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Evaluation of Erythrocyte Sedimentation Rate measurement by IRIA automated method and Westergren manual method at Myungsung Christian Medical Center, Addis Ababa, Ethiopia.

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This is to certify that this thesis prepared by Dawit Tsegaye, entitled: **Evaluation of Erythrocyte Sedimentation Rate measurement by IRIA automated method and Westergren manual method at Myungsung Christian Medical Center, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute.
DRERC	Departmental Research and Ethical Review Committee.
EDTA	Ethylene Diamine Tetra AceticAcid
ENT	Ear, Nose and Throat (Otolaryngology)
HIV	Human Immunodeficiency Virus
IQC	Internal Quality Control
IR	Infrared
ICSH	International Committee for Standardization in Hematology.
LED	Light Emitting Diode
LoA	Limits of Agreement
MCM	Myungsung Christian Medical Center
Mm/hr	Millimeter per hour
NCCLS	National Committee for Clinical Laboratory Standards
PI	Principal Investigator
QC	Quality control
RBCs	Red Blood Cells
SD	Standard Deviation
SOP	Standard Operating Procedure
TB	Tuberculosis

Operational Definitions

Trueness of measurement or Accuracy:-Closeness of agreement between the average values obtained from a large series of results of measurements and a true value or closeness of agreement between a measured quantity value and a true quantity value of a measured.

Precision: - Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified condition

Reference Method: - An exactly defined technique that is used in association with an internationally agreed reference preparation to provide sufficiently precise and accurate data for assessing the validity of other methods.

Selected method : - A method approved by a defined authority as being suitable for routine use, taking account of the limits of its bias and imprecision in the context of its intended (clinical) purpose, economy of materials and labor, ease of performance, and safety; its validity must be verified by Clinical And Laboratory Standards Institute(CLSI).

Reliability:-The ability of a system or component to perform its required functions under stated conditions for specific period of time.

Standardized method: -An alternative to a reference method when an appropriate international reference material is not available; this has all the other characteristics of a reference method.

Elevated ESR: Refers to ESR values above the normal range (above 20 mm/hr).

Agreement: Agreement was considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases in the Bland and Altman.

Correlation:- The way of assessing the relationship between variables.

Correlation Interpretation:-r=0-0.2 very poor or very weak,0.2-0.4 poor or weak, 0.4-0.65 fair or moderate,0.65-0.89 strong or good, 0.90-0.99 very strong/high/good and 1 perfect correlation.

Collagen-Vascular Disease: Any of a group of inflammatory, often autoimmune diseases affecting connective tissue and small blood vessels.

Miscellaneous: Consisting of members or elements of different kinds of mixed character.

Abstract

Background: - Erythrocyte sedimentation rate (ESR) is still a widely used hematological parameter as indicator of disease condition. Westergren method is the standard method based on International committee for Standardization in Hematology (ICSH) recommendation for measuring ESR. But nowadays different automated instruments are available with reduced turnaround time, which increase the quality by reducing bio-hazard risk for the laboratory professionals. However, there is no method comparison study conducted in Ethiopia and still the old method is used in many health facilities.

Objective: -Evaluation of Erythrocyte Sedimentation Rate measurement by IRIA automated method and Westergren manual method at Myungsung Christian Medical Center, Addis Ababa.

Method: -A prospective cross sectional study was conducted at Myungsung Christian Medical Center from February to May 2017 GC. By doubling the ISCH recommendation the samples were tested in 3 different groups of values and the total sample size was 240. Every ESR ordered sample was analyzed using both manual and automated methods. Result was entered and analyzed using SPSS version 20. Linear regression, correlation and Bland Altman analysis were carried out to determine agreement between the reference and the new method was carried out. Agreement was considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases in the Bland and Altman plot.

Result: The comparison between the conventional Westergren and IRIA automated method showed very good correlation in the two methods and agree well for ESR level between 1-20 mm/hr with r=0.934, Mean bias 0.663, SD 2.629,LoA(5.815, -4.490), and ESR level between 21-60 mm/hr r=0.917, Mean bias 0.1875, SD 3.7924 LoA (7.621,-7.246). However, for ESR level greater than 60, it showed good correlation r=0.890 but poor agreement in the Bland-Altman analysis as the mean difference is lying between the limit of agreement for less than 95% of cases Mean difference 1.388, SD 11.346,LoA (23.625,-20.849).

Conclusion: For ESR level below 60 mm/hr, the two instruments can be used interchangeably. However, for ESR level greater than 60mm/hr the result of the automated machine must be confirmed with the conventional westergren method before using it for clinical propose.

Key words: - Erythrocyte Sedimentation Rate, Westergren, IRIA, ESR automated

1. Introduction

1.1. Background

The erythrocyte sedimentation rate (ESR) is also known as the Biernacki reaction due to its inventor of a polish physician Edmund Biernacki in 1897 (1) and published in polish then in 1918 Swedish pathologist Robert Fahraeus presented the results of his analyses of the differences in erythrocyte sedimentation rates in pregnant and non-pregnant women, seeing the test as a possible indicator of pregnancy. Whereas ALF Vilhlm Westergren, a Swedish internist, published his observations of erythrocyte sedimentation in patients with pulmonary tuberculosis in 1935. In 1973 the international committee for standardization in hematology (ICSH) recommended the use of the Westergren method as standard for ESR test (2,3). The erythrocyte sedimentation rate (ESR) is a simple and inexpensive laboratory test. It is commonly used to assess the acute phase response in pathological abnormality and indicate the activity of previously diagnosed condition (4, 5).

ESR has a long standing history and one of the most widely performed laboratory test used in clinical practice as an indicator of disease condition. It measures the settling of erythrocytes in human plasma over a specified time, a typical sigmoid curve. The closed manual ESR measurement delivers the one hour Westergren value after 60 minutes and the two hour Westergren value after 120 minutes. Three phases can be distinguished in the sedimentation process. These Phases are the lag or aggregation phase which reflects the period at which the individual erythrocytes form rouleaux; still there is little sedimentation. The second phase decantation or precipitation phase where the plasma-red cell interface falls more rapidly (increasing sedimentation). In the third phase or packing phase the red cell aggregates pile up on the bottom of the tube or container and sedimentation slows down as a result of mutual interference of the closely packed aggregates. Recently several test methods have been developed, which improve the safety and result reliability of ESR testing procedures (6,7).

The Erythrocyte Sedimentation Rate test is an old but still widely used test and it also has an important role in temporal arthritis, polymyalgia rheumatica, rheumatoid arthritis and myeloma. It have new potential applications in some conditions, such as coronary heart disease, prostate

cancer and osteomyelitis. This test is only one parameter which can be useful in the diagnosis and follow-up of certain inflammatory conditions, and it can also be a prognostic tool in non-inflammatory diseases. The ESR increase for various infectious diseases, infarctions, malignancies, anemia and autoimmune diseases (8,9).

ESR is known to lack specificity and sensitivity. However ,if used correctly and as an adjunct to a good clinical history and physical examination, it plays an important role in clinical practice (10). There are many methods to measure ESR; among those Westergren, is one of oldest methods and currently the most useful method to measure ESR (11). But this method is not very practical in routine analysis because of a longer period required for the results and a higher volume of blood needed. There are also a number of causes of an elevated ESR including vibration of the ESR tube; the tube being non-vertical, temperature and inadequate anticoagulation with clotting of blood sample and others (12).

Technical innovations and semi-automated instruments were introduced day to day to eliminate or decrease the risk of exposure of laboratory workers to potentially infectious material, like blood. The new automated techniques are considered less hazardous, primarily because they are either self-contained or use disposable materials, or both. This new innovation needs to examined both for comparability of results to previously employed methods and to ensure, on an ongoing basis, the quality assurance of the results (13). The new marketed automated instruments should show good correlation to Westergren reference method. Additionally, advantages like safety for operators, reduced turn-around time and reduced analytic imprecision and improvement of working analytical techniques, laboratory workflow, biohazard and standardization are among the added benefits (14).

The traditional Westergren method is not generally used in routine laboratories nowadays, except in some developing countries. Many new technologies and analyzers have been developed for measurement of the ESR. Some of these involve automation of the Westergren method with diluted or undiluted samples while others use very new technologies. The latter tend to use undiluted EDTA samples for ease of use, economy, practicability, closed sample manipulation and speed. The systems that give the results as Westergren method with diluted blood at 60 min or normalized to 60 min as recommended by ICSH-1993 are the only ones that have clinical value. It is important to recognize that the Westergren method is a specific test for the ESR.

Other equivalent tests must establish their own normal reference ranges and levels of clinical utility, sensitivity and specificity. All new technologies, instruments or methodologies must be evaluated against the Westergren reference method before introduction into clinical use (15,16).

Ethiopia is one of the developing countries and we still use the traditional Westergren method in many public and private hospitals.

1.2. Statement of The Problem

According to ICSH, the Westergren's method is suggested as gold standard technique for measuring ESR (15). However, it has many disadvantages like anticoagulant, cleanliness, position, time of the result, vibration and mixing of specimens can affect the result and can causes falsely raised result. The bio hazard or safety issue for the laboratory personnel is also high. On the other hand automated Analysis provides faster results with maintaining the laboratory safety by minimizing contact with blood samples while there is no external influence on the final reading such as temperature, contaminating dust particles, tilting of tube, and ratio of diluents. Many new automated systems have been introduced and have been evaluated for performance with each other as well as with the gold standard Westergren's method (1). Those automated techniques offer more benefits in terms of reduced biohazard risks, speedy processing time, and quicker results, so it is essential to compare this equipment against the standard Westergren's method before using it for clinical purpose.

In Ethiopia as far as my knowledge goes, there is no research conducted on method comparison study on ESR manual and automated method while some of private and public hospitals use automated ESR technique in addition to the widely practiced manual Westergren method. The traditional Westergren method has limitations like the result being not accurate and reliable which will compromise the diagnosis and hence be a challenge for clinicians to rule out appropriate clinical diagnosis. The Myungsung Christian Medical Center (MCM) is also one of private specialized hospital in this country that gives service for many patients every day. The hospital is among the few health facilities which have automated ESR. However, no method comparison was performed when the automated IRIA ESR was introduced in the hospital, in contrary to the international recommendation (16).

This study compared the IRIA linear which is present in MCM with the gold standard reference Westergren Method to fulfill the gap of ISCH recommendation.

1.3. Significance of the study

The Finding of this study will provide necessary information in our country about ESR method comparison of new automated ESR method against the existing Westergren method. It helps for the policy makers adopt this method for public clinical use as a standard ESR method and improving of working analytical techniques and standardization.

Specifically the study finding will improve the quality of the laboratory service by implementing internal quality control (IQC) service of ESR test and in the MCM thus increased customer satisfaction by having good quality, accurate, reliable and timely result for patients as well for clinicians. It also helps to fulfill the ICSH requirement of all new ESR technologies, instruments or methodologies must be evaluated against the Westergren reference method before introduction into clinical use.

Finally the study finding will help to improve the safety of laboratory professionals who are performing ESR by minimizing exposure to blood, and decrease test running time as it will encourage other facilities to introduce the new automated ESR technology. This study will also provide reliable guidance for researchers who want to undertake related study in the future; they can use it as base line information of ESR method comparison.

