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DEPARTEMENT OF MEDICAL LABORATORY SCIENCES

COMPARISION OF HEMOCUE301+ WITH SYSMEX-KX21N AND CELLDYN1800 AUTOMATED HEMATOLOGY ANALYZERS FOR MEASUREMENT OF HEMOGLOBIN LEVELS IN PATIENTS-AT ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE, ADDIS ABABA, ETHIOPIA, 2015.

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This is to certify that the thesis prepared by Mesfin Samuel, entitled:

Comparison of Hemocue 301+ with Sysmex-Kx21N And Celldyn 1800 Automated Hematology Analyzers for Measurement of Hemoglobin Levels in Patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2015 and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (~~He~~ Clinical Laboratory Management and Quality Assurance~~matology and Immunohematology~~) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

CBC Complete Blood Count
CLSI Clinical and Laboratory Standards Institute
CV Coefficient of Variance
EDTA EthyeleneDiamineTetraacetic Acid
FCM Flow Cytometer
FDA Food and Drug Administration
Hb Hemoglobin
HCT Hematocrit
ICC Interclass Correlation Coefficient
ICSH International Committee for Standardization in hematology
ICU Intensive Care Unit
IRB Institutional Review Board
LOA Limit of Agreement
POCT Point of Care Testing
PPV Positive Predictive Value
QC Quality Control
SD Standard Deviation
SLS Sodium Lauryl Sulfate
SOP Standard Operating Procedure
SPHMMC St Paul's Hospital Millennium Medical College
SPSS Statistical Package for Social Sciences
WHO World Health Organization

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Abstract

Background: Hemoglobin concentration is measured routinely using automated hematology analyzers. Even if these automated hematology analyzers are very accurate and reliable, they are expensive and transport of the samples to the laboratory delays the process which may delay treatment. Point of care hemoglobin analyzer like Hemocue301+ has potential to solve such problems. Although method comparison experiment has to be conducted when new instrument are installed, this practice is not common in our country.

Objective: To compare hemoglobin determined by Hemocue301+, with Sysmex-kx21N and Cell-Dyn1800 methods using patients' blood sample at St Paul's Specialized Hospital Millennium Medical College in Addis Ababa, Ethiopia.

Methods:A facility based cross sectional study with convenient sampling method was conducted at St. Paul's specialized teaching hospital starting from May 15 to June 2 2015. A total of 117specimens for agreement study and 3 specimens for precision study were analyzed on Hemocue301+, Sysmex-Kx21N, and cell-Dyn1800 hematology analyzers to compare their Hemoglobin results. This study includes patients of all age group. One sample t-test and bland and Altman plot with limit of agreement were used to make inference. Data analyses were performed using SPSS version 20 Software.

Result: The precision experiment showed within run normal range CV% for Hemocue301+, SysmexKx21N and Cell-Dyn1800 (1.7%, 1.3%, 1.8%, respectively). The one sample t-test showed a non-significant p-value of 0.10 for Hemocue301+ and Sysmex-Kx21N and the limit of agreement from Bland and Altman plot for 95% was (-1.07, 1.25)g/dl with only 1.7% of the cases were out of the limit of agreement. For Hemocue301+ and Cell-Dyn1800, a non-significant p- value of 0.46 and the Bland and Altman plot limit of agreement for 95% was (-1.22, 1.31)g/dl with only 1.7%of the case were out of the limit of agreement.Hemocue301+was agreed with SysmexKx21N and Cell-Dyn1800 within 1 g/dl which was considered as clinically important limit for Hemoglobin measurement.

Conclusion: The Hemocue301+ maybe used interchangeably with Sysmex-Kx21N and Cell-Dyn1800 automated Hematology analyzers in St Paul's Specialized Hospital Millennium Medical College Hematology laboratory.

1. Introduction

1.1. Background

Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status(1). Accurate determination of hemoglobin concentration is a common element in assessing the extent of anemia and making a clinical decision to help patients (2). This decision should be made based on reliable and rapidly assessed laboratory tests (3).

Since hemoglobin concentration is an indicator of a patient's ability to transport oxygen, anemia is routinely monitored or tested in any instance where oxygen transport is thought to be compromised (4). Hematocrit (HCT) and hemoglobin (Hb) concentration are used to monitor patients during procedures with a high risk of blood loss or hemorrhage. The measurements are used in guiding clinical decisions to treat low blood volume, or anemia, through medications or blood transfusion (5).

Hemoglobin (Hb) assessments are the most reliable indicator widely used to screen individuals for anemia, to draw inferences about the iron status of populations and to evaluate responses to nutritional interventions (6). The blood Hb concentration is an important variable in directing transfusion therapy in patients suffering from major blood loss due to accidents, surgery, labour and many other critical conditions(7). Hb concentration is measured routinely using automated hematology analyzers, even if these automated hematology analyzers are very accurate and reliable, they are expensive and transport of the samples to the laboratory delays the process which may delay treatment, resulting in preventable deaths (3). Though automated analyzers give accurate results if properly handled, such devices have to be assessed for their analytical errors (8).

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In principle when new Hb measurement devices introduced to each laboratory, they should be compared against the previous to determine the difference in their measurement value (9).When comparing the new and the reference method there are statistical approaches to compare their performance. This analysis consists of variance of replicated, paired measurements by the two methods and testing for the agreement of two analytical methods (8).

In hemoglobin measurement the principles established and used up to now is comprised of lysing the erythrocytes and the release of their hemoglobin content into a solution (10). As a result, a colored compound is formed by means of chemical reactions, in an amount directly proportional to the hemoglobin content. The traditional method, considered "gold standard" up to the current days, is the Cyanmethemoglobin method. It comprises the transformation of hemoglobin in a stable compound, under the action of Potassium cyanide and Potassium Ferric cyanide. This compound is, then, measured by means of its light absorption in a certain wavelength. As these compounds have toxicity, cyanide-free reagents for hemoglobin count have been developed (11).

More recently, Point of care testing (POCT) devices that require small blood samples have become available for measuring Hb with enhanced speed, simplicity and analytical performance. HemoCue Hemoglobin system (HemoCue, Angelholm, Sweden) is the most popular point of care hemoglobin meter. It consists of disposable micro cuvettes containing reagent in a dry form and a single purpose designed photometer.The reaction in the micro cuvette is a modified Azide-methemoglobin reaction. Sodium deoxycholatehemolyses erythrocytes and hemoglobin is released. Sodium nitrite converts hemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths in order to compensate for turbidity in the sample. The instrument then calculates the Hb and displays the results within 10 seconds (12).

1.2. Statement of the Problems

Hemoglobin (Hb) concentration is measured routinely using automated hematology analyzers.In recent years, the HemoCue Hb photometer also widely used as a point of care testing device to determine the Hb level since it is portable, requires only a small sample of blood, is relatively inexpensive and simple to use. In addition, the device does not require access to electricity, and gives immediate, digitally displayed results (12, 13). Specific to our current local setting, where the power supply is inconsistent, the HemoCue, which is a battery-operated device, due to the above mentioned reasons, becomes highly preferable (14).

Method comparison helps the laboratories to determine whether their different instruments agree or not for the same parameter. It is advisable to compare the new introduced method with the reference methods since the measurement of any analyzer can have different result in different environment or laboratory setup (15).

However, In Ethiopia, only few studies are done regarding comparison of hematological analyzers while Hemocue301+ is not included in the previous studies despite the fact that Hemocue is widely distributed in different laboratories to provide diagnostic test for millions of patients. Besides, different models of Sysmex and Cell-Dyn hematology analyzers are available in a number of public and private health facilities.

Regarding St' Paul's Hospital Millennium Medical college, it is one of the biggest referral hospitals in the country and delivers services for over 200,000 patients annually that come from different parts of the country. In this hospital laboratory, there are hematology analyzers like; Cell-Dyn 1800 and Sysmex Kx-21N and hemocue301+ that perform hemoglobin measurement for over 200 patients daily. However, the laboratory used these instruments interchangeably without doing method comparison. Therefore, this study was conducted to generate information that determines Hemocue301+ with and SysmexKx21N and Cell-Dyn1800 performance agreement.

