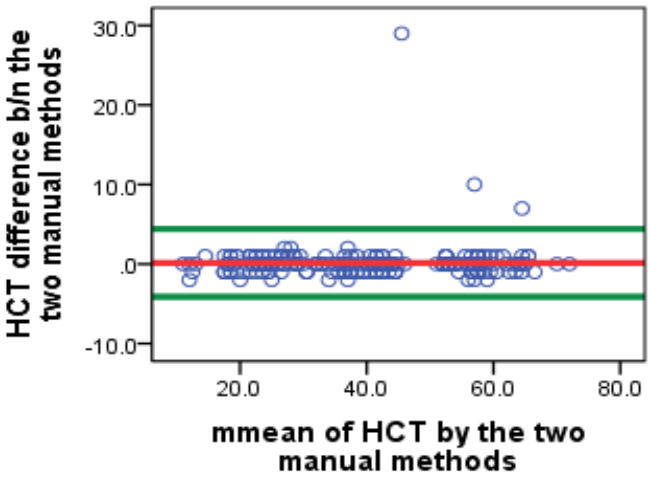
Mean=-2.01, SD=5.69, LoA(-13.16,9.14)



c) r=0.988, y= 0.138+1.01x



Mean=0.63, SD=1.94, LoA(-3.17,4.43)



Agreement test of MCV

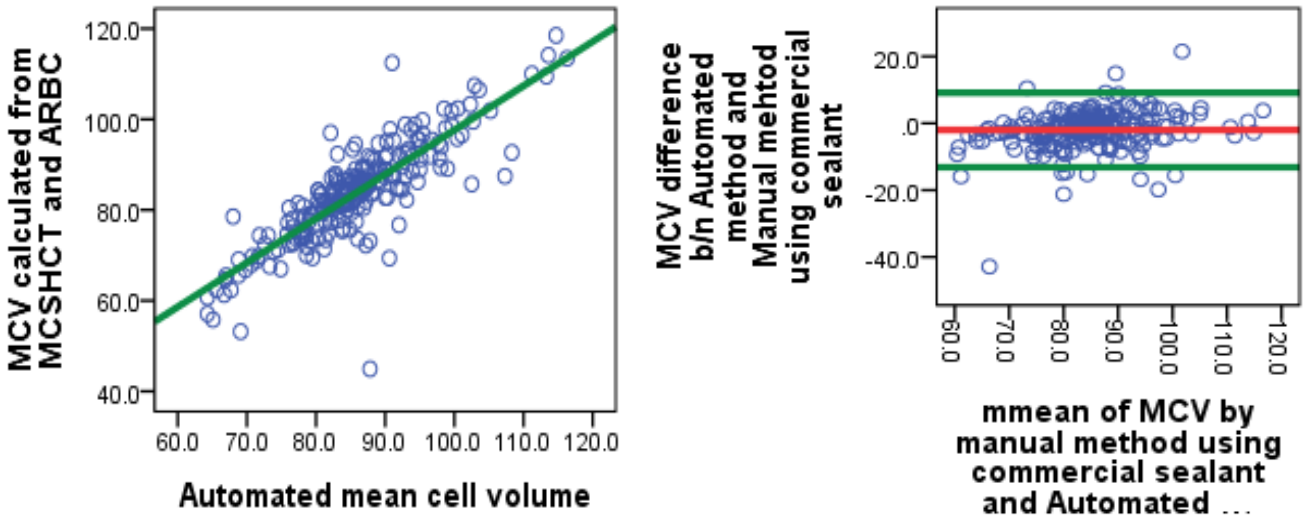
The agreement of Sysmex XT-4000i and Manual method using commercial sealant is unacceptable because in the Bland-Altman plot, the difference is not lying between LoA for 94.17% of cases and from the linear regression graph, we can see an excellent correlation of r=0.844 with the equation of y= 0.1+0.98x, figure 3 (a).

The agreement of Sysmex XT-4000i and Manual method using window clay is unacceptable because in the Bland-Altman plot, the difference is not lying between LoA for 91.67% of cases and from the linear regression graph, we can see an excellent correlation of r=0.870 with the equation of y= 1.61+0.96x, figure 3 (b).