2. Literature Review

A study conducted in Finland Tampere University Hospital in 2010 by Horstia J *etal,* compared the StaRRsed Auto-Compact Erythrocyte Sedimentation Rate instrument and the Westergren method. They included 200 samples. They collected blood with the K_2-EDTA anticoagulant. The intra-assay CV% was 13.0 % (n = 6) in the classic Westergren method (mean 6.0 mm/h) and 0.0% (n = 6) in StaRRsed method (mean 5.0 mm/h). The inter assay CV% was measured by using a commercial SEDRite Plus control and it was 6.1% (n =28) in StaRRsed. The overall correlation coefficient was 0.72 (y = 1.066 x – 0.24, 95% CI: intercept -1.137 to 0.966 and slope 0.980 to 1.166). There were non-linear relationships between the two methods (P value < 0.01). Statistically significant differences were found between ESR values in some samples. With the ESR results over 11 mm/h, 27.5%samples, showed a difference of more than 30% while for healthy controls 12.5% of the results vary. Overall, 24 samples showed increased ESR on StaRRsed while only one had increased ESR on the classic Westergren method. Finally they concluded that ESR determination could lead to different clinical decisions, depending on which instrument was used (17).

In 2010 a similar hospital based study was done in Croatia, by Perovic E *etal,* to Evaluate Ves-Matic Cube 200 an automated system for the measurement of the erythrocyte sedimentation rate on a total of 251 patient samples. They used the K_2 and K_3 EDTA anticoagulant blood sample. Mean ESR value measured with Ves-Matic Cube 200 method was 18.90 mm/h, (95% CI for the mean was 16.28–21.52 mm/h) and they found no significant difference from those measured with the Westergren method (mean was 19.38 mm/h; 95% CI for the mean was 16.89–21.86 mm/h). The mean difference between the two methods was 0.47 mm/h,95% CI for the mean was -0.37 to 1.32 mm/h; P = 0.2734. They obtained Spearmen's rank correlation coefficient of 0.946 (P < 0.001). The obtained linear regression equation was Y=0.0435 + 1.0435*X. In conclusion, the agreement between results obtained by Bland–Altman analysis showed there was no evidence of systemic bias (bias = 0.5) and limits of agreement were 13.0 to 12.9 mm/h (18).

Similar study conducted in Catania hospital in Eindhoven by Curvers J *etal,* 2010 evaluated the accuracy of ESR results using Ves-Matic Cube 200 Erythrocyte sedimentation method with Westergren-based method according to the ICSH protocol using 244 randomly selected patient

samples and they used the EDTA anticoagulant blood sample. The SEDIsystem (n = 92) was compared with the Westergren method. The Passing-Bablok regression analysis show a strong correlation between SEDIsystem and Westergren measurements (*r*=0.96; 95% CI, 0.94-0.97) and no evidence of systemic bias was noted. StaRRsed sedimentation(n = 50) also showed a strong correlation with westergren reference method (r=0.96; 95% CI, 0.93-0.98). The Pearson correlation coefficient between the Ves-Matic Cube 200 and the Westergren reference method was only r=0.83 (95% CI, 0.76-0.88). This poorer correlation is reflected in a negative bias (–5.7) with considerable 95% limits of agreement. Of the 77 samples with an ESR of more than 20 mm/h in the Westergren method, 52 had ESR values obtained with the Ves-Matic Cube 200 that deviated more than 20% from the reference method. In conclusion, The SEDIsystem and StaRRsed Westergren-based methods showed good correlation with the Westergren reference method and an acceptable bias over the entire range of ESR values. The Ves-Matic Cube 200method showed poorer correlation with the reference method, which was mainly caused by a considerable negative bias at low ESR values and a more random bias at higher ESR levels (19).

In 2015 a Comparative study was conducted in Turkey by Bogdaycioglu N *etal,* on two automated systems iSED and Ves-Matic Cube 200 Erythrocyte Sedimentation Rate measurements with Westergren Method. A total of 136 samples they used and collected with EDTA anticoagulant. The iSED sedimentation method showed a poor correlation with the Westergren method (*r*=0.76, *P* <0.0001). According to Bland–Altman analysis, this resulted in a mean bias of 13 (95% limit of agreement between −35.7 and 61.6). On the other hand, the Ves-Matic Cube 200 method showed a moderate correlation with the Westergren method (*r*=0.84, *P* <0.0001). According to Bland–Altman analysis, this resulted in a mean bias of 1.4 (95% limit of agreement between −34.4 and 37.2). They further divided the results (*n*=136) into subgroups according to the Westergren ESR levels as <20, 20–80, >80mm/hr (low, medium, high levels, respectively). The iSED sedimentation method showed poorer correlation with the Westergren method at increased ESR levels (low levels *r*=0.67, medium levels *r*=0.48, high levels *r*=0.27). They concluded that the iSED analyzer cannot be used interchangeably with the Westergren method because of its poor correlation and high bias. The Ves-Matic Cube 200 showed a better but moderate correlation with the Westergren method and had a low bias (20).

A study conducted by Subramanian A *etal,* in India on 200 samples demonstrated a marked discrepancy between the reference Westergren and the Monitor 100® automated methods both for ESR determined within one and two hours after sample collection with EDTA anticoagulant. Out of 200 samples, 79 samples were within the reference range used in their hospital (0-25 mm/hour), while 121 samples had higher ESR values of more than 25 mm/hour. The mean difference between the two methods and 95% limits of agreement at 1 hour was found to be -11.2±35.1 (95% limits of agreement, -46.3 to 23.9). This variation was particularly evident for samples with high ESR readings greater than 25mm/hour. Hence for samples with high manual ESR values, the mean difference was estimated to be -13.4 and the limits of agreement was (-57.3, 30.5) which was markedly different from the corresponding values -7.7 mean of difference; -18.9 to 3.5 limits of agreement for ESR values less than 25 mm/hour. In conclusion, on comparing the manual and automated methods for measuring ESR, marked discrepancy in the ESR results was noted for high ESR values. However, this was not evident for normal ESR values (21).

Suad M *etal,* from Kuwait evaluated erythrocyte sedimentation rate measurement by the automated SEDIsystem TM and the conventional Westergren method in 2005 using 150 samples collected with K_3-EDTA anticoagulant. Pearson's regression analysis between the values of the reference method and the mean of the three runs of the new method showed a good correlation (r=0.91). The mean of the differences between the two methods was –13.18 mm/h. The limits of agreement between the two methods were 11.52 and –37.88. The correlation (r) between the difference and the mean of the two methods was found to be 0.78. The discrepancy was particularly evident in samples with high ESR values (25 mm/h). Thus, for samples with ESR readings > 25 mm/h, the mean of difference (–21.4) and the limits of agreement (–45.2 and 2.26) were markedly different from the corresponding values (–3.9;–13.5 and 5.7, respectively) for samples with ESR values under 25 mm/h. It is apparent from these results that samples with high ESR values varied considerably around the mean difference compared with samples that had normal ESR readings. They concluded that if the SEDIsystem is to replace the Westegren manual ESR method, a correction factor that will correct for the underestimation must be used (22).

A study was conducted in 2011 on Erythrocyte sedimentation rate measurement by VES Matic Cube 80 in relation to inflammation plasma proteins by Cerutti H *etal,* in Italy on a total of 248 samples used and they collected with K_2 and K_3 EDTA anticoagulant. The study reported a mean±SDESR of 23.1±20.9mm/hr (range: 1–110 mm/hr) for the reference method and 21.86±22.1mm/hr (range:1–90mm/hr) for VES Matic Cube 80. There was a significant correlation between Westergren and VES Matic Cube 80 measurements. The same results were also obtained considering the Spearman's rank correlation coefficient (r), which presents a value of 0.951 (95% CI: 0.937–0.961) with a P value of 0.000. The agreement between the results was obtained by different methods was also demonstrated by Bland–Altman analysis. It showed a positive mean bias, equal to 1.2 mm/hr (limits of agreement, 17.4–19.9), indicating that the test method values were slightly lower than the Westergren ones. In conclusion, VES Matic Cube 80 analyzer showed good correlation with Westergren Measurement (23).

All in all, the literature reviewed above demonstrated different level of agreement between various automated ESR measurements methods as compared to the gold standard Westergen method with apparent variability at high ESR values. However, as far as my knowledge goes, there is no published research in Ethiopia. Besides, no research is conducted on method comparison between IRIA automated method though all automated instrument were compared to conventional Westergren method. The current study tried to fill this gap.

3. Objectives

3.1. General objective

To Evaluation of Erythrocyte Sedimentation Rate measurement by linear IRIA automated method and Westergren manual method at Myungsung Christian Medical Center, Addis Ababa, Ethiopia from February to May 2017 GC.

3.2. Specific objectives

- To calculate the correlation between Westergren's and linear IRIA automated methods.
- To determine the agreement of ESR result between manual Westergren and linear IRIA automated method by ESR categories (1-20, 21-60 and >60 mm/hr).

4. Hypothesis

It is hypothesized that there is no significance difference between conventional Westergren ESR method and IRIA automated ESR method.

5. Materials and Methods

5.1. Study Area

This study was conducted at MCM which is located in the southeastern part of Addis Ababa in Bole kefele ketema around Gerji. Historically, the establishment of this hospital is related to Ethiopians support to Korea at time of war In 1951. Ethiopia was the only country to send its troops to the Korean War by Ethiopian Emperor, Haile Selassie. Out of 6,037 Ethiopian soldiers, 123 died, to repay that kindness, the MCM hospital established through the grace of God by Ethiopian government request 1993, after that a lot of activities performed such as building construction of the hospital, the guest house, the medical college and creating partnership to others universities. In 2012 Myungsung Medical College with a 6 year curriculum including internship opened and started to train health professionals with character and qualification in accordance with the international standards.

One of the hospital missions is to play a central role in the fields of medicine and education, following in the footsteps to severance of Hospital in Korea. The hospital provides free medical care service like cardiac surgery and others with collaborated partnership. The service is not limited giving at one place rather through free mobile clinic service for in particular location of Ethiopia.

MCM compound is consisted of two wings; Shalom Wing with the capacity of 161 bed facility; (40) surgical, (25) medical, (20) pediatrics, (7) Obstetrics and gynecology, (10) Emergency Room and (11) Intensive Care and Grace Wing with the capacity of 67 beds. MCM offers ophthalmologic, dental, plastic surgery, ENT, psychiatry and hemodialysis services as part of community health programs, in addition to general medicine, pediatrics and obstetrics and gynecology. Long term expatriate staff include one American Family Medicine Doctor, 4 Korean-American physicians (general surgeon, anesthesiology, radiology and pathology), one Norwegian plastic surgeon, and one Korean dentist. There are approximately 420 staff form those 35 Koreans, 22 form third world, 362 Ethiopian from those 45 GPs including Hematologist and other specialists. The laboratory is also well organized with advanced medical equipment and has parasitological and urinalysis, bacteriology, clinical chemistry, serology, hematology and immunohematology department (24).