1.3. Significance of the Study

This study gives a valuable information for St Paul's Hospital Laboratory personnel and clinicians about the agreement of Hemocue301+ with SysmexKx21N and Cell-Dyn1800 hemoglobin diagnosis methods whether to use or not interchangeably. And it is believed to be that findings of this study will help as milestone for other researchers and professionals who are interested to conduct on related method comparison studies.

2. Literature Review

The comparability of the HemoCue (Hb 301+) measurements with a Sysmex XE 2100 (Sysmex, Kobe, Japan) automated hematology analyzer was assessed by Morris*et al* by analyzing more than 300 routine venous blood samples. A bias greater than 7% occurred in 4.3% of cases (13/300), and in only 3 cases (i.e., 1%), the bias exceeded 10%, which was considered the threshold for clinical significance by the authors. These results supported that the HemoCue may be a suitable strategy for blood donor screening. Additional advantages were listed, including the lower cost of Hb 301 microcuvettes as compared with other models and the robustness against adverse climatic conditions (16).

In a study from Ghana, Nkrumah *et al* using three different methods (HemoCue, Sysmex-Kx21N and Cyanmethemoglobin). Agreement between the test methods was assessed by the method of Bland and Altman. Comparing the hemoglobin determined by the HemoCue to sysmex Kx21, the Bland and Altman's limit of agreement was (-0.389, 0.644 g/dl)with the mean difference being 0.127 and a non-significant difference in variability between the two measurements (p=0.843).Hemoglobin determined by the HemoCue method is comparable to that determined by the other methods. The HemoCue photometer was, therefore, recommended for use as on-the-spot device for determining hemoglobin in resource poor setting (17).

A contradicting data was obtained from a study which was conducted to evaluate the accuracy and precision of point-of-care testing for blood hemoglobin concentration measurements in critically ill patients. Blood samples from 50 post-operative patient were taken and analyzed using the HemoCueA system (Mallinckrodt Medical,Germany), and an automated hematology analyzer (M-2000A,Sysmex, Germany). The cyanmethemoglobin method served as the reference 'gold standard' procedure. HemoCue systems were tested using heparinzed blood samples and their conclusion was Hb measurement method differ in agreement to a laboratory hematology analyzer (18).

A prospective observational study was conducted by Mimoz *et al* in three adult surgical intensive care units of a university hospital. One hundred and ninety-eight consecutive patients were included, and a total of 1166 hemoglobin concentrations were measured. Samples were analyzed in the laboratory (HbLAB) and using a portable hemoglobin meter (HemoCueHb201+). The result shows mean difference (bias) between HemoCueHb and HbLAB was 0.2 g/dL (95%CI, 0.1, 0.3), In their conclusion, HemoCue is not sufficiently accurate to make a therapeutic decision (19).

Ziemann and colleagues compared venous blood from whole blood donation. Hb testing was performed on venous blood samples with the Sysmex SE 9000. In the vast majority of blood donors, the capillary (HemoCue) and venous (SE 9000) Hb values differed less by than 10 g/L, whereas the difference exceeded 20 g/L in 86 donors (1.0%) and 30 g/L or more in 10 donors (0.1%) (20).

In a study by Kim *et al* Hb was measured using a portable hemoglobin meter (HemoCue; HemoCue AB, Sweden), and an automated hematology analyzer (LH500; Beckman Coulter, USA). Hb measurements were compared among 3 different Hb level groups. The result shows mean Hb values of 506 blood donors were 14.0 g/dl by the LH500, and 14.3 g/dl by the HemoCue. The correlation between the LH500 and the HemoCue agreed almost perfectly (ICC=0.86) (21).

Another study by Miller *et al* compared the accuracy of the HemoCue with a Beckman Coulter co-oximeter in arterial blood samples of patients who received general anesthesia for spine surgery. In 77 of the 78 Hb measurements (i.e., 98.7%) obtained in these patients, the HemoCue values exhibited a bias lower than 10 g/L as compared with the co-oximeter. This led the authors to conclude that arterial HemoCue values might be virtually interchangeable with those obtained with standard co-oximetry (22).

Rechner *et al* tried to determine whether HemoCue may be useful in a neonatal unit to measure Hb as compared with a laboratory method (Beckman Coulter). Samples were collected by vein puncture. Again, the concordance of measures between the HemoCue and laboratory testing was excellent (limits of agreement of the two methods were between −4.8 and +9.8 g/L) over a broad range of Hb values (23).

A study was conducted by Zhao and Yin to estimate hemoglobin by venous blood samples from 71 children aged 12-13 years by using Hemocue and cyanmethemoglobin methods. The results showed that the two methods had good correlations in estimating hemoglobin. The results from HemoCue method were higher than the cyanmethemoglobin method by both the capillary blood samples and venous blood samples. The results indicated that HemoCue method has been found to be easy in operation, less in training and portable size, and can be used in the field work that the cyanmethemoglobin method was not available (24).

Adult patients presenting to an outpatient research clinic were tested for total blood hemoglobin measurement by different methods: on a point-of-care device, and venous sample on a laboratory hematology analyzer (as reference device). Samples from 152 patients were assessed (average age, 46 years; 69% female).The Bland-Altman plots assessing agreement of the test methods to the reference method had limits of agreement of -1.7 to 2.3 g/dl for HemoCue 201+ (13).

Seguin *et al* carried out a study in which Hb values measured using the HemoCue (Hb 201+) were compared with those obtained with the Beckman Coulter LH 750. Venous blood was analyzed using both HemoCue and the automated laboratory analyzer.The mean absolute bias between the HemoCue and Beckman Coulter LH 750 was 1 g/L (95% CI, −25 to 26 g/L) in Hemocue, and 11 g/L (95% CI, −36 to 58 g/L) in Beckman Coulter LH 750. These results led the authors to advice against the use of the HemoCue in critically patients, especially in the presence of edema and for capillary blood (25).

Accuracy of Hb measurement using HemoCue was assessed in 140 surgical blood samples using 7 HemoCue devices in comparison with a CO-Oximeter (IL 482, Instrumentation Laboratory, Lexington, MA). HemoCue underestimates the Hb concentration by 2-5% and exhibits a 8-10 times higher variability with only 86.4% of HemoCue being within +/- 10% of CO-Oximeter Hb. Although the mean bias between CO-Oximeter Hb and HemoCue Hb was relatively low, Hb measurement by HemoCue exhibited a significant variability (26).

Venous samples were tested using four different methods of Hemoglobin (Hb) estimation (hematology cell analyzer, Hemoglobin Color Scale, Copper sulphate (CuSO4) specific gravity method and hemocue). Hemoglobin Color Scale was found to be comparable to cell analyzer but more subjective. Considering cost effectiveness, accuracy, and suitability of a method for donor screening, the authors recommended continuation of donor screening with CuSO4 (27).

A study was performed to evaluate the utility of point-of-care Hb measurement with the HemoCue device to compare the analytical performance of the HemoCue against the Coulter LH 750 automated hematology analyzer. Evaluation was carried out with regard to accuracy and precision in the measurement of Hb in adult and pediatric patient samples, referred for routine laboratory testing. The results were compared using standard scatter and difference plots. The mean Hb value of the HemoCue(11.3 g/dl; range 4.6-16.7) was comparable to the Coulter LH 750 (11.3 g/dl; range 4.7-17.2). The Bland-Altman difference plot revealed good correlation. The imprecision was within acceptable limits. The study, therefore, concluded that HemoCue may be used to provide accurate and reliable Hb measurements with a smaller sample volume and improved turnaround time (28).

Taken together, the studies reviewed above revealed conflicting data. Though the majority confirmed that the HemoCue has an excellent agreement with the various automated hematological analyzers studied, a number of others including Gehring *et al*(18), Mimoz *et al* (19), Seguin *et al* (25) and Rippmann *et al* (26) generated evidences against the use of the HemoCue device in some patient population. Despite these, as far as my literature review goes no published data is available from Ethiopia comparing HemoCue against the widely distributed hematological analyzers like Sysmex Kx-21N and Cell-Dyn 1800.

3. Objective of the Study

3.1. General Objective:

To compare hemoglobin method agreement determined by Hemocue301+ with Sysmex Kx-21N and Cell-Dyn1800 methods using patient's whole blood at St Paul's Specialized Hospital Millennium Medical College in Addis Ababa, Ethiopia, 2015.