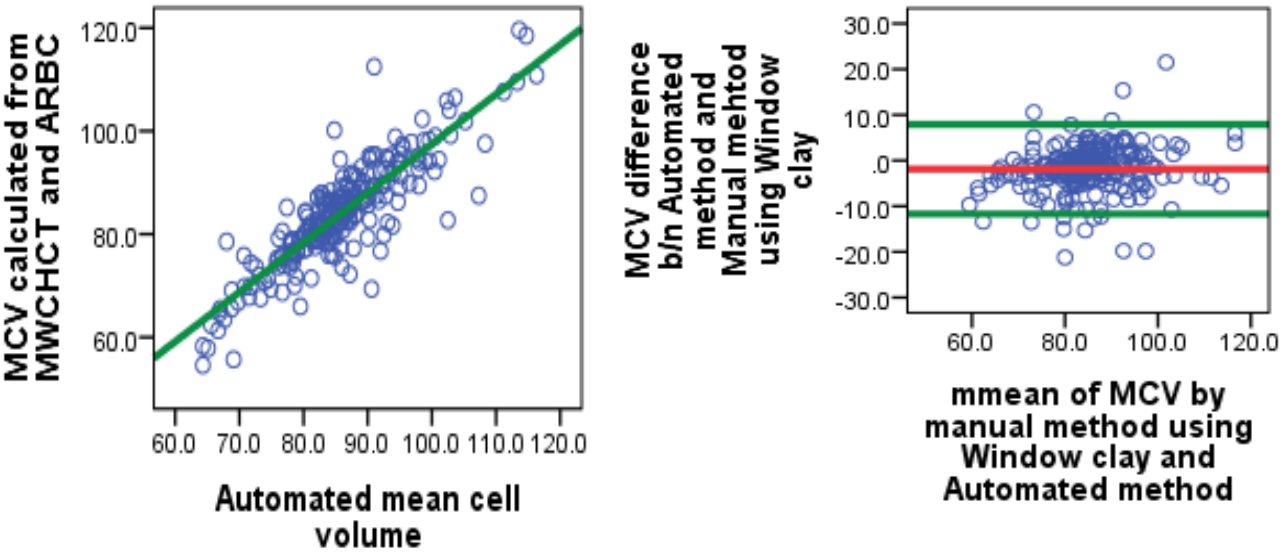
The agreement of Manual method using commercial sealant and Manual method using window clay is acceptable because in the Bland-Altman plot, the difference is lying between LoA for 96.67% of cases and from the linear regression graph, we can see an excellent correlation of r=0.934 with the equation of y= 9.18+.89x, figure 3 (c).

Figure 3a, b and c: Correlation coefficient and Bland Altman Plot for automated and manual methods for MCV

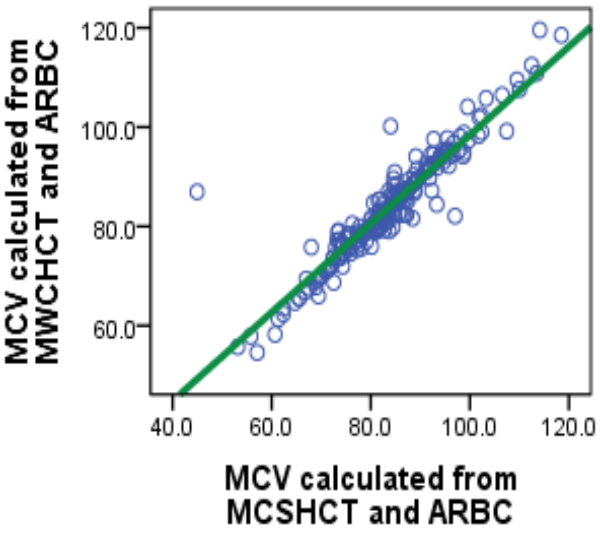
a) r=0.844, y= 0.1+0.98x Mean=-1.89, SD=5.00, LoA(-11.70,7.92)