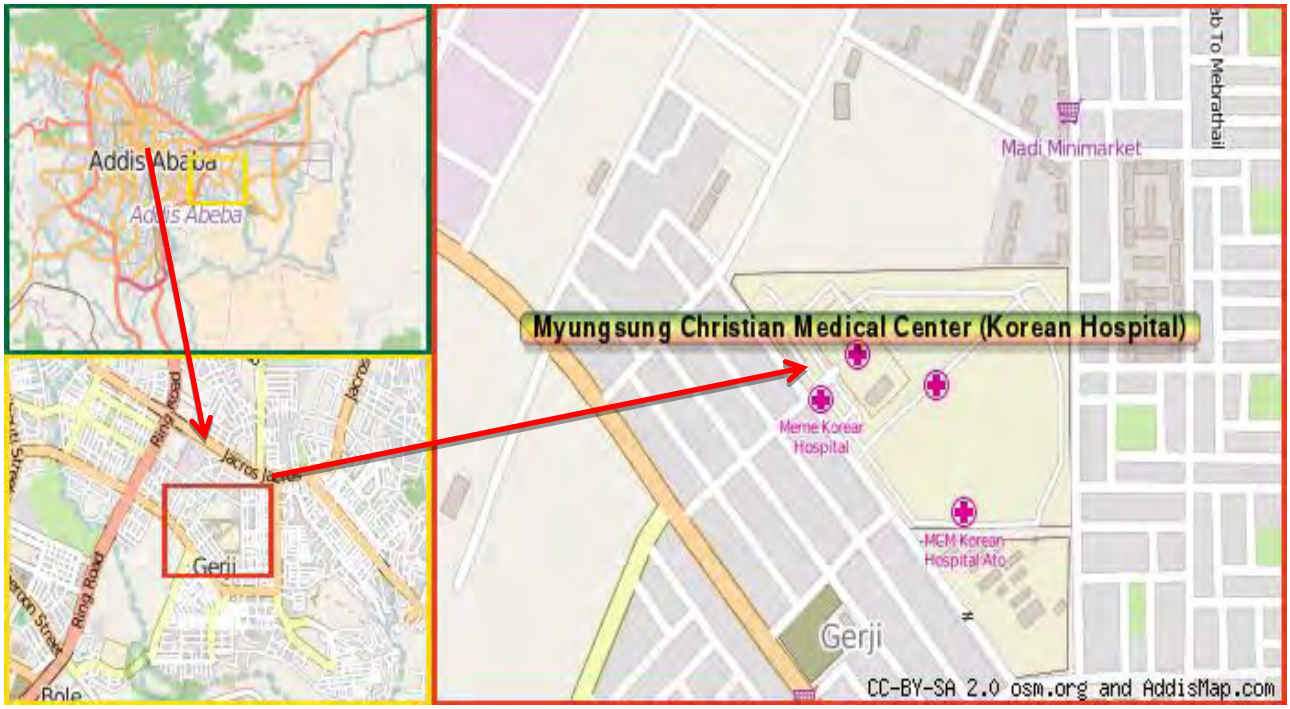

Figure 1: Location of Myungsung Christian Medical Center (MCM) in Addis Ababa, Ethiopia.

Figure 2: Top view Myungsung Christian Medical Center (MCM) Addis Ababa(25).

5.2. Study Design and Period

A hospital based cross sectional study was conducted at Myungsung Christian Medical Center (MCM) Addis Ababa starting from February to May 2017 GC.

5.3. Population

5.3.1. Source Population

The source population ware those patients who visit Myungsung Christian Medical Center (MCM) laboratory during the study period.

5.3.2. Study Population

The study population was those who have ESR examination order in the study period.

5.4. Inclusion

5.4.1. Inclusion Criteria

- All volunteers whose blood samples are within the acceptable criteria of the laboratory sample collection and Rejection SOP.

5.5. Study Variable

5.5.1. Dependent Variable

- Agreement between manual and automated ESR
- Correlation between manual and automated ESR

5.5.2. Independent Variable

- Conventional Westerngren and IRIA instruments
- ESR levels (Low, Normal, High)
- Sex, and Age

5.6. Measurement and Data Collection

5.6.1. Sample Size Determination

By doubling the ICSH sample size recommendation for ESR method evaluation with conventional Westergren method test system and automated method a minimum of 80 samples are tested in three different groups of values: 1–20, 21–60 and more than 60 mm/hr involving

wide variety of diseases and with ESR results distributed evenly (14). Thus a total of 240 samples, 80 from each category, were utilized for parallel testing on the two methods.

5.6.2. Sampling Method

Convenient sampling technique was used to select study participant presenting to MCM laboratory with ESR test request

5.6.3. Laboratory Analysis

5.6.3.1. Conventional Westergren Method Principle

The basic principle of the ESR is that when anti-coagulated blood is placed in a vertical column, the RBCs normally settle quite slowly. This occurs for two main reasons; the first one is RBCs repel each other due to the negative charges on their surfaces, or zeta potential, and the second one is large surface-area-to-volume ratio of normal RBCs resists settling.

The aggregation of RBCs into rouleaux, which happens slowly under normal conditions, markedly accelerates sedimentation by decreasing the surface-area-to-volume ratio. Various factors will affect the ESR, such as the size and shape of the red cells, plasma fibrinogen, and globulin levels, as well as mechanical and technical factors. Conditions that promote the formation of rouleaux produce an elevated ESR. The ESR expresses in mm per hour reflecting the rate at which red blood cells settle when anti-coagulated blood is allowed to stand in a narrow tube (Westergren) (6).The detail standard testing procedure is found in the Annex.

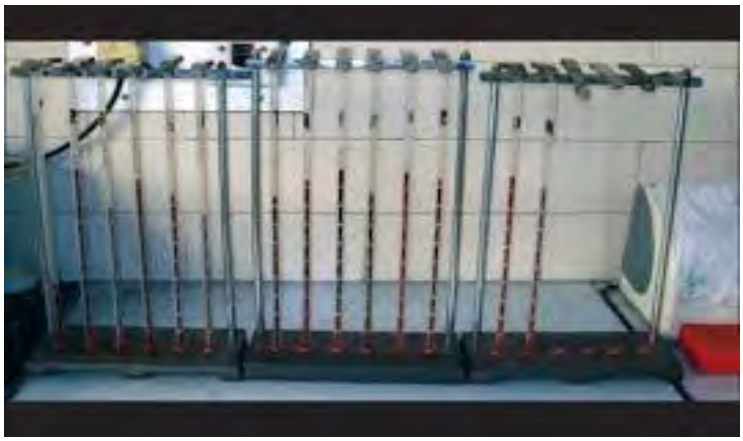

Figure 3. Westergren ESR Instrument

5.6.3.2. IRIA Automated ESR Method Principle

IRIA is a random access auto analyzer to determine the ESR, capable of processing up to 5 samples simultaneously by means of an Infrared (IR) Light Emitting Diode (LED) optical system. This technique requires a minimal handling of the sample, as the test is performed directly on the test-tube, admitting both open and vacuum tubes then spill 1.5ml blood using the ESR tubes (the instrument only works with tubes machine manufacture because they have a special dimension). Then the ESR tube is agitated/mixed(around 5 minutes) and plugged into the instrument. The instrument detects immediately the sample and start to read it. After 17 minutes, the result is displayed on the result screen for the 1st and 2nd hour at the same time. As it is a Random access instrument it is capable to process 25 samples per hour, with uninterrupted sample loading, as test positions are left free (26). The detail standard operating procedure is found in annex.

Figure 4. ESR Analyzer IRIA (Linear, Spain)

5.7. Data Quality Assurance

5.7.1. Pre-analytical Phase

In the pre-analytical phase proper sample collection handling and transporting SOP was strictly followed to ensure the quality. Starting from proper patient/client preparation, proper patient identification, briefly explaining about the tests and why they participate in this study, proper sample collection and labeling. Finally by checking the rejection criteria whether it fulfill or not, the sample was transferred to analytical Hematology department.

5.7.2. Analytical Phase

In the analytical phase before the test is performed the daily quality control were done; in this case we used the fresh whole blood sample according to National Committee for Clinical Laboratory Standards (NCCLS) recommendation (6,27,28).When performing the ESR in Westergren and IRIA automated method, the analysis procedure was strictly followed as per the manufacture's instruction and standard operating procedure. Any disturbance around the testing area like vibration was avoided and the room temperature was monitored. The quality control has been neglected and considered unimportant for test such as ESR (28). Fresh blood samples quality control for the measurement of ESR is feasible, inexpensive, and easy to perform (29) so that the sample were analyzed each day after quality control sample running. The quality control sample were obtained from fresh patient sample with PCVs 0.35 or less or grater 0.35 PCV by adjusting packed cell volume. In this study fresh samples were used within two hours of collection.

5.7.3. Post-Analytical Phase

Finally all the results were checked and recorded appropriately on the registration work sheet of the two test methods daily then transferred in two registration book and computer.

5.8. Data analysis and Interpretation

Every ESR sample was measured by both methods manual and automated method and result was entered and analyzed using SPSS version 20. Patients were divided in to three groups on the basis of the ESR values obtained by Westergren method: Group1: ESR 1-20;Group2: ESR 21-60; Group 3: ESR more than 60 mm/hr. Means and standard deviation of results obtained from manual and automated methods were compared in all samples and in the three groups using independent t test; p values Value of <0.05 was taken as statistically significant. Linear regression analysis was employed to analyze the correlation coefficient(r) between the reference and the new method and presented on regression graph. In the comparison, 95% of the differences should be 5 mm/hr or less (16), with larger differences associated with higher ESR values. The Bland and Altman plot was used to assess agreement between the two methods. In the Bland Altman method the differences in ESR values between the two methods was plotted against mean values. Agreement was considered acceptable when the difference is lying between mean $\pm$ two standard deviation (Mean$\pm$1.96SD) for 95% and above of cases(29).

5.9. Ethical Considerations

Before starting the research work, ethical clearance was obtained from the Addis Ababa University, College of Health Sciences, School of Allied Health Science, Department of Medical Laboratory Sciences, Department Research and Ethical Review committee (DRERC). A formal letter of cooperation was sent to MCM. The study aim, benefit, risk and right for withdrawal anytime from the study was explained to the study participants and informed consent was obtained. Samples were coded by continuous and unique code number and confidentiality of patient data was maintained throughout the study.

5.10. Dissemination of The Result

The final finding of this study will be presented and submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Science, Department of Medical Laboratory Sciences to serve as a reference material in the library, for MCM to serve as a reference material for other instruments method comparison, and for the respective hospital having the same analyzers. Finally the study finding will also be disseminated and presented at local and international workshops and seminars. Manuscript will be submitted to peer reviewed journals for possible publication.

6. Result

6.1. Demographic and Clinical Characteristics of The Study Participants

A total of 240 samples were collected within ICSH recommendation. From this 43(53.8%) females and 37(46.3%) males were enrolled in the category of ESR value 1-20 mm/hr. For ESR level from 21-60mm/hr41(51.3%) females and 39(48.8%) males and for ESR level greater than 60 mm/hr41(51.3%) males, 39 (48.8 %) female were included as summarized in Table 1.The age range of the study participants was between 18 and 93 years and the range of ESR level was from 1 up to156 mm/hr.