3.2. Specific Objectives

- To compare the overall method agreement between HemoCue301+ and SysemxKx-21N hemoglobin measurement methods.
- To compare the overall method agreement between HemoCue301+ and Cell dyn1800 hemoglobin measurement methods.

4. Hypothesis

There will be no significant difference between Hemoglobin result from Hemocue301+, with Sysmex Kx-21N and Cell-Dyn 1800.

Ho: Hohemocue301+ = Hosysmexkx21N = 0, $P \geq 0.05$

Hohemocue301+ = HocellDyn1800= 0 , $P \geq 0.05$

5. Materials and Methods

5.1 Study Design

A facility based cross sectional study was conducted at St Paul's Specialized Hospital Millennium Medical College (SPHMMC) in Addis Ababa Ethiopia.

5.2 Study Site

SPHMMC is one of the largest hospitals serving the people referred from different parts of the country. The main Hospital departments include the Pediatrics, Surgical, and Medical, Obstetrics and Gynecology and the laboratory departments. The laboratory department is fully equipped and has functional Microbiology, Hematology, Parasitological, and Clinical Chemistry departments. The laboratory participates in various External Quality Assessment programs.

5.3 Study Period

The study was conducted from May 15-June 2, 2015.

5.4 Population

5.4.1 Source Population

All patients who gave blood sample for the laboratory department of St. Paul's Hospital Millennium Medical College within the study period.

5.4.2 Study Population

Blood samples from patients who were referred to hematology laboratory department of St. Paul's Hospital Millennium Medical College within the study period.

5.5 Inclusion and Exclusion Criteria

5.5.1 Inclusion Criteria

Blood samples collected in SPHMMC laboratory within the study period and within the acceptable sample quality criteria as per the laboratory sample collection SOP (with proper specimen collection, with non hemolyzed sample, non-clotted sample) were included in the study.

5.5.2 Exclusion Criteria

Leftover samples which were insufficient to carry out the study were excluded.

5.6 Variables

- Precision
- Agreement between two methods

5.7 Sample Size Calculation and Sampling Techniques

5.7.1 Sample Size calculation

For method comparison studies, Clinical and Laboratory Standard Institute (CLSI) 2013 guideline recommends a minimum of 40 specimens to carry out evaluation between analyzers. However, in this study using convenient sampling techniques 117 specimens were analyzed in duplicate on each analyzer (29).

5.7.2 Sample Collection Techniques

From patients who are referred for hematological investigation to the hospital laboratory, about 3 ml fresh whole blood sample was collected with EDTA anticoagulant containing tubes by phlebotomists at SPHMMC reception as per the laboratory sample collection SOPs. Samples were transported immediately to the hematology section of the laboratory. From these samples, we used the leftover blood sample for this study as they arrive consecutively.

Total samples used in this study were one hundred twenty out of which three samples were used for precision within sample study and the rest one hundred seventeen samples were used for the agreement study of the analyzers. The samples were heterogeneous having Low, Normal and High Hb value for all analyzers.

5.8 Data collection and Processing

5.8.1 Sample Processing

The collected samples were analyzed on Hemocue 301+, Sysmex Kx-21N and Cell-Dyn 1800 Hematology analyzers by the principal investigator following SOPs.

The Clinical and Laboratory Standard Institute (CLSI) guideline recommends using the manufacturer's procedures for method comparison experiment. Hemoglobin values that cover Low, Normal and high value interval were used (29).

5.8.2 Laboratory Analyses

The Specimen was checked for the presence of any clot and aliquot sample were obtained from a well mixed EDTA whole blood specimen. Before performing the test all quality control procedures specified in the Hemocue301+, SysmexKx21N and CellDyn1800 operators Guide were followed. for Hemocue301+ excess blood on the outside tip were carefully wiped away without removing any of the sample and specimens were run on the analyzer and result was documented. The specimen were run on SysmexKx21 and CellDyn1800 on whole blood mode any flag or error massage were traced and solved immediately and result are documented when the procedure were completed according with the manufacturer's instructions.

5.9 Quality Assurance

- Samples were checked whether they were in the acceptable criteria like; hemolysis, clotting, volume and collection time.
- Manufacturer procedures and SOPs were strictly followed.
- Prior to process samples were checked for time of collection.
- Prior to process, samples were homogenized and inverted 10-15 times.
- Three levels hematology cell controls (Low, Normal and High) for the respective machines were run.

In addition, all instruments were operated in accordance with manufacturers' instructions. Before performing an accuracy test, thoroughly familiarization with the correct operation of the Hemocue301+,Sysmex Kx21N and CellDyn1800 System were confirmed and all quality control procedures specified in the Hemocue301+,Sysmex Kx21 and cellDyn1800 Operator's Guide were performed.

The function of the HemoCue301+ photometer was checked on a daily basis by measuring the control cuvette. A three set (Low, Normal and High) Hb status were run daily to ensure the function of the SysmexKx21N and Cell-Dyn1800. Samples were only processed when the QC material had passed. Finally results from all the analyzers were properly recorded and documented to ensure post-analytic quality.

5.10 Data Analysis and Interpretation

Data were entered and analyzed using SPSS version 20 (SPSS INC, Chicago, IL, USA). Standard deviation, coefficient of variation, proportions, percentages, figures, and tables were used for the description of the data as appropriate. By using SPSS, Pearson´s correlation coefficient and Bland-Altman plot were calculated to assess agreement between each two analyzers.

Agreement between Hemocue301+ and Sysmex Kx-21 and between Hemocue301+ and Cell-Dyn 1800 was calculated for Hemoglobin measurement by using one hundred seventeen samples that covered the Low, Medium and High range of the analytic interval range and were run in duplicates. In the Bland Altman method, the differences in hemoglobin value between the two methods were plotted against mean values. Agreement was considered acceptable when the difference is lying between mean bias $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95%CI and above of cases when t-test p values < 0.05 were considered statistically significant.Hemoglobin measurement of 1g/dl was considered as clinically significant limit of agreement in many literatures (16, 30, 31).

For each measurements generated first the difference was determined by subtracting the result of Hemocue301+ minus SysmexKx21for all of the 117samples and this difference is shown on the y- axis of the Bland and Altman plot, while the average of the values was put on the x axis. One sample t-test was used for testing the difference by taking zero as a test value. Difference plot were employed to determine the scatter of each observed values against their difference. These procedures were repeated for the determination of Hemocue301+ and Cell-Dyn1800 agreement study.

For the precision study the analyzers' capacity to reproduce the results for Hemoglobin in a given sample was determined using the coefficient of variation. Analyses were carried out by the same operator, within a short period of time, same location and with the same reagents. The prepared samples were analyzed 20 consecutive times in HemoCue301+, Sysmex Kx-21and Cell-Dyn 1800. The mean, the standard deviation (SD) and the coefficient of variation (CV) were calculated for the hemoglobin value. Precision was computed to check how the results of a sample that is carried out in similar conditions of same reagent, same location of analyzers and same operator are dispersed.

5.11 Ethical Considerations

Before conducting the research work, ethical clearance and approval was obtained from St. Paul's Hospital Millennium Medical College Institutional Review Board (IRB) and from Research ethical committee of Addis Ababa University; Department of Medical Laboratory Sciences to use leftover blood samples collected for routine examination. Samples were coded and also confidentiality of patient data was maintained throughout the study.

5.12 Dissemination of Result

The final paper will be disseminated to St. Paul's Hospital Millennium Medical College laboratory following public defense at the Department of Medical Laboratory Sciences of Addis Ababa University. In addition, a copy of this material will be given to, Ministry of Health, Addis Ababa Health Bureau, and respective hospitals that use the same hematology analyzers. The result will be published in peer reviewed international journal.

5.13 Operational Definition

- **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.
- **Limit of agreement:** It represents the range of values in which agreement between methods will lie for approximately 95% of the sample.
- **Precession:** closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions and usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.
- **Accuracy:** the extent to which a value from a test reflects or agrees with the reference value of the analyte being tested.