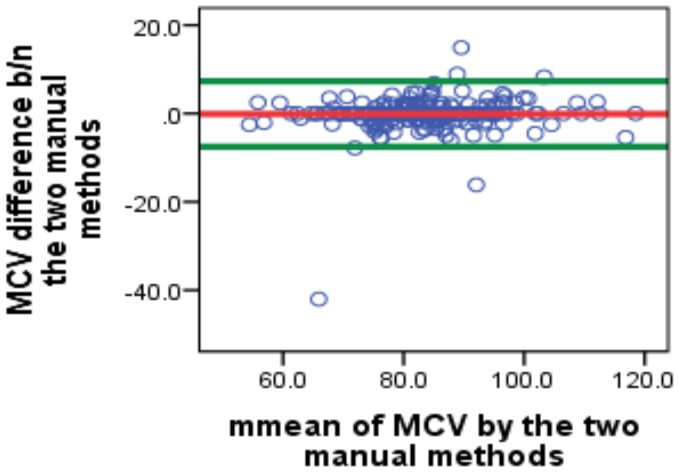
b) r=0.870, y= 1.61+0.96x Mean=0.15, SD=2.17, LoA(-4.12,4.41)



c) r=0.934, y= 9.18+.89x



Mean=-0.12, SD=3.78 LoA(-7.53,7.30)



8. DISCUSSION

In this study, three methods were utilized to determine HCT and MCV on the same day and hours. HCT and MCV results which comprise different levels (low, normal, high) determined by the three methods were compared to each other. The three methods comply with the predetermined precision values (coefficient of variation). Also the measurements for all HCT values found ±3% for the automated and both manual methods, but not for the MCV.

In the current study we found much strong positive correlation between automated and manual methods using commercial clay (r=0.984) and window clay (r=0.992) for hematocrit compared to the study from Hawassa University by Gebretsadkan G et al (26). Both studies also similarly showed the automated results are more precise from both manual methods based on the calculated coefficient of variation (CV).

A similar study from Iraq found high correlation (R=9.75) between manual procedure and automated methods for hematocrit values (21), but in the current study we found more strong positive correlation between automated and manual methods using commercial clay (r=0.984) and window clay (r=0.992) determine the hematocrit.

Another similar findings by Ode SA et al (strong positive correlation (r=0.946) (22) and Ike SO et al (positive correlation (r = 0.9496)) (24) which are still lower correlational values compared to current study between the automated hematocrit and microhematocrit values (r=0.984).

A comparable and similar finding by Babadoko AA et al showed there was no statistically significant difference between mean hematocrit 37.5 ± 7.2% for manual and 37.2 ± 7.3% automated method (p>0.05) with a positive correlation (23) which also comply to our findings (automated: 40.9±14.91 and manual: 40.1±14.84 (r=984, p>0.05).

A study by Charuruks N et al and from University of Khartoum which included MCV beside HCT, they similarly found statistically significant difference between manual and automated methods for MCV (20,25), but in the current study there is no statistically significant difference between automated and both the manual methods showed statistically significant different (p>0.05).

9. STRENGTHS & LIMITATION

9.1 Strength

- Each sample was run in duplicates for the agreement studies.
- Total number of samples for the agreement study was made doubled from that of the minimum CLSI guideline requirement.
- Two manual methods were considered for the agreement against each other and between automated
- Manual method for MCV determination was also tried using automated RBC value

9.2 Limitation

- Lack of suitable reference range to categorize low, normal, and high levels of HCT and MCV parameters, we used the reference range on the analyzer given by Sysmex Corporation for Sysmex XT- 4000i.
- Limited studies were available on similar analyzer models, making comparison difficult.
- There was lack of recommended manual methods for determining RBC.

10. CONCLUSION & RECOMMENDATION

10.1 Conclusion

Sysmex XT-4000i comply with the manufacturer's specification and the HCT values performed by these analyzer is in good agreement with both manual methods, but not for MCV. Huge and referral hospitals like Black Lion Hospital need to generate reliable results associated with hematological disorders and patients waiting clinical decision for immediate transfusion or phlebotomy drawing of blood. Also both manual methods could be used interchangeably due to good agreement for HCT determination in case of inadequate blood drawn for example infants. Therefore, the current finding gives confidence on using both the automated and manual methods to decide whether the results are due to clinical conditions or machine failure.