Table 1. Sex frequency, percent and Age (mean, SD) distribution of 240 participants with in the ESR level categories at Myungsung Christian Medical Center Addis Ababa, February to May, 2017

ESR Level	Sex	Frequency	Percent	Age Mean (SD)
1-20 mm/hr (n=80)	Female	43	53.75	44.8 (15.7)
	Male	37	46.25	
21-60 mm/hr (n=80)	Female	41	51.25	47.0(17.1)
	Male	39	48.75	
>61 mm/hr (n=80)	Female	39	48.75	45.6(17.0)
	Male	41	51.25	
Total		240		

The total samples were categorized in to three ESR levels. We also categorized the disease in to two as infectious and noninfectious by the three ESR levels as shown in Table 2 to 4. In the first ESR level from 1-20 mm/hr the infectious disease rate was 15.0% of which Pneumonia was the highest accounting11.25%, Tuberculosis 2.5% and HIV accounted 1.25%. The large proportion had noninfectious diseases accounting 85.0% of the diseases occurred; from those 21.25% had

collagen-vascular disease (rheumatoid arthritis, osteoarthritis and arthritis) and next to that chronic disease (Diabetic mellitus, hypertension and liver cirrhosis) were the dominant other than those with miscellaneous diseases (Table 2).

Table 2. Disease Category By ESR Level 1-20 Mm/Hr In The Study Participants At Myungsung Christian Medical Center Addis Ababa, February To May, 2017.

Disease category	Types	Frequency	Percent	Total percent
Infectious	HIV	1	1.25	
	Pneumonia	9	11.25	15%
	Tuberculosis	2	2.5	
Non infectious	Collagen-vascular	17	21.25	
	Neoplastic	5	6.25	
	Gastrointestinal	4	5.0	85%
	Renal	4	5.0	
	Chronic disease	10	12.5	
	Miscellaneous	28	35.0	
Total		80	100	

In the group with ESR level of 21-60 mm/hr, 17.5% had infectious diseases from those the highest was Tuberculosis 8.75%.Whereas from noninfectious was chronic disease account for 25% as shown in Table 3. For ESR level greater than 60 mm/hr, infectious disease percentage was 22.5% of which Tuberculosis was again the highest with 11.25%. Similarly, for noninfectious again chronic disease account the highest with 33.75% as displayed in Table 4.

Table 3. Disease Category By ESR Level 21-60 Mm/Hr In The Study Participants At Myungsung Christian Medical Center Addis Ababa, February To May, 2017.

Disease category	Types	Frequency	Percent	Total percent
Infectious	HIV	2	2.5	
	Pneumonia	5	6.25	17.5%
	Tuberculosis	7	8.75	
Non infectious	Collagen-vascular	4	5.0	
	Neoplastic	4	5.0	
	Gastrointestinal	4	5.0	82.5%
	Renal	9	11.25	
	Chronic disease	20	25.0	
	Miscellaneous	25	31.25	
Total		80	100	

Table 4. Disease Category By ESR Level >60 Mm/Hr In The Study Participants At Myungsung Christian Medical Center Addis Ababa, February To May, 2017.

Disease category	Types	Frequency	Percent	Total percent
Infectious	HIV	2	2.5	
	Pneumonia	4	5.0	
	Tuberculosis	9	11.25	22.5%
	Malaria	1	1.25	
	Typhus and Typhoid	2	2.5	
Non infectious	Collagen-vascular	7	8.75	
	Neoplastic	11	13.75	
	Gastrointestinal	5	6.25	77.5%
	Renal	6	7.5	
	Chronic disease	27	33.75	
	Miscellaneous	6	7.5	
Total		80	100	

6.2. Comparison of IRIA Automated ESR with the conventional Westergren manual ESR method.

Correlation coefficient, linear regression and Bland-Altman statistical data analysis methods were employed to see the association and agreement in each level of ESR category. A total of 80 patients samples were analyzed in ESR level 1-20 mm/hr; the linear regression analysis showed very good correlation r=0.934, as depicted in Figure 5. The Bland-Altman plot for the difference against ESR values obtained with Westergren method and IRIA automated method against the Mean of the two ESR methods. The mean difference was 0.663 with SD 2.629and LoA(5.816, -4.490) *p*=.000 indicating the agreement is acceptable because the difference is lying between LoA for more than 95% of cases is shown in Figure 6.

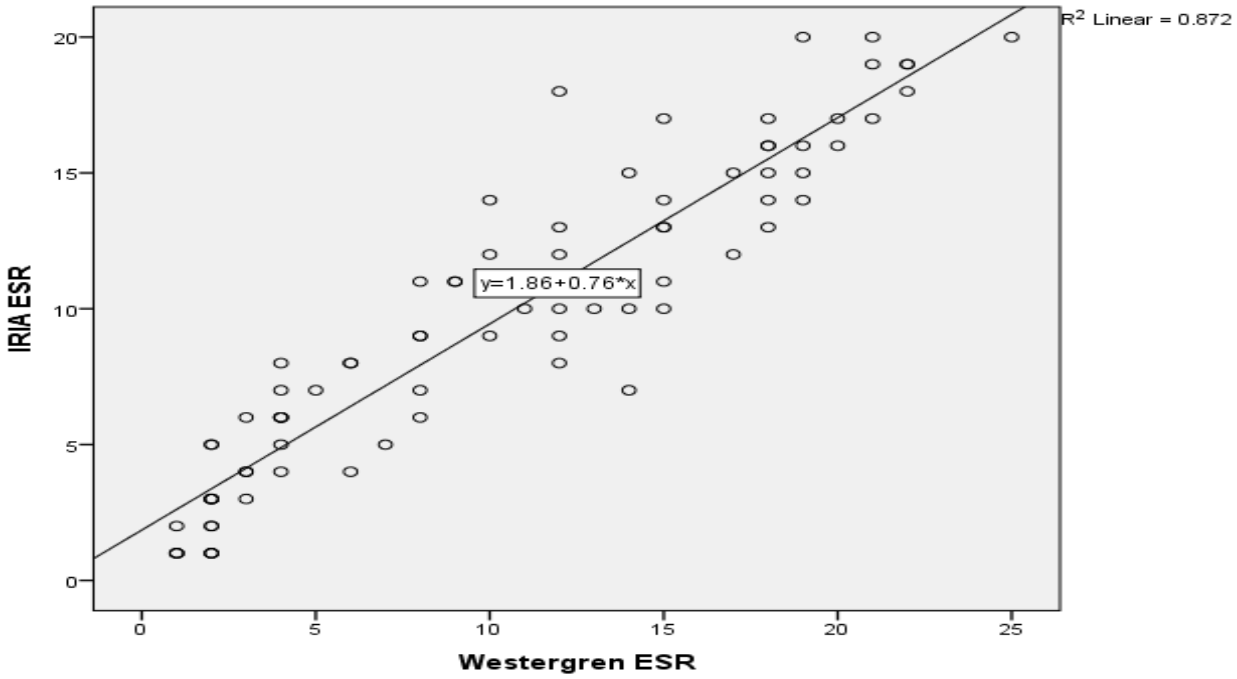

Figure 5. Erythrocyte Sedimentation Rate (ESR) Regression Line Between Standard Westergren And IRIA Automated ESR Methods For ESR Level 1-20 Mm/Hr (N=80).

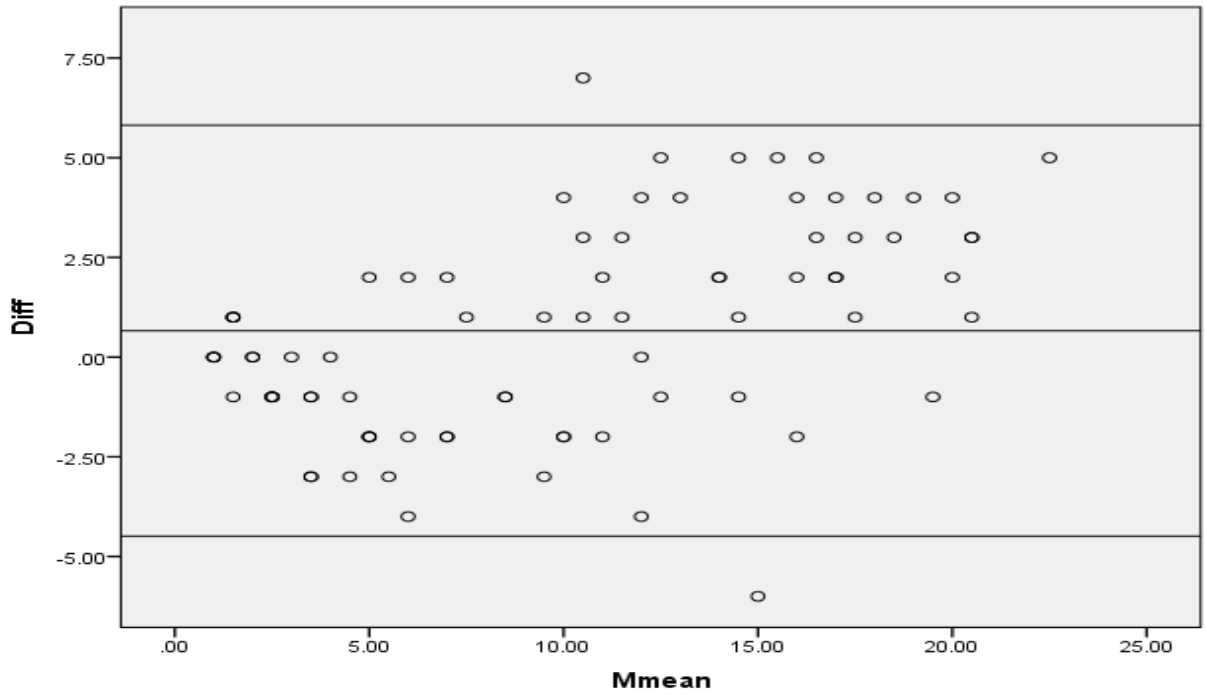

Figure 6. Bland-Altman Plot For The Difference Against Mean By The Two Methods For ESR Level Of 1-20 Mm/Hr (N=80).

On the second ESR level of 21-60 mm/hr a total of 80 patients' samples were analyzed and showed very good correlation r=0.917, as shown in Figure 7.The Bland-Altman plot for the difference against Mean of the two ESR methods revealed a Mean of 0.1875 with SD 3.7924 and LoA (7.621,-7.246) *p*= 0.040. Thus, the agreement between the two methods is acceptable because the difference is lying between LoA for more than 95% of the cases show in Figure 8.