6. Result

In this study 120 venous blood specimens were collected. Three specimens were used for precision study and the rest one hundred seventeen samples were used for agreement study. From a total of 117 samples; 66(56.4%) were collected from female patients and 51(43.6%) from males. The age ranges of patients were from 5-78 years.

6.1 Precision of the Analyzers

Precision studies were carried out using low, normal, and high value of Hemoglobin for the three methods and compared against the manufacturer's claim. Each sample was run 20 times. The table below summarizes the calculated coefficient of variation values of Hemocue301+, SysmexKx21N and CellDyn1800 for the hemoglobin values. As shown in the table, the precision values of the Hemoglobin for SysmexKx21only lie within the verification specification of the manufacturer (Table 1).

Table 1: Imprecision (within run sample variation) of hemocue301+, SysmexKx21N and Cell-Dyn1800 for Hemoglobin measurement. St. Paul's Hospital Millennium Medical College Hematology laboratory, Addis Ababa, May 15-June 2, 2015

	Imprecision		
Low Hb values (g/dl)	Hemocue301+	SysmexKx21N	CellDyn1800
Mean	5.1	4.1	4.1
SD	0.12	0.11	0.14
(%CV)	1.6	1.3	2.2
Manufacturer's specification(%CV)	0.82		

	Imprecision		
Normal Hb values (g/dl)	Hemocue301+	SysmexKx21N	CellDyn1800
Mean	15.5	14.6	14.5
SD	0.12	0.11	0.13
(%CV)	1.7	1.3	1.8
Manufacturer's specification(%CV)	≤0.8	≤1.5	≤1.2

	Imprecision		
High Hb values (g/dl)	Hemocue301+	SysmexKx21N	CellDyn1800
Mean	19.6	18.6	18.6
SD	0.12	0.12	0.14
(%CV)	1.6	1.6	2.2
Manufacturer's specification(%CV)specification	0.78		

6.2 Agreement of the Analyzers

Agreement between Hemocue301+ with each of Sysmex Kx-21N and Cell-Dyn 1800 was evaluated using Bland Altman method. Differences in hemoglobin values between two analyzers, Hemocue301+versus sysmexkx21 and Cell-Dyn1800, were plotted against the mean values of the results generated by the two analyzers for the same specimen as stated by Bland and Altman. Agreement is considered acceptable when the difference lies between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases (32).

6.2.1 Agreement between Hemocue301+ and Sysmex KX-21N for Hemoglobin measurements

As shown in Table 2, the result from the correlation coefficient Pearson correlation r value was 0.98, which showed good correlation between SysmexKx21 and Hemocue301+ for measuring Hb. From the t-test the mean differences of the results from the two analyzers were 0.09 for Hemocue301+ and SysmexKx21. Since the P value (0.103) was greater than 0.05 for hemocue301+ and sysmexKx21which means the difference of the two analyzers was not statistically significant. To validate this finding and to check for any bias, we plotted difference (Hemocue301+ generated value minus Sysmex Kx-21 generated Hb values) versus the mean values generated by both machines (Hb generated by Hemocue301+ plus Hb value generated by sysmexKx21divided by 2) (Figure 1).

Table 2: One sample T-test of zero test value for hemocue301+ and sysmexKx21Hb parameter at St. Paul's Hospital Millennium Medical College Hematology laboratory, Addis Ababa, May 15-June 2, 2015

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Measurement	Pearson correlation coefficient(r)	Mean of difference	Std. dev. of difference	95% C.I.	Sig. two tailed
Hemoglobin (g/dl)	0.98	0.09	0.593	(-0.0184,0.198)	0.103

6.2.2 Agreement between Hemocue301+ and Cell-Dyn1800 for Hemoglobin measurements

As shown in Table 3, the result from the correlation coefficient Pearson correlation r value was 0.98, which showed good correlation between Hemocue301+ and Cell Dyn1800 for Hb.from the t- test the mean differences of the two analyzers was0.04for hemocue301+ and cell dyne1800.Since the P value (0.46) were again greater than 0.05 for Hemocue301+ and CellDyn1800, which means the difference of the two analyzers was not statistically significant. To validate this finding and to check for any bias, we plotted difference (Hemocue301+ generated Hb value minus Cell-Dyn 1800 generated Hb values) versus the mean values generated by both machines (Hb generated by Hemocue301+ plus Hb value generated by Cell-Dyn1800 divided by 2) (Figure 2).

Table 3:One sample T-test of zero test value for hemocue301+ and cell dyne1800Hb parameter

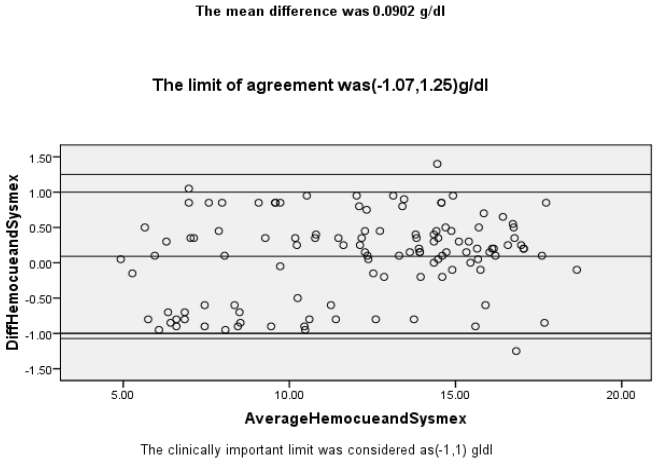
At St. Paul's Hospital Millennium Medical College Hematology laboratory, Addis Ababa, May 15-June 2, 2015

Measurement	Pearson correlation coefficient(r)	Mean of difference	Std. dev. of difference	95% C.I. of the diff.	Sig. two tailed
Hemoglobin (g/dl)	0.98	0.044	0.646	(-0.074,0.162)	0.46

6.2.3 Bland Altman plot of Hb between Hemocue301+ and SysmexKxN-21

Limit of agreement from Bland and Altman plot was (-1.07, 1.25)g/dl. The clinically important limit was considered as (-1, 1) g/dl. From the Bland-Altman scatter plot (2)1.7% of the cases were out of the limit of agreement (-1.07, 1.25) g/dl and 3(2.5%) cases were out of 1g/dl limit which is the clinically important limit of agreement for Hb measurement (10,24,25). The values were still within the range for 95% of the cases and should be within the limit of agreement. There were 74 cases above the mean difference and 43 cases below the mean difference indicating the Hemocue301+ measure higher Hb value than SysmexKx21.The 95% limit of agreement was-1.07g/dl and 1.25g/dl. Bland-Altman analysis defines, "if two methods are to agree, then the mean of the difference between every paired determination will not be statistically different from zero and a limit of agreement can be established." Our results showed Hemocue301+ might read 1.07g/dl below and 1.25g/dl above the mean difference values in 1.7%of the cases (32).

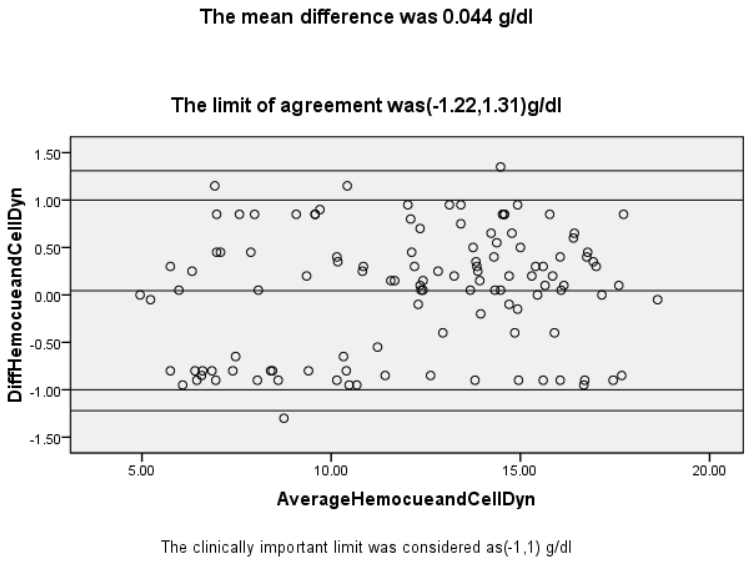
Figure 1: Bland Altman plot of Hb between Hemocue301+and Sysmex KX-21 at St. Paul's Hospital Millennium Medical College Hematology Laboratory, Addis Ababa, May 15-June 2, 2015.