10.2 Recommendation

Based on the findings from this study, we recommend further studies on MCV determination by manual methods that are precise, reliable, with less cost and small blood sample in the case of infants and patients in difficulty to draw enough volume of blood. We also encourage the use of manual hematocrit methods for checking hematocrit results by the automated analyzer.

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ANNEXES

Annex-I SOP For Microhematocrit

Purpose This procedure provides clear instruction to measure haematocrit value

Abbreviations
HCT- Hematocrit
Hgb- Hemoglobin
RBC- Red blood Cell
WBC- white blood cell

Materials

Reagents
N/A

Reagents preparation: N/A

Reagents stability and storage: N/A

Supplies
• Disposable Gloves • Microhematocrit tube • cotton swab • sealer

Equipment
• Microhematocrit Centrifuge.

Sample

Sample type	**Amount required**	**Transport and Storage**	**Stability**
Anti-coagulated Whole Blood	Minimum of 100Ul	Keep at room Temperature.	8 hrs at room Temperature.

Limitations: Hemolaysed sample,
High volume of Anti-Coagulant
Inadequate volume of samples i.e less than 2/3.

Sample retention: Samples are discarded after 8 hrs

Special Safety Precautions
- Use universal laboratory safety practice.
- Need Precautions to avoid microhematocrit tube breakage. it will cause serious injury.

Maintenance **N/A**

Calibration

Calibrator	Level	Stability	Frequency	Preparation (y/n)
N/A				

Calibrator preparation: N/A

Note:

Microhematocrit centrifuge should be checked by using Tachometer for its constant speed, once in a Month

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
● perform duplicate tubes,	Normal	2 hrs		N

Control preparation:

Take the sample and run duplicate and the result should agree with in +/- 1.5 of each other.

Note:

Procedure

Step	Action
1	Collect a well-mixed blood samples into a microhematocrit capillary tube at least ¾ full
2	seal at one end by using the sealer
3	Place each tube in the centrifuge with the sealed end away from the Centre and resting against the peripheral Rim.
4	Centrifuge for 4 Minutes at 10.000 RPM
5	Remove the capillary tube from microhematocrit centrifuge at the end of 4 minutes
6	put the microhematocrit capillary tube into the reader accordingly
7	Read the RBC volume in percent from the scale directly beneath the marked line indicator of the reader

Calculation: **N/A**

Result Interpretation	The result is interpreted the percentage of Paced Red Cells Against the plasma.

Expected Values If different sample type used, more than one table may be required.

Analyte	Reference Range		Toxic range	Analytical Range	Units
	Male	Female			
Whole blood	36%-50%	34%-44%	>60%		%

Limitations:	Haemoconcentraion, Faulty centrifuge, heamolyzed samples, Inadequate sealing of capillary tube, Improper reading of results from the reading device.

Procedural Notes	N/A
Principle	To determine HCT from extremely small volume of blood specimens in a microcapillary tube after centrifuged in a special device (Microhematocrit Centrifuge) to obtain paced cells from the total volume which is directly Related to the percentage occupied packed cell volume or hematocrit value.
Clinical Utility	It is a useful test for the screening of Anemia and to diagnose polycythemia vera and to monitor treatment.
Related Procedures and Documents	N/A

Reference

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Source: Addis Ababa University College of Health Sciences Tikur Anbessa Specialized Hospital Laboratory SOP for Microhematocrit Method, September 23 2014.

Annex-II SOP for Sysmex XT-4000i

a. Purpose

This SOP provides general information about sysmex 4000*i* automated machine. The XT *4000i* can provide results of 39 parameters.

b. Scope

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

c. Abbreviation

CBC	Complete Blood Count
dL	decilitre (0.1 litre)
EPK	CELLPACK
FBA	STROMATOLYSER-FB
FCM	Flow cytometry
FFD	STROMATOLYSER-4DL
FFS	STROMATOLYSER-4DS
fL	femtolitre (10-15 litre)
LL	lower limit
PD	pre-diluted mode
Pg	picogram (10-12 gram)
QC	Quality Control
RED	RET SEARCH (II)
SLS	SULFOLYSER
SRV	Sample Rotor Valve
UL	upper limit
WB	whole blood mode
µL	microlitre (10-6 litre)

d. 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.

e. Principle

Hydro dynamic focusing (DC detection)

Inside the detector, the sample nozzle is positioned in front of the aperture and in line with the center. After diluted sample is forced from the sample nozzle into the conical chamber, it is surrounded by front sheath reagent and passes through the aperture center.