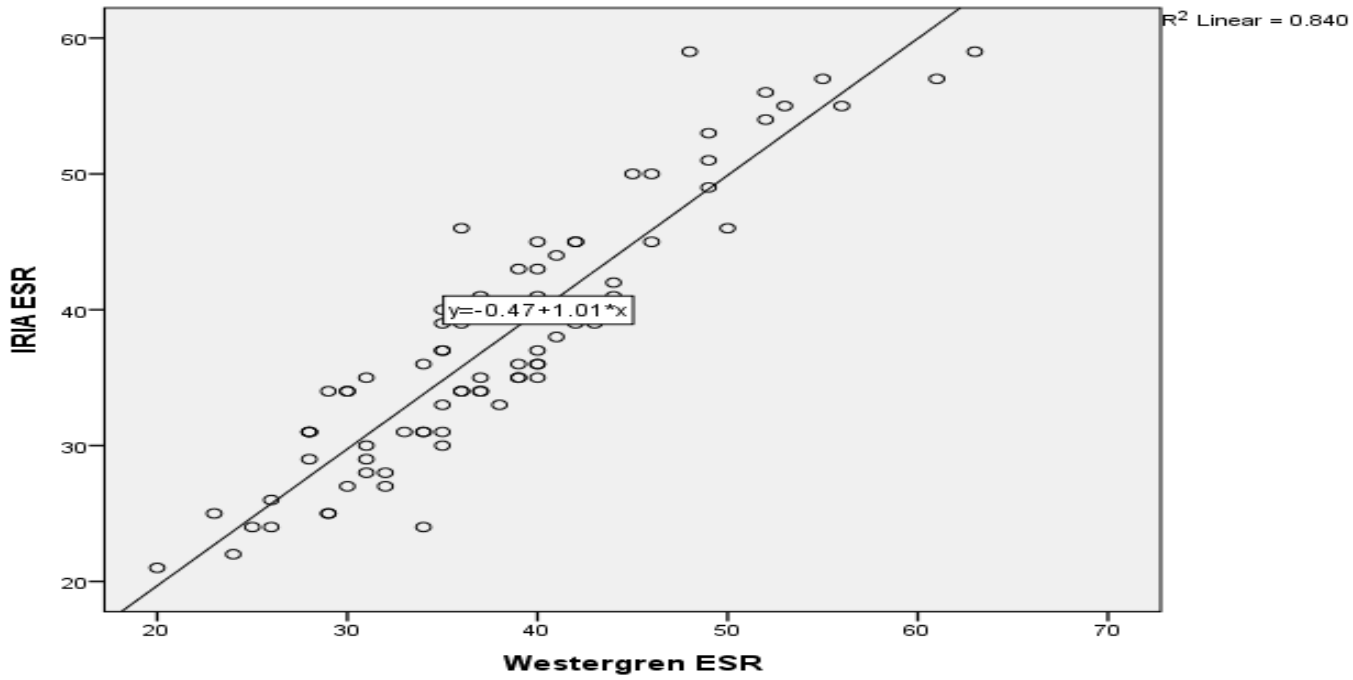

Figure 7. Erythrocyte Sedimentation Rate (ESR) Regression Line Between Standard Westergren And IRIA Automated ESR Methods For ESR Level 21-60 Mm/Hr (N=80)

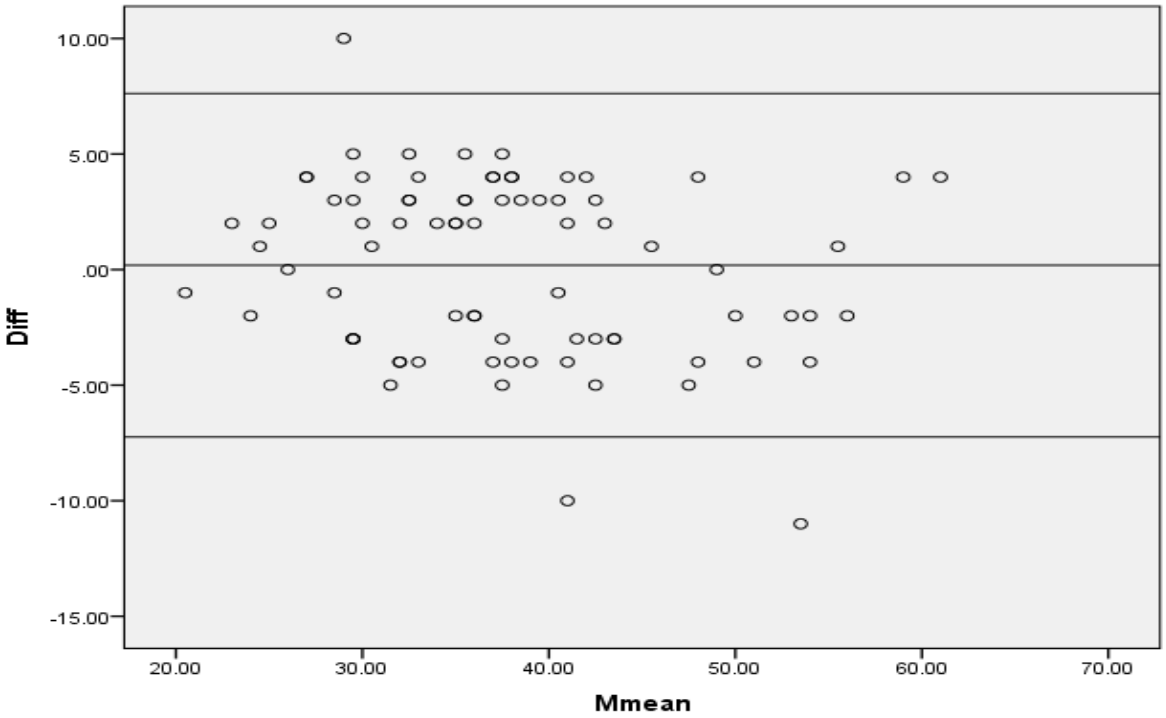

Figure 8. Bland -Altman Plot For The Difference Against Mean By The Two Methods For ESR Level Of 21-60mm/Hr (N=80).

When analysis was done for the group with ESR level greater than 60, there was a good correlation of r=0.890, as shown in Figure 9. However, the Bland-Altman plot for the difference between the two methods against Mean of ESR value by the two ESR methods revealed Mean 1.388, SD 11.346 and LoA (23.625,-20.849) *p*=0.584. This indicates poor agreement and it is not acceptable for clinical purposes because the difference is lying between LoA for less than 95% of cases show in Figure 10.

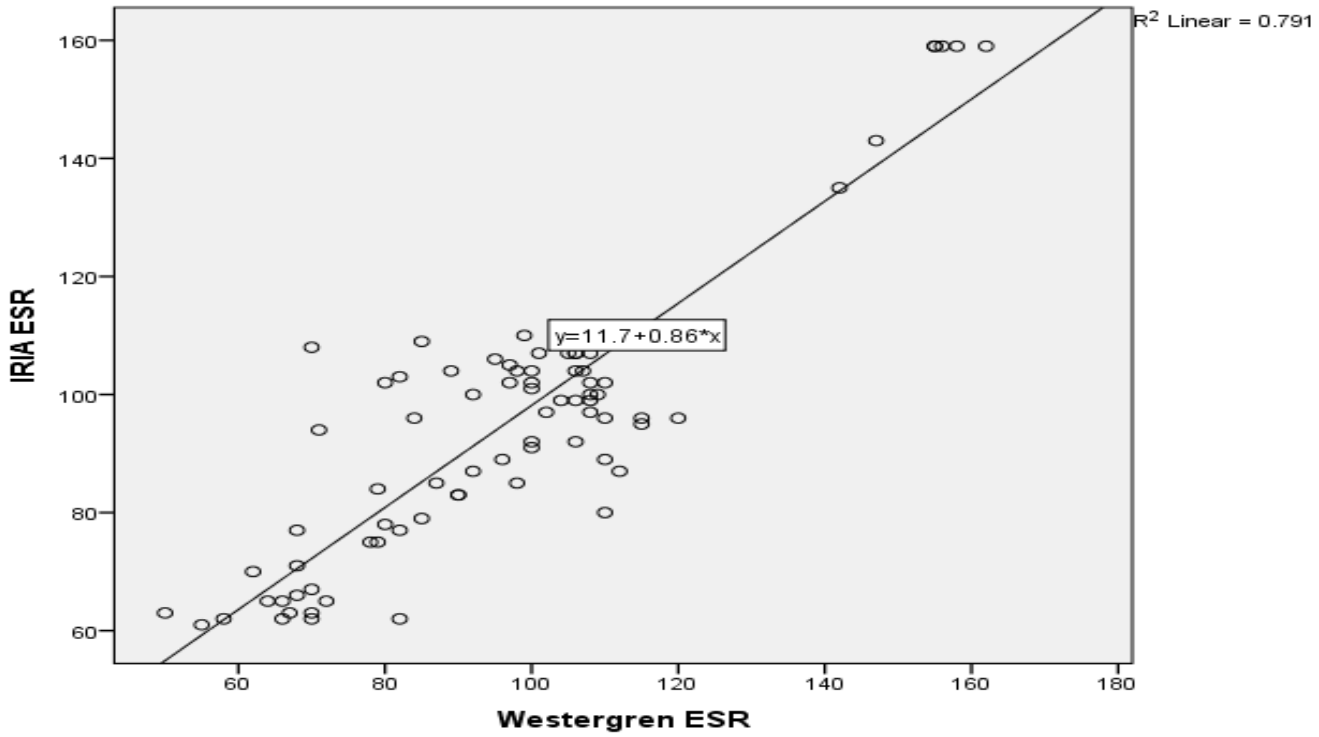

Figure 9. Erythrocyte Sedimentation Rate (ESR) Regression Line Between Standard Westergren And IRIA Automated ESR Methods For ESR Level Greater Than 60 Mm/Hr (N=80).

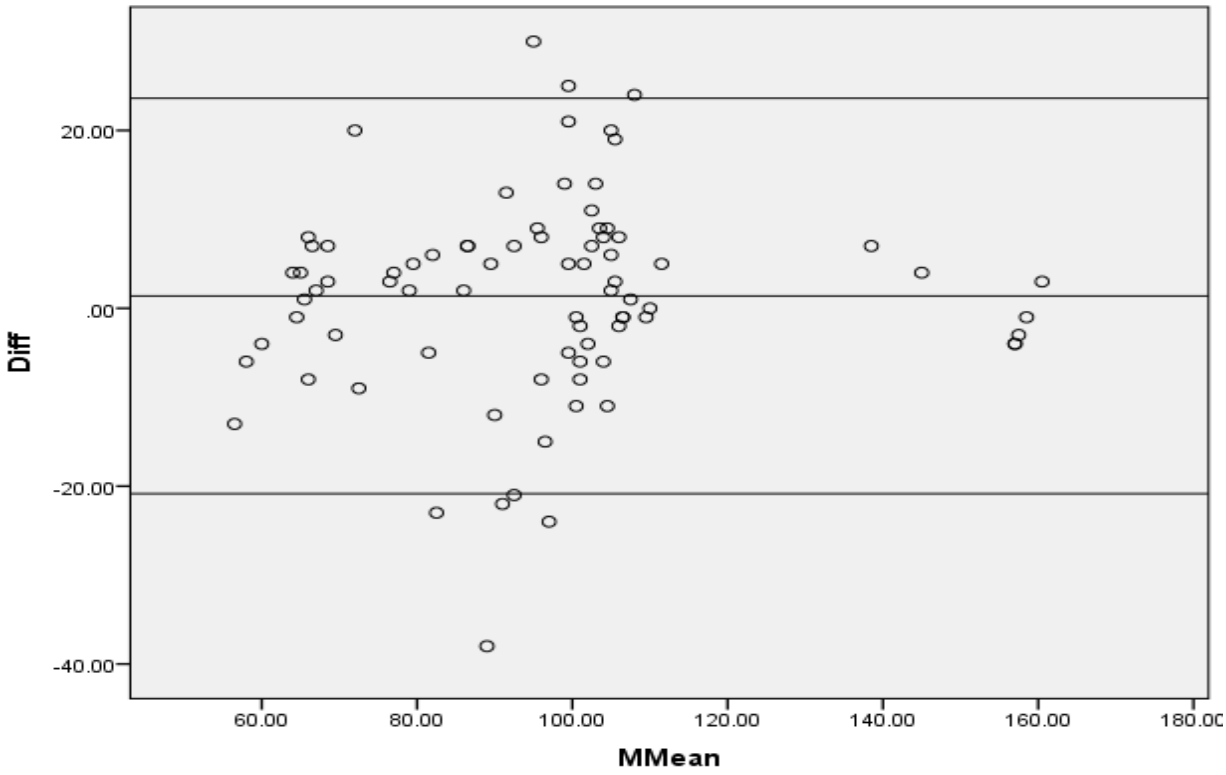

Figure 10. Bland -Altman Plot For The Difference Against Mean By The Two Methods For ESR Level Greater Than 60mm/Hr (N=80).