6.2.4 Bland Altman plot of Hb between Hemocue301+ and Cell-Dyn1800

Limit of agreement for Hemocue301+ and Cell-Dyn1800 obtained from the Bland and Altman plot was (-1.22, 1.31g/dl). The clinically important limit was considered as (-1, 1) g/dl. From the Bland-Altman scatter plot (2)1.7% of the cases were out of the limit of agreement (-1.22, 1.31) g/dl and 4(3.4%) cases were out of 1g/dl which is the clinically important limit of agreement for Hb measurement (10,24,25). These values were still within the range for 95% of the cases and should be within the limit of agreement. There were 73 cases above the mean difference and 44 cases below the mean difference indicating Hemocue301+ measure higher Hb value than CellDyn1800.The 95% limit of agreement was -1.22g/dl and 1.31g/dl. Bland-Altman analysis defines, "if two methods are to agree then the mean of the difference between every paired determination will not be statistically different from zero and a limit of agreement can be established." Our results showed Hemocue301+ might read 1.22g/dl below and 1.31g/dl above the mean difference in 1.7% of the cases (32).

Figure 2: Bland Altman plot of Hb between Hemocue301+and Cell-Dyn 1800 at St. Paul's Hospital Millennium Medical College Hematology Laboratory, Addis Ababa, May 15-June2,2015.



The clinically important limit was considered as(-1,1) g/dl

7. Discussion

The present study evaluated the Hb measurement of the Hemocue301+ POCT device against a reference laboratory method. Hemoglobin determination by Hemocue301+ has advantage of time, cost, and simplicity. This study focuses on the method agreement with SysmexKx21N and CellDyn1800, which were reference methods. The manufacturers claim that these instruments give good agreement of hemoglobin levels. This study documents within run precision and agreement studies of Hemocue301+with Sysmex Kx-21N and Cell-Dyn1800 hematology analyzers.

When results were evaluated against manufacturer's specifications of %CV, our result in normal range demonstrated that SysmexKx21N fulfills the manufacturer specifications with CVof1.3%. However, the precision was not within the manufacturer's specification for Hemocue301+ and Cell-Dyn1800.

A similar study for the precision specifications for Hemocue by Gomez-Simon *et al* (33) and Schapkiitz *et al* (28)for hemoglobin value within the normal range showed %CVs of 2.3%, 1.75%,respectively. These findings plus the value obtained in our study which was 1.7%, are all outside the specifications of the manufacturers. The normal range CV% for Cell-Dyn1800 in our study was 1.8%.Similarly; some studies report a CV% which meets the manufacturer specification a study by LardiA.M *et al* evaluates Hemocue using different level of samples; the precision check revealed a CV% of 0.74% which contradicts with the current study (34).

However, a study by Ghys*et al* evaluated the precision of Sysmex XS-1000i by using patient samples of different levels and the specifications of the manufacturer were not met with a CV% of less than 3% (35).According to the current study results, the HemoCue301+(CV%1.7)has a lower precision than the SysmexKx21N(CV=1.3%).On the other hand, HemoCue301+ (CV=1.7%)exhibited similar reproducibility when compared with the Cell-Dyn1800+ (CV%1.8).

In this study, the one sample t-test for mean differences of Hb results generated from test and reference methods revealed a difference of 0.09g/dl with P-value of 0.103 for Hemocue301+ versusSysmexKx21N and a mean difference of 0.04g/dl (p=0.46) for Hemocue301+ versus Cell-dyne1800 comparisons. This means there was some level of agreement between Hemocue301+ and the reference methods (SysmexKx21N and Cell-Dyn1800) as there was no statistically significant mean difference between the test and reference methods. Moreover, the Pearson

correlation coefficient showed strong correlation between Hemocue301+ and the reference methods (SysmexKx21N and Cell-Dyn1800).

Similar result was reported by Nkrumah *et al* in Ghana on the comparability of the HemoCue Hemoglobin measurements with SysmexKx21. They recorded a mean bias of 0.13 g/dl and a non-significant difference in variability between the two measurements (p= 0.391) indicating some level of agreement between Hemocue301+ and SysmexKx21N (17).

In the present study the mean difference and the correlation coefficient(r) between Hemocue301+and SysmexKx21N was 0.09g/dl, 0.98, respectively. Similar finding was reported by Van de *et al* who reported a bias of 0.1g/dl (31) and Mimoz, *et al* who reported a similar bias of 0.1g/dl and a correlation coefficient(r)of 0.88(19).

Moreover, Hemocue301+ was compared with Sysmex XE 2100 by Morris *et al* (16) and only in 1%of the cases was the value of Hb out of limit of agreement (LOA).Similarly a study by Nkrumah, *et al* (17) reported acceptable agreement with LOA between-1.4 to 1.6g/dl. Schapkaitz *et al* recommends the use of hemocue with LOA -0.396 to 0.549 g/dl (28). This was similar with our result which showed only three cases (2.5%) were out of the LOA. In all cases the authors concluded that the methods comply with their respective limit of agreement. The finding support that the Hemocue301+ may be a suitable strategy for Hb measurement in their limit of agreement (16, 17, 28).

Unlike our result a study from Sudan in pregnant women by Ishaga *et al* and a study by Gehring *et al* report Hb measurement method differ in agreement to a laboratory hematology analyzer. The study differ may be due to the fact that their study subject were in range of low hemoglobin value and the agreement may not work at low hemoglobin value (30, 18).

Concerning Hemocue301+and Cell-Dyn1800 our result showed that 1.7%of the values were out of limit of agreement unlike a study by Morris, *et al* who found 9% out of limit of agreement. A study by Neufeld *et al* showed HemoCue has higher bias (+0.5 g/dl) than that of Cell Dyn;in our case the mean deference was 0.04g/dl (16, 37).

According to Spielmann*et al* the HemoCue for Hb determination were compared with results obtained on a Sysmex XE 2100.Potential significant differences were observed beyond a range

of 2 g/dl in only 2 cases that was 0.8% (36). This was still within the LOA similar to our result that only three cases (2.5%)out of one hundred seventeen were out of the limit of agreement.

Based on the findings of this study, agreement of Hemocue301+ point of care testing with automated hematology analyzerSysmexKx21N and Hemocue301+ with Cell-Dyn1800,should have to be used in their limit of agreement LOA (-1.07to 1.25) g/dl and (-1.22,1.31)g/dl, respectively, for Hemoglobin measurement.

8. Strength and Limitation of the Study

8.1 Strength:

- Samples run in duplicates

8.2 Limitation:

- Limited studies were available on similar analyzer models, making comparison difficult for our work and yet justifies the need for this study.

9. Conclusion and recommendation

9.1 Conclusions

Hemoglobin measurement by the Hemocue301+ method showed good agreement with automated hematology analyzer (SysmexKx21, CelDyn1800) in their limit of agreement for Hemoglobin measurement. Hence, Hemocue301+system may be a convenient method for measuring blood Hb in St Paul's Specialized Hospital Millennium Medical College Hematology Laboratory.

9.2 Recommendations

- The Hospital Laboratory can use the Hemocue301+ and reference methods (SysmexKx21 and CellDyn1800) hematology analyzer interchangeably for Hb determination as needed.

- The Hospital Laboratory should have to conduct method comparison experiment periodically and when there is spare part and reagent change.

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11. Annexes

I: Principle of the instruments

Principle of Hemocue301+

Principle: Hemoglobin concentration is determined by measuring the absorbance of whole blood at an Hb/HbO2 isobestic point. This method correlates well with the reference method for hemoglobin determination (the ICSH method). The analyzer uses a double wavelength measuring method, 506 nm and 880 nm, for compensation of turbidity (12).

Sample material: Capillary, venous or arterial whole blood.

Measurement range: 0-25.6 g/dL (0-256 g/L or 0-15.9 mmol/L).

Results: In about 10 seconds.

Sample volume: 10 µL.

Power: AC adapter or batteries.