After passing through the aperture, the diluted sample is sent to the catcher tube. This prevents the blood cells in this area from drifting back, and prevents the generation of false platelet pulses.

The Hydro Dynamic Focusing method improves blood count accuracy and reproducibility. And because the blood cells pass through the aperture in a line, it also prevents the generation of abnormal blood cell pulses.

Flow Cytometry Method Using Semiconductor Laser

A semiconductor laser beam is emitted to the blood cells passing through the flow cell. The forward scattered light and lateral scattered light are received by the photodiode, and the lateral fluorescent light is received by the photomultiplier tube. This light is converted into electrical pulses, thus making it possible to obtain blood cell information.

SLS-Hemoglobin Method

SLS hemoglobin detection method uses cyanide-free sodium lauryl sulphate (SLS). The reagent lyses red blood cells and white blood cells in the sample. The chemical reaction begins by altering the globin and then oxidizing the heme group. Now the SLS' hydrophilic groups can bind to the heme group and form a stable, colored complex (SLS-HGB), which is analyzed using a photometric method.

An LED sends out monochromatic light and by moving through the mixture light is absorbed by the SLS-HGB complexes. The absorbance is measured by a photo sensor and is proportional to the haemoglobin concentration of the sample.

f. Reagents and supplies

- Cell clean
- Cell pack
- Stromatolyzer-4DL
- Stromatolyzer-4DS,
- Stromatolyzer-FB
- Sulfolyzer
- RET-search (II) diluents
- RET-search (II) dye
- Control
- Calibrator
- A4 size paper

g. Equipment

- Sysmex XT 4000*i* machine

- Printer

h. Sample and container type

- About 2-3 ml of venous blood collected into EDTA tubes.
- For micro sampling the blood can be obtained from the ear lobe or finger of adult or from the heel of an infant.

i. 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).

j. 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.

k. Quality Control

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.

l. Procedure

1. Check to see that the reagents needed for the number of the samples to be processed for the day are available.
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

Permissible background counts

WBC	0.1[x 10^3/μL]
Diff-WBC	0.2 [x 10^3/μL]
RBC	0.02 [x10^6/μL]
HGB	0.1 [g/dL]
PLT	10 [x10^3/μL]
PLT-O	10x10^3/μL]

4. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material
5. If the result of quality control is inacceptable range, run the blood samples. Samples can be run in manual mode, Sampler mode or Capillary mode.

Manual mode

- Click the manual icon at the top of the computer.
- Input the sample ID number or use bar code reader.
- Check the discrete setting. If it is necessary to change the setting, click the appropriate "Discrete Mode" to set it.
- When all settings are completed, click ok.
- Mix the sample tube gently.
- Hold the opened sample tube under the sample probe and press the start switch button.
- When the READY LED turns off (and two short beeps sound), remove the sample tube.
- Automatic print of the result will be printed out when analysis finished.

Sampler mode

- Click the sampler icon at the top of the computer.
- Prepare and put the samples on a rack.
- Position the tubes so that all the bar codes are visible through the slits in the rack.
- Check the rack number and tube position number in the Rack Number/Tube Position Confirmation dialog box.
- When all of the racks have moved to the left rack pool of the sampler, the READY LED turns on.
- Press the START switch to start analysis. The rack is automatically transported to the aspiration position. The sample is aspirated and analyzed.
- Automatic print of the result will be printed out when the analysis is finished.

Capillary mode

- Prepare the sample for capillary analysis (dilute the sample in 1:5 ratios with cell packs).
- Input the sample ID number, and set for capillary mode and discrete.
- Hold the opened sample tube under the sample probe and press the start switch button.
- When the READY LED turns off (and two short beeps sound), remove the sample tube.
- When analysis finished the result will be automatically printed.

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).

m. Calculations

Not applicable

n. Performance Characteristics

Method was verified for intended use.

o. Uncertainty measurement

p. Interferences/Limitations

The following is a list of possible substances that may interfere with the listed parameters.