7. Discussion

The erythrocyte sedimentation rate test is still used widely in clinical practice as an indicator of inflammation, infection, trauma, or malignant diseases (30). This study aimed to compare the conventional Westergren ESR method with one of the new technologies, IRIA Automated ESR method at MCM Addis Ababa, in a diverse patient population. In this study the diseases were categorized by ESR levels (1-20, 21-60 and > 60 mm/hr) as infectious and non-infectious. There were different disease distribution pattern by ESR values categories. Some of infectious disease like tuberculosis, neoplastic (breast, prostate and blood cancer), chronic disease (Diabetic, hypertension and liver cirrhosis), collagen-vasccular (poymyalgia rheumatica, osteoarthritis arthritis) were common in the high ESR>60 mm/hr category. Studies showed by Mahalakshmama *etal,* 2016 and others showed that the ESR is significantly increased in pulmonary tuberculosis, HIV and others diseases (31-33). This showed the increased ESR values are vital for clinical purposes.

In this study Pneumonia was higher in percentage from greater than 20mm/hr ESR Levels group. This agrees with similar study by Melbye H *etal* who demonstrated ESR values is >20mm/hr in their Pneumonia patients (34) demonstrating the role of increased ESR value for patient diagnosis and monitoring.

On the other hand, a comparative study conducted in Tampere University Hospital in Finland by Horstia J *etal,* on 200 sample reported correlation coefficient of 0.72. There were non-linear relationships between the two methods in StaRRsed and westergren and statistically significant differences were found between ESR values in some samples. With the ESR results over 11 mm/h, 27.5% of samples showed a difference of more than 30% for healthy controls and also they did not categorized the ESR level (17). However in our case the correlation and agreement was not good for ESR levels below 60 mm/hr but we categorized the ESR in three level and get agreement in below ESR 60mm/hr but with ESR level >60 we get in agreement with the two methods.

In this study a total of 240 samples were analyzed in three different groups of ESR levels and the ESR levels of 0-60mm/hr have very good correlation r=0.934 for ESR level 1-20, r=0.917 for

ESR level 21-60 in this group the Bland-Altman plot also showed agreement between Westergren method and IRIA automated method but for ESR level greater than 60 mm/hr r=0.890 but the Bland and Altman plot revealed poor agreement because the LoA contains less than 95% of the cases but a study conducted in Croatia, by Perovic E *etal,* they using a different instrument of ESR which is Ves-Matic Cube 200 to Evaluate an automated system with a total of 251 patient sample and they compare with Westergren ESR without ESR level category and got r=0.946 and the Bland–Altman analysis showed agreement between the two methods (18).

In a comparative study conducted using 244 randomly selected patient sample by Curvers J *etal,* the SEDIsystem they found r=0.96 and StaRRsed Westergren-based r=0.83 methods this showed good correlation with the Westergren reference method and an acceptable bias over the entire range of ESR values but they got for Vas -Matic 200 poor correlation (19). When we comes to in our study with ESR levels of 0-60 mm/hr we got a very good correlation and agreement as well. For ESR level greater than 60 mm/hr, we got good correlation but disagreement with the Westergren method and correspondingly we categorized the ESR in three levels.

In a total of 136 patient sample a Comparative study were done by Bogdaycioglu N *etal,* in 2015. Their comparison study revealed that correlation of the Westergren method with The iSED sedimentation method have poor correlation and agreement (r = 0.76, P <0.0001). According to Bland–Altman analysis, this resulted in a mean bias of 13 (95% limit of agreement between 61.6and −35.7) But The Ves-Matic Cube 200 method showed a moderate correlation with the Westergren method (r =0.84, P <0.0001. Their subgroups according the Westergren ESR levels was <20, 20–80, >80mm/hr (low, medium, high levels, respectively) (20). however, in this current study we use different instrument model and ESR level category and for ESR level 0-60mm/hr we got Avery good correlation and agreement but for ESR level greater than 60mm/hr good correlation and poor agreement.

ESR correlation and agreement study by Subramanian A *etal,* in India on 200 samples demonstrated a marked discrepancy between the reference Westergren and the automated Monitor 100* methods both for ESR determined within one and two hours after sample collection. Out of 200 samples, 79 samples were within the reference range used in their hospital (0-25 mm/hour), while 121 samples had higher ESR values of more than 25 mm/hour. On

comparing the manual and automated methods for measuring ESR, marked discrepancy in the ESR results was noted for high ESR values (21). In this study we used different instrument model as well ESR level category and we found for ESR level 0-60 a very good correlation and agreement in addition for level greater than 60mm/hr ESR we found good correlation and poor agreement.

In our study we used IRIA automated method instruments model and compare to Westergren method and the ESR level is categorized in three and we take total sample size of 240 and we got the correlation and agreement is different in each ESR level. On the other hand, correlation and agreement study of ESR determination methods by Suad M *etal.* using 150 samples revealed that the correlation (r=0.91) between the difference and the mean of the two methods of SEDIsystem and westergren, they got r= 0.78. For ESR reading of >25 mm/hr the automated SEDI System and the conventional Westergren methods showed no agreement which do not agree with our study with ESR level 0-60 but it was similar with ESR level >60 and they classify ESR level >25mm/hr and <25mm/hr (22).

Moreover, comparative study carried out by Cerutti H *etal*, on VesMatic Cube 80 analyzer using 248 samples showed good correlation r=0.951 with Westergren measurement (23). unlike to study done by Cerutti H *etal*, even if we compare the manual and automated method of ESR the instrument model and ESR level category is different we got a very good correlation and agreement for ESR level 1-60 but for ESR level greater than 60 we found poor correlation and disagreement between the two methods.

In the above study all uses EDTA anticoagulant as a diluents for blood collection but in this study we used sodium citrate which is the best diluents to be used in the contemporary laboratory to set ESR(16,35). Taken together, studies on the evaluation of automated and the conventional manual Westergren methods give conflicting results for the different ESR categories. In those studies which demonstrated better agreement at lower ESR values, the methods poorly agree at ESR values above 60 mm/hr. Technological variations, sample size different, ESR level of category and type of diluents as well as diversity of patient population studied could partly explain the differences.

8. Strength And Limitation

8.1. Strength

- As far as my knowledge goes this is the first study of its kind which has never been investigated in this country as well with the same instrument model.
- Divers population is involved in this study.
- Internal quality control were done before comparative analysis; the study demonstrated fresh whole blood can be used as internal quality control, which can be scaled up to other facilities in the country which have automated ESR.
- Doubling the ICSH recommendation sample size were used for comparative studies.

8.2.Limitation

- No commercial quality control material available; however ICSH recommended fresh samples were used as QC.
- No similar study conducted with the same analyzer model.
- No similar study conducted with the same ESR level category.

9. Conclusion and Recommendation

9.1.Conclusion

The ESR is widely utilized laboratory examination for assessment of infectious and non infectious diseases and it was helpful for patients evaluation and management. To improve the laboratory quality work, as demonstrated in the current study the internal quality control with fresh blood sample can be used. This good practice can be scaled up to other facilities which use automated ESR method. The conventional Westergren and IRIA automated ESR methods show very good correlation and agreement in level of ESR 0-60mm/hr. When the ESR is greater than 60mm/hr, the two ESR determination methods showed good correlation but have poor agreement as demonstrated by the Bland-Altman analysis, which is a recommended method for method evaluation. These findings indicate that IRIA are reliable and suitable system for ESR level between 0-60 mm/hr and can be used for clinical laboratory interchangeably with the Westergren method. But for ESR above 60 mm/hr, the result must be confirmed with the standard method before use for patient clinical management.

9.2. Recommendation

We recommend that every new automated ESR machine before being introduced for clinical purpose, it must be compared to standard method to confirm its agreement. Finally, we recommend that if commercial quality control material is not available the fresh whole blood sample can be used as the Internal quality control and this will improve the laboratory quality work.

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11.Annex

Annex I English Versions of Participant Information sheet and consent form

Participant Information sheet

Addis Ababa University, College of Health Sciences, School Of Allied Health Science

Department of Medical Laboratory Sciences

You are invited to participate in a study to be conducted by Master's student Dawit Tsegaye at Addis Ababa University, College of Health Sciences, School Of Allied Health Science and Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of this study is Comparison of Erythrocyte sedimentation rate between conventional Westergren method and IRIA automated methods in Myungsung Christian Medical center Addis Ababa, Ethiopia. Participation in this study is exclusively voluntarily. If you or your child are not interested to participate, there was done no consequences. If you/your child decide to participate, you/your child have to sign on the consent form.

What is expected from me as participant of the study?

As a participant of this study, no additional blood sample was done taken from you/your child. The left over sample was done used for this study.

Potential benefits to participant and/or to the society

Based on the results obtained from the result, corrections was done taken in interpreting results of different analyzers. Hence, you are indirectly benefiting other patients and the society.

Compensation for participation

You will not receive any payment for you or your child's participation in this research study.

Confidentiality

On the request paper your/your child's name identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact

Please direct any questions you may encounter during this study to the principal investigator.

Dawit Tsegaye

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Cell phone: +251- 09 11 46 22 26

Email: contactmedve@gmail.com

Consent form

This page contains an agreement signature to participate in the study entitled "Comparison of Erythrocyte sedimentation rate between conventional Westergren method and IRIA automated methods in Myungsung Christian Medical center Addis Ababa, Ethiopia."So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study in "Comparison of Erythrocyte sedimentation rate between conventional Westergren method and IRIA automated methods in Myungsung Christian Medical center Addis Ababa, Ethiopia"
2. I know that the left over sample (blood) that I/my child gave is going to be used for this study only.
3. I understand that, all the information and the results are confidential.
4. I understand that I will not get any money for my/my child's participation.
5. All the information is explained by phlebotomist and Principal investigator.
6. I understand that my/my child's participation is voluntary and can withdraw anytime from the study and this will not affect the service I am/my child getting from the hospital.