Operating temperature: 10-40 °C (50-104 °F).

Quality control: Built-in "self test" control cuveete.

Calibration: The system is factory calibrated against the ICSH reference method for hemoglobin and needs no further calibration.

Storage for the HemoCueHb 301 Analyzer: The analyzer can be stored at temperature 0-50 °C (32-122 °F).

Storage for the HemoCueHb 301+ Microcuvette: The microcuvettes are to be stored at 10-40 °C (50-104 °F). Short-term storage (6 weeks) -18-50 °C (0 – 122 °F). Once the container is opened the microcuvettes are stable for three months. Always keep the container closed.

Principle of Sysmex Kx-21 Hematology analyzer

Sysmex KX-21 employs two basic technologies to achieve precise measurements at the micro-liter level. These are sheath flow DC detection in which a constant electric current is passed through a solution; this method measures the changes in electrical resistance that occur when blood cells pass through detection aperture. The second one is flowcytometry which is the measurement of cellular properties as they are moving in a fluid stream (flow), past a stationary set of detectors. The chemical action principle use SLS (38).

Principle of Cell-Dyn 1800 Hematology Analyzer

Cell-Dyne 1800 hematology analyzer follows a method of counting and volumetric sizing based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid as it is drawn through a small aperture. It employs the non conductive nature of blood cells. The chemical action principle use modified Cyanomethahemoglobin methods (39).

II: Quality Control

All instruments were operated in accordance with manufacturers' instructions. Before performing the test, thoroughly familiarize with the correct operation of the Hemocue301+, Sysmex Kx21 and CellDyn1800 System is confirmed and perform all quality control procedures specified in the Hemocue301+, Sysmex KX21, cellDyn1800 Operator's Guide.

Quality Control for Hemocue301+

The function of the HemoCue301+ photometer was checked on a daily basis by measuring the control cuvette. Low Medium and cuvettes control run each day before any client samples are tested. It ensure that the instrument is functioning properly, that the microcuvettes are capable of accurately detecting critically low and unusually high hemoglobin levels, and that the analyst was performing the test accurately.

Low, medium, and high control was run on Hemocue301+ analyzer in the laboratory prior to client testing. Low, medium and high control was run to check each new vial of microcuvettes before they are used for client testing. Low, medium and high control was run to check each new lot number of microcuvettes before they are used for client testing.

Quality Control for SysmexKx21N

Before starting sample analysis, quality controls were performed to monitor an instruments performance over time. Three control levels which were Low, Normal and High Hb status were run daily to ensure the function of the Sysmex KX21N. Blood controls were analyzed to obtain highly reliable quality data. Control was run as the constant monitoring of the instrument for early detection of problems. Samples were only processed when the QC material had passed, Heamolyzed sample, coagulated sample in general in appropriate sample were rejected. Any data collection errors where corrected at the time of collection. Using L-J control program the data from control blood analysis were used to evaluate analyzer performance.

Low, Normal, and High Control: was used to display QC information such as Standard deviation, and coefficient of variation If the control results fall within acceptable limits, review

the data for shift or trends, record the results, and begin to process patient samples. Since three levels of control are used to monitor the analyzer, it is reasonable to consider all three runs at the same time. The westgard rules were used in combination the number of the rule is displayed if that rule is violated.

Quality Control for CellDyn1800

The function of the Cell-Dyn 1800 was checked on a daily basis by measuring the control. before starting sample analysis, quality controls were performed to monitor an instruments performance over time. Three control levels which were Low Normal and High Hb status were run daily to ensure the function of the CellDyn1800. Blood controls were analyzed to obtain highly reliable quality data. Control was run as the constant monitoring of the instrument for early detection of problems. Samples were only processed when the QC material had passed, Heamolyzed sample, coagulated sample in general in appropriate sample were rejected. Any data collection errors where corrected at the time of collection. Using L-J control program the data from control blood analysis were used to evaluate analyzer performance.

Low, Normal, and High Control: was used to display QC information such as Standard deviation, and coefficient of variation If the control results fall within acceptable limits, review the data for shift or trends, record the results, and begin to process patient samples. Since three levels of control are used to monitor the analyzer, it is reasonable to consider all three runs at the same time. The westgard rules were used in combination the number of the rule is displayed if that rule is violated.

III: English version of participant information sheet, consent form

Participant information sheet

Department of Medical Laboratory Science, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Title of the Research Project: Comparison of Hemocue301+ with SysmexKx21N and CellDyn1800 automated Hematology analyzer hemoglobin measurement in patient blood at St Paul's specialized teaching hospital, Addis Ababa Ethiopia, 2015.

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Background information

Background: Anemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status

Aim of the study comparing hemoglobin determined by Hemocue301+, sysmex kx21N and cellDyn1800 in patient blood in st Paul's specialized teaching hospital

Benefits for participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the diagnosis result you will be treated accordingly. Most importantly, the result of the study will be beneficial when effective diagnostic method is available in the health facility; you are indirectly benefiting other patients and the society in this respect.

Risks and complication

There are no anticipated risks to your participation. Except little discomfort when you give blood sample.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. Only interested participants can retrieve their own lab result using their code number. The information collected about you will be coded using numbers. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Mesfin Samuel

Signature: _____ Date: _____

Note: If you have any questions about this study, you should feel free to ask now or anytime

Throughout the study by contacting:

Address: Mesfin Samuel: Department of Medical Laboratory Sciences, Collage of Allied Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

E-mail: mesfin9803@gmail.comTell 0913625554

Informed consent

I have been informed about the objective of the study entitled "comparison of method in hemoglobin measurement by Hemocue301+, sysmexKx21N and cellDyn1800 in patient blood in St Paul's specialized teaching hospital, Addis Ababa Ethiopia" I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

Therefore, with full understanding of the situations I agree to give the entire necessary information and blood sample for laboratory analysis. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also told that results for the hemoglobin analysis will be given to the health facility and that I may ask the information if I want.

I _____ hereby give my consent for giving of the requested

Information and specimen for this study.

Participant code: _____ Signature: _____

Date: _____

IV: Amharic Version of the participant information sheet and Consent

I. የተሳታፊዎች የመረጃ ቅፅ

አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ዲፓርትመንት

አርስት፡-□ደም ውስጥ ሄሞግሎቢን መጠንን በሂሞኪዶ□ በሲስሚክስ ና በሴልዳይን ማወዳደር

አጠቃላይ መረጃ፡-

በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት ይህን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ፤እንዲሁም ግልጽ ያልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ

ስለጥናቱ መረጃ፡-□ደም ውስጥ ሄሞግሎቢን መጠንን ማወቅ ብዙ ጥቅም ያሉት ሲሆን ከነዚም ውስጥ ዋነኛው በተለምዶ የደም ማነስ የሚባለውን በሽ□ አስቀድሞ ለማወቅ ያስችላል::

የጥናቱአላማ፡-□ደም ውስጥ ሄሞግሎቢን መጠንን በሂሞኪዶ/በሲስሚክስ ና በሴልዳይን አወዳድሮ ልዩነ□ችውን ማጥናትና ማወቅ ነው፡፡

ጥናቱ ለተሳታፊዎች ያለው ጥቅም፡-በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም፣ ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖሮታል፡፡ በተጨማሪም የጥናቱ ውጤት የደም ማነስ ህመምን ለመቆጣጠርና ለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁም ህብረተሰቡን የመጥቀም እድል ያገኛሉ፡፡

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር

በዚህ ጥናት በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ለዚህ ጥናት የሚያገለግል □□□ ናሙና የሚወሰድ ሲሆን ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ ወቅትም ሆነ ከዛ በኂላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም□□ □□ □□□□□□□□□□□□□□□□□□□□□

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□□□mesfin9803@gmail.com □□□ 0913625554

V- Laboratory test results register

1. code no of participant_____

2. Ages _____

3. Sex:

☐ Male

☐ Female

Code no of participants	Test Results					
	Hemocue301+		SysmexKx21		CellDyne1800	
001						
002						
003						
004						
005						

VI: Manufacturer's Specifications

Manufacturer's Specifications

Imprecision

Imprecision of Cell-Dyn1800 hematology analyzer

Parameters	Rang	Pooled CV%	CV% 95%(confidence limit)
Hemoglobin	**12.8 -16.0 g/dl**	**0.6**	**≤1.2**%

Linearity

Linearity of Cell-Dyn 1800

Parameters	Printed Report range	Analytical measurement range	Absolute deviation	Relative deviation
Hemoglobin	**0.0 -24.0 g/dl**	**0.8 -22.7 g/dl**	**±0.3**	**≤2.0**%

Carry over

Carry over	Level tested	Accepted limit
Hb	22.3g/dl	≤ 0.8%

Imprecision

Imprecision of Sysmex Kx-21 hematology analyzer

Parameters	CV%
Hemoglobin	≤1.5

Linearity

Linearity of Sysmex Kx-21

Parameters	Printed Report range	Absolute deviation	Relative deviation
Hemoglobin	**0.0 -25.0 g/dl**	**0.2**	**2**%

Carry over

Carry over	Accepted limit
Hb	**≤ 1**%

Hemocue301+

Proprietary Name: HemoCue 301+Hb system

Common Name: Hemoglobin analyzing system

data in table was obtained for within-run and total precision of the HemoCueHb 301 system.