WBC

Platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse resistant RBCs in patients with hemoglobinopathies and severe liver disease.

RBC

Cold agglutinins, severe microcytosis, fragmented RBCs, large numbers of giant platelets, in vitro hemolysis.

HGB

Lipemia, abnormal proteins in blood plasma, leukocytosis (above 100,000/µl).

HCT

Cold agglutinins, leukocytosis (above 100,000/µl), abnormal red cell fragility.

PLT

Pseudo thrombocytopenia, giant PLTs, PLT aggregation, microcytosis.

q. Critical values

WBC <2,000 or >40,000 x 10^3/ µL

HGB <7g/dl

Platelet <50,000/mm^3

r. Result reporting

Results are reported from automated printing and through computer

s. Result Interpretation

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

t. Biological Reference Interval

Parameter	Female	Male	Unit	Parameter	Female	Male	Unit
WBC	4.23 - 9.07	3.98 - 10.04	$x10^3/\mu L$	Lymph#	1.18 - 3.74	1.32 - 3.57	$x10^3/\mu L$
Neu%	34.0 - 71.1	34-67.9	%	Mono#	0.24 - 0.36	0.30-0.82	$x10^3/\mu L$
Lymph%	19.3 - 51.7	21.8 - 53.1	%	Eo#	0.04-0.36	0.04-0.54	$x10^3/\mu L$
Mono%	4.7-12.5	5.3-12.2	%	Baso#	0.01-0.08	0.01-0.08	$x10^3/\mu L$
Eo%	0.7 - 5.8	0.8 - 7.0	%	RBC	3.93-5.22	4.63-6.08	$x10^6/\mu L$
Baso%	0.1-1.2	0.2-1.2	%	HGB	11.2-15.7	13.7-17.5	g/dl
HCT	34.1-44.9	40.1-51.0	%	RDW-SD	36.4-46.3	35.1-43.9	%
MCV	79.4-94.8	79.0-92.2	fL	RET#	0.0164-0.0776	0.026-0.095	$x10^6/\mu L$
MCH	25.6-32.2	25.7-32.2	pg	IFR	3.0-15.9	2.3-13.4	%
MCHC	32.2-35.5	32.3-36.5	g/dl	PLT	182-369	163-337	$x10^3/\mu L$
RDW-CV	11.7-14.4	11.6-14.4	%	MPV	9.4-12.3	9.4-12.4	%

u. Supporting documents

Document Development/Amendment Form. (AAU/TASHL/ALS/F4.3-01)

v. References

a) Sysmex XT-4000*i* user manual.

b) World Health Organisation. Guidelines on Standard Operating Procedures for Haematology. WHO 2000.

c) Hematology Standard Operating Procedures (SOPs).*http://www.moh.gov.ps/portal/wp-content/uploads/Hematology-SOPs.pdf.*Acessed on NOV. 2017.

d) Sysmex Educational Enhancement and Development | July 2017. Seed Hematology.

Source: Addis Ababa University College of Health Sciences Tikur Anbessa Specialized Hospital Laboratory SOP for CBC using sysmex xt-4000i, September 1 2018.

Annex III: Check list

Result reporting form

Serial no	Card no	Age	Sex	Clinical diagnosis	A1	A2	B1	B2	C

Name of health facility----------

Date: _____

Signature of the laboratory technologist_____

Remarks

A1 and A2= duplicate manual Hematocrit value (capillary tube sealed by clay)

B1 and B2= duplicate manual hematocrit value (capillary tube sealed by commercial sealant)

C= automated HCT Value

Annex IV: Dummy Table-1

Mean ± standard deviation (SD) of HCT and MCV result by automated and manual methods

Parameter	Automated	Microhematocrit (capillary tube sealed by window clay)	Microhematocrit (capillary tube sealed by commercial sealant)	p-value
HCT (%)				
MCV(fL)				

Dummy Table-2

The Precision of Manual and Automated Methods for Hematocrit and MCV Determination

	HCT			MCV		
	Mean	SD	CV	Mean	SD	CV
1						
2						
3						
4						
5						
7						
8						
9						
10						

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: Haymanot Birhanu (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with my approval as university based advisor.

Advisor: Aster Tsegaye **(MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mikias Negash **(MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.