Therefore, with full understanding of the situations I agree to give blood for laboratory analysis.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Parent consent for children aged 12-17 years: I agree the sample from my child can be used for this study provided my child gives assent. Parent signature:_____

Assent from children aged 12-17 years: With full understanding of the situations I agree the sample can be used for this study provided my parents give their consent.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Annex II Amharic version of Participant Information sheet and consent form

በአዲስ አበባ ዩኒቨርሲቲ፤የጤና ሳይንስ ኮሌጅ በኣላይድ የጤና ሳይንስ ት/ቤት

የህክምና ላቦራቶሪ ት/ክፍል

በአዲስ አበባ ዩኒቨርሲቲ፤የጤናሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል በሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ላይ ከመሳተፍዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡ/ይመልሱ፤ግልፅ ያልሆነ ነገር ካጋጠመዎት ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ “በሚዩንግ ሳነግ ሜዲካል ሴንተር ውስጥ የሚገኘውን Westergren የዌስተርንግሪን ማንዋል ኢኤስአር ማሽን እና IRIA ኣይአርኣይኤ የአውቶሜትድ ማሽኖች መመርመሪያዎች የሚሰጡትን ውጤት ማወዳደር እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ እርሶም ሆኑ የእርሶ ልጅ/ልጆች ሙሉ ለሙሉ በበጎ ፈቃደኝነት ላይ የተመሠረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ከወሰኑ እርሶም ሆኑ የእርሶ ልጅ/ልጆች በዚህ የህክምና ቦታ ዉስጥ የሚሰውን አገልግሎት አይቋዋረጥም፡፡ በጥናቱ ለመሳተፍ እርሶም ሆኑ የእርሶ ልጅ/ልጆች የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሑፍ ወይም በጣት ፊርማዎትን ማስቀመጥ ይጠበቅቦታል፡፡

የጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድን ነዉ?

የጥናቱ ተሳታፊ በመሆንዎ ምንም ዓይነት ተጨማሪ የደም ናሙና እንዲሰጡ እርሶም ሆኑ የእርሶ ልጅ/ልጆች አይጠየቁም። እርስዎ ለምርመራ በሚሰጡት ደም ጥናቱ የሚካሂድ ይሆናል እንጂ አዲስ ናሙና እንዲሰጡ አይጠየቁም፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች

በጥናቱ ውጤት መሰረት የላብራቶሪ ውጤቶቹን ለመረዳት ማስተካከያ ይደረግበታል፡፡ ስለዚህም በማሽኖች መቀያየር ምክንያት የሚመጣውን ውጤት መለያየት ያስቀራል፡፡ በጥናቱ በመሳተፍዎ እርሶም ሆኑ የእርሶ ልጅ/ልጆች በተዘዋዋሪ መንገድ ለሌሎች ህሙማን ብሎም ለህብረተሰቡ ይጠቅማሉ ማለት ነው።

በዚህ ጥናት በመሳተፍ የሚከፈል ክፈያ

በዚህ ጥናት ስለ ተሳተፉ እርሶም ሆኑ የእርሶ ልጅ/ልጆች ምንም ዓይነት ክፍያ ኣይከፈልም

የተሳታፊዎች ሚስጢር ስለ መጠበቅ

በመጠየቂያው ወረቀት ላይ የእርሶም ሆነ የእርሶ ልጅ/ልጆች ስም ወይም ማንነት ኣይገለጽም። በተሳታፊዎች የሚሰጥ ናሙና ለዚህ ጥናት ጥቅም ብቻ የሚያገለግል ይሆናል።

ጥያቄ ካሎዎት

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ዳዊት ፀጋዬ

የሕክምና ላብራቶሪ ሳይንስት/ክፍል፤የጤና ሳይንስ ኮሌጅ፤አዲስ አበባ ዩኒቨርሲቲ

ሞባይል: +251- 09 11 46 22 26

ኢ-ሚይል: contactmedve@gmail.com

የስምምነት መጠየቅያ ቅጽ

የጥናቱ ተሳታፊ መለያ ቁጥር፡ _____

የዚህ ጥናት ርእስ“በሚዩንግሳነግ ሜዲካል ሴንተር ውስጥ የሚገኘውን Westergren የዌስተርንጌሪን ማንዋል ኢኤስአር ማሽን እና IRIA ኣይአርኣይኤ የአውቶሜትድ ማሽኖችን መመርመሪያዎች የሚሰጡትን ውጤት ማወዳደር“ጥናቱ የሚካሄደው በሚዩንግሳነግ ሜዲካል ሴንተር አዲስ አበባ፣ ኢትዮጲያ ይሆናል። እባክዎትን ከዚህ በታች የተዘረዘሩ ነጥቦች በጥሞና ያንቡቡ እና በመጨረሻ በተሰጠው ክፍት ቦታ ይፈርሙ::

1. በሚዩንግሳነግ ሜዲካል ሴንተር ውስጥ የሚገኘውን Westergren የዌስተርንጌሪን ማንዋል ኢኤስአር ማሽን እና IRIA ኣይአርኣይኤ የአውቶሜትድ ማሽኖችን መመርመሪያዎች የሚሰጡትን ውጤት ለማወዳደር የሚካየሄደውን ጥናት ዓላማ ተረድቻለሁ።
2. እኔም ወይም የእኔ ልጅ የንምሰጠው ናሙና ለዚህ ጥናት ብቻ እንደሚዉል አውቂያለሁ።
3. ለጥናቱ የምሰጠው ናሙና እንዲሁም ውጤቱ በሚስጥር እንደሚያዝ ተረድቻለሁ።
4. በጥናቱ በመሳተፌ እኔም ወይም የእኔ ልጅ የሚከፈለን ክፍያ እንደሌለ ኣውቂያለሁ።
5. ሁሉም የሚያስፈልገው ነገር በተመራመረሪው ይብራራልኛል።
6. በዚህ ጥናት ላይ የሚኖረኝ ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሠረተ መሆኑን፣ በማንኛውም ጊዜ ማቋረጥ እንደምችልና በዚህ ጥናት ዉስጥ በመሳተፍ እኔም ወይም የእኔ ልጅ በዚህ የህክምና ቦታ ዉስጥ የሚሰጠኝ/ን አገልግሎት እንደማይቋረጥ ተረድቻለሁ፡፡

ስለዚህ ከላይ የተጠቀሱትን ነጥቦች በመረዳት ናሙና(ደም) ለመስጠት ተስማምቻለሁ።

የተሳታፊ ፌርማ፡_____

ቀን፡_____

እድሜአቸው ከ 12-17 ለሆኑ ልጆች ቤተሰብ ፍቃድ የሚሰጡበት

ልጄ በዚህ ጥናት ላይ ለመሳተፍ ፉቃዱን የሚሰጥ ከሆነ ከልጄ የሚወሰደው የደም ናሙና ለዚህ ጥናት እንዲውል ተስማምቼአለው፡፡

የቤተሰብ ፊርማ፡_____

እድሜአቸው ከ 12-17 የሆኑ ልጆች ፍቃድ የሚሰጡበት

ቤተሰቦቼ በዚህ ጥናት ላይ እኔ እንድሳተፍ ከተስማሙ አኔ ስለጥናቱ ሁኔታ በመረዳት ለመሳተፍ ፍቃደኛ ነኝ፡፡

የተሳታፊው ፊርማ፡ _____

የተሳታፊው አድራሻ፡ _____

ቀን፡_____

Annex III Standard operating procedure(SOP)

Specimen collection, handling and Transport

Purpose

This procedure provides instructions on specific procedures for blood specimen collection from adults with proper specimen identification and handling and transportation.

Abbreviations

- ESR= Erythrocyte sedimentation rate
- EDTA = Ethylene diamine Tetra acetic acid
- RBC=Red blood cells

Materials

- **Reagent**
    - 5% bleach solution, 70% alcohol
- **Supplies**
    - Gloves
    - Spirit swabs
    - Tourniquet
    - Collection needles compatible with the Vacutainers
    - EDTA Vacutainer tubes
    - A tube rack which fits the Vacutainer tubes
    - Gauze Sponges
    - Band-aid
    - Sharps disposing Container
    - Cool packs (not frozen)

Equipment

- Vaccine carrier cool box or

- Cold box/or sample packaging container/box

Sample

- Sample type: - Whole blood collected in EDTA anticoagulant tube and citrate tube
- Amount required:- 7ml form this 1.5 and 1.5ml for the two citrate tube and 4ml for EDTA tube
- Transport and storage:- Ambient temperature (15-25 ^{0}C)
- Stability:- 8hrs after collection

Limitation:-Heamolysed and clotted samples interfere with ESR test results.

Sample retention: Samples are discarded after 1day of collection.

Special Safety Precautions:-Refer to the Laboratory Safety Manual for standard safety procedures

- Follow infection prevention principle during sample collection
- Wear gloves for handling blood or serum
- Change gloves when they become contaminated
- Wash hands after handling specimens
- Wear protective clothing
- Decontaminate working area with 5% bleach solution

Any forms that are sent in contact with the specimens (i.e. wrapped around the specimen container) should be discarded after the proper information is obtained. They are not to be filed.

Procedure

- Label tubes with the client's name/identification number. (Labeling can also be done immediately after the specimen is obtained).
- Explain the blood drawing procedure to the client and reassure him/her.
- Wear the rubber gloves and make the patient a comfortable position
- Prepare the vacutainer tube, citrate tube and needle

- Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
- Tell the patient to clench his/her fist
- Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture
- Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.
- Insert the needle directly into the vein and withdraw peripheral blood of approximately 6 or 7 ml in the syringe and dispense 4ml in EDTA vacutainer tube and 1.5 and 1.5 ml in two citrate tubes.
- Tell the patient to open his/her clenched fist
- Release the tourniquet
- Withdraw the needle from the vein and cover the puncture site cotton swab and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate haemostasis is visible.
- Properly discard the used materials in a safe container and tell the subject to do so if handled the cotton swabs to stop the bleeding

Sample Handling and Storage

- Mix the blood properly by inverting the tube 6-8 times immediately after collection to avoid formation of small clots
- Label the tube with patient's identification, date and time of collection as well as the name of the collecting personnel.
- Double check the tube labels for accuracy with the sample request form
- Sample should reach the laboratory within 2 hours of phlebotomy.
- Do not refrigerate or freeze the specimens.
- Keep the tubes at ambient temperature (15 -25^{0}C) and avoid extremes of temperature so that specimens do not freeze or get heated above 32 ^{0}C
- The samples should be rejected if they are clotted, hemolysed or frozen

Sample transportation

- Make sure that the lab request & specimen transports forms are filled properly.
- Make certain that the test tube labeled properly and clearly.
- Ensure that the outside of the specimen container is clean and uncontaminated.
- Check if the test tube (container) closed tightly so that its contents do not leak during transportation.
- Place test tube into test tube rack aseptically in appropriate manner.
- Pace tube rack in the transportation bag close tightly
- Deliver to main laboratory without shaking and mixing

Clinical Utility

Hematology analysis gives useful information for the patients and the sample must be handling carefully as infectious material

Erythrocyte Sedimentation rate Westergren method

Purpose

This procedure provides instruction on how to measure Erythrocyte Sedimentation Rate performed in Westergren method

Principle

The ESR expresses in mm per hour the rate at which red blood cells settle when anti-coagulated blood is allowed to stand in a narrow tube (Westergren).