Control Level	Number	Mean g/dl	Within run precision SD	CV%	Total Precision SD	CV%
Low	400	7.3	0.059	0.82	0.066	0.91
Normal	400	13.2	0.106	0.80	0.122	0.92
High	400	17.2	0.135	0.78	0.152	0.88

Summary of Linearity study

The linearity of the HemoCue Hb 301+ system was tested according to the NCCLS document EP6-A "Evaluation of the Linearity of Quantitative Measurement Procedure" using one batch of HemoCueHb 301 Microcuvettes, five HemocueHb 301 Analyzers and three EDTA whole blood samples each prepared to seven haemoglobin concentrations in the range 0-25 g/dL. The EDTA whole blood samples were analyzed with four replicates on each HemoCue Hb 301 analyzers. As a reference method all levels were as well analyzed in duplicates with the international reference method ICSH. The HemoCueHb 301 system is linear between 2-25 g/dL (within 4% difference at 2-5 g/dL and within 3% difference at 5-25 g/dL).

Summary of Accuracy studies

The accuracy for the HemoCueHb 30 1 system was determined by analyzing three batches of HemoCueHb 301 Microcuvettes on four HemoCueHb 301 Analyzers. Eight operators were performing the measurements. The International reference method ICSH was used for the comparison. With correlation coefficient $r = 0.998$

VII: Test Results

Experiments Results

Impression

No	Hemocue30 low	SysmexKX21N low	Celldyne1800 low	Hemocue301 normal	SysmexKX21N normal	Celldyne normal	Hemocue30 lHigh	SysmexKX21N high	Celldyne1800 High
1	5.4	4.4	4.4	15.7	14.7	14.9	19.8	18.9	18.9
2	5.3	4.4	4.3	15.7	14.7	14.7	19.8	18.9	18.9
3	5.3	4.3	4.3	15.7	14.7	14.6	19.8	18.7	18.8
4	5	4.3	4.3	15.7	14.7	14.5	19.7	18.7	18.5
5	5.3	4.2	4	15.5	14.7	14.6	19.7	18.7	18.9
6	5.2	4.1	4.1	15.7	14.6	14.5	19.7	18.7	18.6
7	5.3	4.1	4.3	15.7	14.7	14.7	19.5	18.5	18.9
8	5.3	4.2	4.4	15.6	14.7	14.4	19.6	18.6	18.6
9	5.2	4.1	4.2	15.4	14.7	14.5	19.6	18.6	18.6
10	5.1	4.1	4.2	15.7	14.6	14.5	19.6	18.6	18.6
11	4.9	4.3	4.3	15.4	14.7	14.4	19.6	18.6	18.6
12	5.1	4.2	4.3	15.5	14.5	14.4	19.6	18.6	18.6
13	5.2	4.2	4	15.5	14.6	14.5	19.6	18.6	18.6
1	5.2	4.2	4	15.7	14.5	14.5	19.6	18.6	18.6

4									
15	5.2	4.2	4.2	15.7	14.6	14.4	19.6	18.6	18.7
16	5.3	4.3	4	15.5	14.5	14.5	19.5	18.6	18.7
17	5.1	4.1	4	15.3	14.3	14.4	19.5	18.6	18.5
18	5.2	4	4	15.5	14.5	14.5	19.4	18.5	18.5
19	5.1	4	4.2	15.5	14.5	14.5	19.4	18.4	18.5
20	5	4.2	4	15.5	14.5	14.3	19.4	18.4	18.5

Agreement study results

N0	age	sex	Hm1	Hm2	Sysmx1	Sysmx2	CDyne1	CDyne 2	Hemocue Average	Sysmex average	avegceldyn
1	34	f	8.1	8.1	9	8.9	9.3	9.5	8.1	8.95	9.4
2	28	f	7.8	8.3	8.6	8.7	8.8	8.9	8.05	8.65	8.85
3	34	m	5.5	5.7	6.5	6.6	6.6	6.5	5.6	6.55	6.55
4	27	m	16.2	16.2	17.4	17.5	17.1	17.2	16.2	17.45	17.15
5	29	f	10	10	10.9	11	11	10.9	10	10.95	10.95
6	31	f	10.1	10.3	11	11	11.1	11.2	10.2	11	11.15
7	27	m	15.1	15.2	16.1	16	16.1	16	15.15	16.05	16.05
8	28	m	8.1	8.2	8.8	8.9	9	9.1	8.15	8.85	9.05
9	35	f	7.5	7.7	8.5	8.6	8.5	8.5	7.6	8.55	8.5
10	22	f	6	6	6.8	6.9	6.9	6.9	6	6.85	6.9
11	5	m	13.5	13.2	14.1	14.2	14.2	14.3	13.35	14.15	14.25
12	40	m	6.5	6.5	7.2	7.2	7.4	7.4	6.5	7.2	7.4
13	5	m	14.5	14.5	14.6	14.8	15.4	15.4	14.5	14.7	15.4
14	32	f	9.6	9.8	9.7	9.8	10.6	10.6	9.7	9.75	10.6
15	22	f	15.4	15.8	16.2	16.2	16.5	16.5	15.6	16.2	16.5
16	18	m	16.2	16.3	16.1	16.2	17.1	17.2	16.25	16.15	17.15

17	42	m	17.1	16.9	16	17	17.9	17.9	17	16.5	17.9
18	20	m	17.2	17.3	18	18.2	18	18.2	17.25	18.1	18.1
19	25	m	11	11	11.8	11.8	11.8	11.9	11	11.8	11.85
20	23	m	12.2	12.2	13	13	13	13.1	12.2	13	13.05
21	18	f	6.3	6	7.1	7	7	7	6.15	7.05	7
22	24	f	8	8	8.9	8.9	8.8	8.8	8	8.9	8.8
23	42	f	10	10	10.9	10.9	10.8	10.8	10	10.9	10.8
24	19	m	9	9	9.9	9.9	9.8	9.8	9	9.9	9.8
25	33	m	5.2	5.5	6.1	6.2	6.2	6.1	5.35	6.15	6.15
26	29	f	7	7	7.9	7.9	7.8	7.8	7	7.9	7.8
27	21	m	6.4	6.5	7.2	7.3	7.3	7.2	6.45	7.25	7.25
28	34	f	6.2	6.2	7	7	7	7	6.2	7	7
29	40	m	6	6	6.7	6.7	6.8	6.8	6	6.7	6.8
30	35	f	7.2	7.1	7.7	7.8	7.8	7.8	7.15	7.75	7.8
31	35	m	10	10	10.5	10.5	10.6	10.7	10	10.5	10.65
32	30	f	10.9	11	11.5	11.6	11.5	11.5	10.95	11.55	11.5
33	24	f	15.7	15.7	15.8	15.8	16.1	16.1	15.7	15.8	16.1
34	70	m	14.5	14.8	14.2	14.2	15	15.1	14.65	14.2	15.05
35	25	f	12.8	12.7	12.9	13	13.1	13.2	12.75	12.95	13.15
36	55	f	13.8	13.9	14.1	14	14	14.1	13.85	14.05	14.05
37	26	f	14.8	14.9	15	14.9	15	15	14.85	14.95	15
38	32	f	14.8	14.5	14.5	14.6	14.8	14.7	14.65	14.55	14.75
39	28	f	12.4	12.1	12	12	12.2	12.5	12.25	12	12.35

40	60	m	5.2	5.2	5.4	5.3	5.3	5.2	5.2	5.35	5.25
41	30	m	18.6	18.6	18.7	18.7	18.6	18.7	18.6	18.7	18.65
42	21	m	15.5	15.4	15.4	15.5	15.4	15.5	15.45	15.45	15.45
43	38	f	4.9	5	4.9	4.9	5	4.9	4.95	4.9	4.95
44	29	m	17.4	16.9	17	16.9	17	17.3	17.15	16.95	17.15
45	27	f	8.2	8	7.9	8.1	7.8	8.3	8.1	8	8.05
46	34	f	12.4	12.4	12.3	12.3	12.4	12.3	12.4	12.3	12.35
47	47	f	13.8	13.6	13.6	13.5	13.5	13.8	13.7	13.55	13.65
48	71	m	6	6	5.9	5.9	6	5.9	6	5.9	5.95
49	35	f	12.3	12.6	12.8	12.4	12.2	12.6	12.45	12.6	12.4
50	22	m	14.3	14.4	14.3	14.4	14.3	14.3	14.35	14.35	14.3
51	30	f	14.5	14.5	14.4	14.5	14.4	14.5	14.5	14.45	14.45
52	33	f	15.9	16.3	16	15.9	16.1	16	16.1	15.95	16.05
53	33	m	17.8	17.5	17.5	17.6	17.6	17.5	17.65	17.55	17.55
54	35	f	12.4	12.4	12.3	12.4	12.3	12.3	12.4	12.35	12.3
55	23	f	15.7	15.7	15.7	15.6	15.6	15.6	15.7	15.65	15.6
56	37	f	16.3	16.1	16	16	16.1	16.1	16.2	16	16.1
57	31	m	14.2	13.8	13.9	13.8	13.8	13.9	14	13.85	13.85
58	36	f	11.9	11.6	11.7	11.3	11.4	11.8	11.75	11.5	11.6
59	24	f	12.5	12.5	12	12.1	12.4	12.3	12.5	12.05	12.35
60	25	m	11.8	11.5	11.1	11.5	11.3	11.7	11.65	11.3	11.5
61	36	f	15.2	15.6	15.2	15.3	15.2	15.2	15.4	15.25	15.2
62	34	f	9.7	9.2	9.1	9.1	9.2	9.3	9.45	9.1	9.25

63	50	f	16	15.9	15.4	15.5	15.8	15.7	15.95	15.45	15.75
64	25	f	14.9	14.7	14.6	14.7	14.6	14.6	14.8	14.65	14.6
65	19	f	13.3	13.4	13.3	13.2	13.1	13.2	13.35	13.25	13.15
66	78	f	14	14	13.8	13.8	13.7	13.8	14	13.8	13.75
67	27	m	6.2	6.7	5.9	6.4	6.5	5.9	6.45	6.15	6.2
68	32	f	10.8	11.1	10.4	10.8	10.9	10.5	10.95	10.6	10.7
69	23	m	13.1	12.8	12.3	12.7	12.5	12.9	12.95	12.5	12.7
70	67	m	17	17.3	16.9	17	16.8	16.9	17.15	16.95	16.85
71	25	m	12.3	12.4	12	12.4	12	12.1	12.35	12.2	12.05
72	40	f	15.7	15.8	15.6	15.5	15.4	15.5	15.75	15.55	15.45
73	21	m	15.8	15.3	15.3	15.2	15.2	15.3	15.55	15.25	15.25
74	27	m	11.1	10.9	10.4	10.8	10.5	10.9	11	10.6	10.7
75	69	f	14.2	13.8	13.6	13.6	13.7	13.7	14	13.6	13.7
76	28	m	5.8	6	5.5	5.3	5.7	5.5	5.9	5.4	5.6
77	72	f	14	14	13.8	13.9	13.6	13.7	14	13.85	13.65
78	68	m	17.3	16.9	16.8	16.9	16.8	16.7	17.1	16.85	16.75
79	32	m	10.3	10.4	10.2	10	9.9	10.1	10.35	10.1	10
80	21	m	16.3	16.2	16	16.1	15.8	15.9	16.25	16.05	15.85
81	40	f	16.7	17.2	16.6	16.6	16.6	16.5	16.95	16.6	16.55
82	48	f	14.3	14.7	14.2	14.2	14.1	14.1	14.5	14.2	14.1
83	31	f	10.5	10.2	9.8	10.2	10.1	9.8	10.35	10	9.95
84	20	m	7.1	7.3	6.8	6.9	6.7	6.8	7.2	6.85	6.75
85	33	m	17	17	16.4	16.5	16.5	16.6	17	16.45	16.55

86	23	m	7.5	7.1	6.7	7.2	6.6	7.1	7.3	6.95	6.85
87	21	m	12.2	12.5	11.8	12.2	11.7	12.1	12.35	12	11.9
88	32	m	7.9	8.3	7.9	7.4	7.9	7.4	8.1	7.65	7.65
89	50	f	15.4	15.1	14.9	15	14.7	14.8	15.25	14.95	14.75
90	16	m	13.8	14.2	13.7	13.6	13.5	13.5	14	13.65	13.5
91	48	f	14.8	14.5	14.3	14.3	14.1	14.1	14.65	14.3	14.1
92	30	f	16.8	16.6	16.4	16.5	16.1	16.1	16.7	16.45	16.1
93	42	m	16.8	16.7	16	16.2	16	16.2	16.75	16.1	16.1
94	23	f	14.7	14.4	14.2	14.1	13.9	13.9	14.55	14.15	13.9
95	25	f	14.9	15.3	14.6	14.7	14.4	14.5	15.1	14.65	14.45
96	35	m	12.4	12.6	11.8	11.6	11.6	11.8	12.5	11.7	11.7
97	37	m	16.2	16.2	15.4	15.6	15.4	15.3	16.2	15.5	15.35
98	27	f	18.2	18.1	17.3	17.3	17.3	17.3	18.15	17.3	17.3
99	23	f	14.7	15.2	14.5	14.4	14.1	14.1	14.95	14.45	14.1
100	23	m	8	8	7.1	7.2	7.2	7.1	8	7.15	7.15
101	26	m	7.4	7.4	6.6	6.5	6.5	6.6	7.4	6.55	6.55
102	21	f	8.4	8.4	7.5	7.6	7.6	7.5	8.4	7.55	7.55
103	24	f	9.5	9.5	8.6	8.7	8.7	8.6	9.5	8.65	8.65
104	35	f	10	10	9.1	9.2	9.2	9.1	10	9.15	9.15
10	37	f	15	15	14.2	14.1	14.1	14.2	15	14.15	14.15

5											
106	28	f	10.1	10.2	9.3	9.3	9.2	9.3	10.15	9.3	9.25
107	28	f	12.5	12.5	11.6	11.5	11.6	11.5	12.5	11.55	11.55
108	24	f	13.9	13.9	13	13	12.9	13	13.9	13	12.95
109	27	f	15.4	15.4	14.4	14.5	14.4	14.5	15.4	14.45	14.45
110	27	m	13.6	13.6	12.7	12.6	12.6	12.7	13.6	12.65	12.65
111	14	f	7.5	7.5	6.4	6.5	6.3	6.4	7.5	6.45	6.35
112	25	f	11	11	10	10.1	9.9	9.8	11	10.05	9.85
113	36	f	14.5	15.8	13.7	13.8	13.8	13.8	15.15	13.75	13.8
114	42	m	13.8	13.8	13	13	13.1	13	13.8	13	13.05
115	50	m	12.7	12.7	11.9	12	12	12	12.7	11.95	12
116	35	f	10	10	9.1	9.2	9.2	9.1	10	9.15	9.15
117	37	f	15	15	14.2	14.1	14.1	14.2	15	14.15	14.15

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: **Mesfin Samuel(B.Sc.)**

Signature: _____

Date of submission: _____

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

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

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.