Abbreviation

- ESR = Erythrocyte sedimentation
- HCT = Heamatocrite
- Hgb = Hemoglobin
- RBC = Red blood cell
- QC = Quality control

Material required

- Reagents
    - 3.8% Trisodium Citrate Solution
    - Reagent Preparation
        - Tri-sodium Citrate, anhydrous..... 3.8 g
        - Distilled water..... up to 100 ml
        - Reagent storage and stability
            - Keep the solution in the refrigerator. The solution is stable up to the expired date
- Equipment
    - Westergreen rack

    - Westergren tubes, internal diameter 2.5mm graduated from 0 to 200mm(often marked 1 to 20: 1 corresponds to 10mm, 2 to 20mm, etc.)

- Dilution bottles
- Graduated syringe, 10ml
- Graduated pipette, 5ml
- Timer

Sample

The sample was collected under all aseptic precautions, from the antecubital vein using a 10-ml syringe with 24G needle

- Sample type: - Whole blood
- Amount required: - 2ml of whole blood
- Transport and storage: - store under refrigerator
- Stability: - Stable for 24 hours

Limitation:

- specimens more than 24 hours post-collection
- hemolysis,
- clotted specimens

Sample retention

- Maximum 8 hrs. at room temperature
- If it is refrigerated to 2-8 0c it will stay for 24 hrs.

Procedures

- Add exactly 0.4 ml of the 3.8% Tri-sodium citrate solution into a clean and dry small bottle.
- Draw 2ml of venous blood and immediately place 1.6 ml into the Tri-sodium citrate solution.

Note: You can also use EDTA blood. If kept at 4°C, it can be used after up to 24 hours. In this case mix the EDTA blood well, and place 1.6 ml into the Tri-sodium citrate solution.

- Mix the blood and Tri-sodium citrate solution well.
- Fill a clean and dry Westergreen ESR Tube with the mixture up to the 0 mark.

NB. Do not use mouth pipette. Use a pipetting device.

- Wipe the outside of the Westergren tube with a tissue.
    - Make sure no air bubbles enter the tube.
- Recheck that the tube is filled up to the 0 mark, exactly.
- Close the top of the tube firmly while you place the tube into the tube holder, otherwise the mixture could escape the tube.
- Immediately set your timer for 1 hour or write down the time on a sheet of paper
- Exactly after 1 hour read how far the red cell layer has fallen. Give the result in mm per hour.

Results Interpretation

- The height of the column of clear plasma at the end of one hour
- The results are reported in terms of mm/hr.

Expected value

- Male:-0 - 15 mm /hour
- Female: 0 - 20 mm /hour

Limitation

- Test should be done within 2 hours after blood collection or up to 12 hours after collection if blood is kept refrigerated at 4 C
- Specimen older than specified time after collection and before testing
- Incorrect proportion of anticoagulants
- Incorrect type of anticoagulant
- Haemolysed sample

- Contaminated sedimentation tube
- Air bubbles in the tube will interfere with accuracy. Careful pipetting and diluting are critical.
- Tubes tilted during sedimentation (every 1 degree tilt ESR increases by 3 mm)
- Test set up near central heating or direct sunshine
- Test set up adjacent to centrifuge or other instrument causing vibration
- Failure to read at exactly one hour

Quality control: -Fresh patient sample with PCVs 0.35 or less (1).

Packed cell volume adjustment

To have A PCV less than 0.35 can also be obtained by following technique by adding autologous plasma**.**

1. Divide the specimen into two equal aliquots of about 3.5 mL by using suitable centrifuge tubes.
2. Centrifuge one of the aliquots at a speed (relative centrifugal "force") and for a sufficient length of time to ensure separation into cells and cell-free plasma.
3. Add plasma to the second aliquot to obtain a sample with a PCV equal to or less than 0.35. A convenient formula to calculate the least amount of plasma that must be added to 3.5 mL of blood to adjust the PCV is as follows:

$$3.5\left(\frac{PCV\,(as\ fraction)}{0.35}\right) - 3.5 = mL\ plasma\ required$$

After this manipulation, check that the PCV of the adjusted sample is =0.35.

Clinical utility

Are found with all diseases associated with a modification of the plasma proteins like globulin, albumin and fibrinogen.ESRshows especially high values in.

- Tuberculosis
- Leishmaniases
- Malignant condition

- Hepatic Amoebiasis
- Acute and Chronic Inflammation
- Indicates Leukemia.

Safety Precaution

Personal Protection

- While in the laboratory; all persons working in technical areas must wear gown, or full-length laboratory coat that is buttoned closed.
- Whenever handling and processing sample personnel must wear gloves.
- Personal protective clothing was done changed, and it should be changed immediately if it is contaminated.
- Shoes should be comfortable, and cover the entire foot.
- Hands must be washed frequently during the day.
- Foods, drinks, smoking, and like substances are strictly prohibited in technical work areas.

Erythrocyte Sedimentation rate IRIA automated method

Purpose

This procedure provides instruction on how to measure Erythrocyte Sedimentation Rate performed in IRIA automated method.

Principle

IRIA is a random access auto analyzer to determine the Erythrocyte Sedimentation Rate (ESR), capable of processing up to 5 samples simultaneously by means of an IR LED optical system this technique was requires a minimal handling of the sample, as the test is performed directly on the test-tube, admitting both open and vacuum tubes then Spill 1.5ml blood using the ESR tubes (the instrument only works with tubes machine manufacture because they have a special dimension) Then agitate/mix the ESR tube (around 5 minutes) and just plug into the instrument. It will detect immediately the sample and start to read it. After 17 minutes it will displayed on the screen the results (in mm westergren) for the 1^{st} and 2^{nd} hour at the same time. As it is a Random access instrument it is capable to process 25 samples per hour, with uninterrupted sample loading, as test positions are left free.

Abbreviation

- ESR = Erythrocyte sedimentation
- HCT = Hematocrit
- Hgb = Hemoglobin
- RBC = Red blood cell
- QC = Quality control

Material required

Material

- IRIA automated ESR Machine

- Citrated test tube

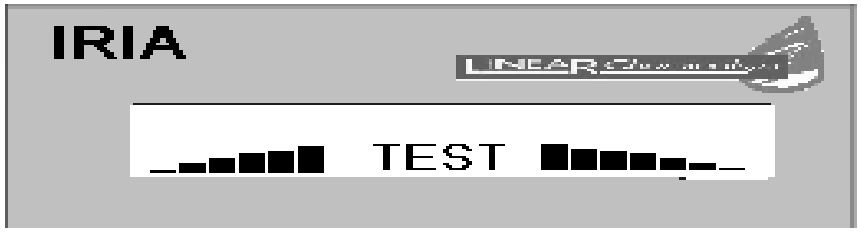
Sample

The sample was done collected under all aseptic precautions, from the antecubital vein using a 10-ml syringe with 24G needle

- Sample type: - Whole blood
- Amount required: - according to the level marking of the tube
- Transport and storage: - store under refrigerator
- Stability: - Stable for 24 hours

Operation

- Switching on the instrument.
    - The "TEST" message was done displayed alternate in both lines

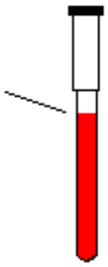


    - Around 1 minute and it will check its different electronic and mechanical components and the instrument doesn't have backup memory.

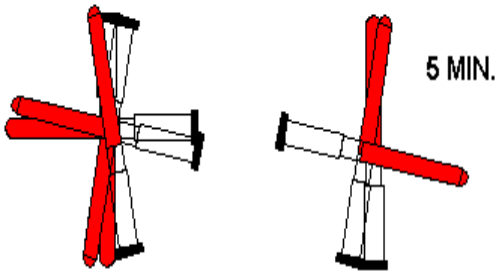
    - The checking being completed (1 minute approx.), the instrument printout de presentation and the "LOAD" message was done displayed alternate in both lines.

- The Instrument is now ready to begin an analysis cycle. If during the test the instrument detects a tube in some position they show the **"EXTRACT"** message. **Then we must to extract that tube.**

- Tubes must be **correctly labeled** with the blood sample, according to the **level** marking on the tube.



- In open and vacuum tubes system Spill 1.5ml blood in the ESR tubes
- The sample should be shaken by slowly inverting the tube during approximately 5 minutes, before inserting it in the Instrument.



- The test starts when the tube is inserted in the Instrument.
    - **Do not remove the tube from its position until the test is completed**. If you do so, the test was done aborted and the **"S.E."** message will appear on the results printout.
- After **17 minutes** have elapsed from the start of the test, **the end of the test** is reported showing in the first line the **1H results** and in the second line the **2H results** in the corresponding position on the display.
    - This results remains in the corresponding position of the display until we insert another tube in that position.
- At the same time the instrument send trough the serial port this result to the printer or a computer.
- Then we can take off the sample, and when a dot "." Appears in the corresponding position we can reload this position with a new tube.

    - Note: It's very important to wait until the instrument shown a dot "." In the corresponding position, because in that way the instrument recognize the position empty and it's able to accept another sample.

- The result of the last analysis of one position remains in the display until we load a new sample in that position or switch off the instrument.

The IRIA has been designed to operate under certain environmental conditions:

- Room temperature 15º-32ºC
- Max. relative humidity 80% at 32ºC

Analytical Errors

Low results:

a. Possible blood clot in the sample. Repeat the test with a new sample.
b. When more than 2 hours have elapsed from the taking out of the sample until the test.
c. Insufficient sample amount, which alters the proportion between blood and anticoagulant, affecting the test result.

High results:

a. Sample not shaken correctly.
b. If the Instrument has been installed on a surface that is not perfectly level. A 3% inclination can increase the ESR by 30%.
c. Excessive sample amount, which alters the proportion between blood and anticoagulant, affecting the test result.

Safety requirements

- In case the cable or the power supply begins to get damaged or broken, they should immediately be replaced
- This Instrument is an electro mechanic device of which the user may not handle the internal parts.
    - **WARNING**: The Instrument must be disconnected from the power supply before starting any technical corrective work. Operators should not attempt to repair faults or breakdowns.
- In case of accidental spillage of the analyzed samples, the spill must immediately be cleaned with an appropriate disinfecting solution, to avoid the possible contamination of the laboratory personnel and Equipment.

- **Note:** The Instrument can be cleaned with a water and soap solution or with 70% alcohol. Do not use a larger alcohol concentration, as it could damage the cover of the Equipment.

Result interpretation

- The results (in mm westergren) for the 1st and 2nd hour at the same time

Annex IV Data collection Log Sheet

Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences

Data collection Log Sheet

No	Code No	ID N0	Age	Sex	Result				Clinical Diagnosis Rule Out	Remark
					Westergren		IRIA			

Declaration

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

Name: **Dawit Tsegaye (BSc, MSc candidate)**
Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia
Date of submission _____

This thesis has been submitted with our approval as University Advisors.
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Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.
Name:- **Jemal Alemu (MSc, PhD candidate)**

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.
Name:- **Mikias Negash(MSc)**

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia.