**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCES**

# Establishment of reference interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Demiraw Bikila, entitled "**Establishment of reference interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia"** submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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**List of Abbreviations**

| | |
|---|---|
| AAU | Addis Ababa University |
| AU | African Union |
| CE | Capillary Electrophoresis |
| CLSI | Clinical and Laboratory Standards Institute |
| ENAO | Ethiopian National Accreditation Office |
| EOF | Electro-Osmotic Force |
| EPHI | Ethiopian Public Health Institute |
| FDA | Food and Drug Administration |
| HsCRP | Highly sensitive C-reactive protein |
| IFCC | International Federation of Clinical Chemistry and Laboratory Medicine |
| IMWG | International Myeloma Working Group |
| ISO | International Organization for Standardization |
| MC | Monoclonal component |
| pI | Isoelectric Points |
| SOP | Standard Operating Procedure |
| SPE | Serum Protein Electrophoresis |
| SPSS | Statistical Package for Social Sciences |
| UNECA | United Nations Economic Commissions for Africa |

**Abstract**

**Background:** Serum protein electrophoresis is a laboratory technique routinely employed for the identification of patients with multiple myeloma and other disorders of serum protein fractions. Even though, several factors affect reference intervals, company derived values are currently in use, and little or no data is available in resource limited setting countries like Ethiopia. Therefore, the current study will establish reference interval for serum protein fractions which serve as baseline data for the diagnosis, interpretation and monitoring of problems associated with serum proteins fractions in clinical laboratories.

**Objective**: To establish reference interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia.

**Method:** A cross-sectional study design was conducted on a total of 297 apparently healthy adults from April-October 2019 in four selected sub cities (Akaki, Kirkos, Arada, Yeka) of Addis Ababa, Ethiopia. Socio-demographic data were obtained from previously collected reference interval study data while laboratory analysis of collected samples was performed using Capillarys 2 Flex Piercing analyzer. Data was entered into Microsoft Excel and exported to SPSS version 23 and med-cal software for analysis. Data was explored and tested by Kolmogorov-Smirnov (K-S) and Shapiro-Wilk test of normality and Mann-Whitney test to check Partitions. For $p \geq 0.05$, the data was considered as normally distributed. Non-parametric method of reference range establishment was performed as per CLSI guideline EP28A3C. The lower and upper limits, including 95% distribution with 90% confidence intervals, were calculated. The results were expressed as percentages and absolute concentration and presented by tables accordingly.

**Result:** The established RIs were: Albumin 53.83-64.59%, 52.24-63.55%; Alpha-1 globulin 3.04-5.40%, 3.44-5.60%; Alpha-2 globulin 8.0-12.67%, 8.44-12.87%; and Beta-1 globulin 5.01-7.38%, 5.14-7.86%. Moreover, Albumin to globulin ratio was 1.16-1.8, 1.09-1.74 for male and female, respectively. The combined RIs for Beta-2 globulin and Gamma globulin were 2.54-4.90% and 12.40-21.66%, respectively.

**Conclusion:** The current study established reference interval for serum protein fractions and revealed gender specific differences in reference interval except for Beta-2 globulin and Gamma globulin.

**Keywords**: Serum protein electrophoresis, reference interval, Addis Ababa, Ethiopia

# 1. Introduction:

## 1.1 Background:

Proteins are the major structural and functional molecules made up of amino acids (contains an acidic carboxyl group (-COOH), a basic amino group (-NH2) and R groups (can be neutral, acidic, or basic)) which are necessary for host defense, cell structure, movement, and as regulatory molecules. Due to their ability to exist as either positively or negatively charged molecules, proteins are considered ampholytes, which is characterized by varying numbers of ionizable amino (-NH2) or carboxyl (-COOH) groups. The overall charge on the protein will depend on its isoelectric point (pI), i.e., the pH at which the net charge is neutral. If a protein is in a buffer that is more acidic than its pI, the protein will be positively charged; conversely, if the buffer is more alkaline, the net charge will be negative (1, 2).

The introduction of Capillary Electrophoresis (CE) as an attractive alternative to the gel-based methods gives rise to the advancement of serum protein electrophoresis in the last decades (3).

Protein electrophoresis (separation of serum protein fractions) is a technique used in clinical laboratory for screening samples for protein abnormalities. The Capillary's 2 flex piercing (SEBIA; Lisses, Evry Cedex, France) has been developed to provide complete automation of this testing with fast separation and good resolution (4-9).

The two major types of proteins that can be separated with SPE are albumin (major component of serum proteins produced by the liver under normal physiologic conditions) and globulins (a-1 globulin, a-2 globulin, b-1 globulin, b-2 globulin and g-globulin) which comprise a much smaller fraction of the total serum protein content. To interpret the results, the main focus is to set on the relative and absolute quantity of the various subsets of these proteins **(**10-12**)**. Although changes in each of these bands can be correlated with a patient's health status, it is the changes that occur in beta and gamma fractions where monoclonal component (MC) proteins are normally found that are of the most clinical interest.

The six serum protein fractions are albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma-globulin.

Among the six serum protein fractions, Albumin (about 69kDa) is the single most abundant protein present in serum, which is produced by the liver. The main role of albumin encompasses maintenance of oncotic pressure and a transport protein for steroids, fatty acids, hormones, bilirubin, as well as various drugs in the circulation. Compounds like penicillin and bilirubin can influence the migration of albumin by binding to it (13).

The second serum protein fraction, α1 Fraction**,** is composed of a number of proteins like α1-antitrypsin, α1- lipoprotein, α1-fetoprotein, α1-acid glycoprotein and α1-antichymotrypsin. In the serum protein electrophoresis, A1-Antitrypsin is the most abundant and clinically useful acute-phase reactant component of α1 fraction which readily changes in response to inflammation. Its deficiency is a genetically inherited autosomal co-dominant disorder associated with various clinical conditions like liver disease (hepatitis, cirrhosis, hepatoma) (14).

The constituents of third serum protein fraction, α2 fraction, include α2-macroglobulin, haptoglobin, ceruloplasmin and complement proteins. The most relevant acute phase reactant of α2 fractions are α2-macroglobulin and haptoglobin. Among α2 Fraction, the most abundant one is α2-Macroglobin which serves as plasmin inhibitor as well as other serum proteases like serine-, cysteine-, aspartic- and metalloproteases (15). The elevation of α2-macroglobulin is observed in nephrotic syndrome due to increased synthesis and inability to pass through the glomerulus. The role of Haptoglobin is to bind free hemoglobin released by intravascular hemolysis to protect renal damage and retain iron. The clearance of hemoglobin-haptoglobin complex from the circulation can be achieved with an estimated half-life of 12h (16).

The other serum protein fraction, β-globulins, can migrate as a single β-fraction or can be resolved into β1 and β2 fractions depending on the electrophoretic system utilized. Increased synthesis results in an elevated β1 fraction consistent with iron deficiency. Even-though IgA is the most common β migrating monoclonal protein, monoclonal IgM and IgG can also be observed. IgG, IgA and IgM (IgD and IgE at low concentration) along with C-reactive protein (CRP) and Fibrinogen are the constituents of the sixth serum protein fraction called γ fraction (17).

Even-though, western country based reference ranges for capillary electrophoresis (CE) in healthy adults were reported (8, 18-21), no data is available in resource constrained laboratory setting countries like Ethiopia.

As per the recommendation of the guidelines developed by Clinical and Laboratory Standards Institute (CLSI) (22), every country must establish reference intervals for its own population that aid in accurate diagnosis and interpretation of laboratory test results. Therefore, determination of reference interval for serum protein fractions plays a crucial role in the diagnosis of problems associated with of serum proteins fractions in clinical laboratories. As a result, the proposed current study will fill the gap to some extent.

## 1.2 Statement of the Problem:

In many African countries, the clinical laboratory reference intervals have not been established, and western country based reference intervals are usually being used in many diagnostic laboratories and clinical trials to screen, diagnose and monitor different disease states (23). In poor setting country like Ethiopia, almost all available clinical laboratories rely on reference intervals obtained either from textbooks or package kit inserts provided by the manufactures.

The considerable difference within the established reference intervals in several countries and population groups may increase the chance of either unnecessary additional investigations or failure to detect the specified abnormalities related underlying disease states or mismanagement of patients (24).

One of the most important applications/indication of serum protein electrophoresis is detection and quantification of monoclonal and polyclonal gammopathies in patient serum (3). Multiple myeloma (cancer of plasma cells in bone marrow) which is treatable but incurable serum protein related abnormality is characterized by spike-like peak in a gamma globulin region and responsible for 1% of all cancers and is the 2$^{nd}$ most common hematologic malignancy after lymphoma with an estimated 24, 2802 to 30,330 new cases and 12,650 deaths to occur for 2016 (25, 26). Serum protein electrophoresis is one of the main tools for diagnosis, management of multiple myeloma and to screen for the presence of monoclonal proteins.

Even though laboratory tests represent 5% of a health system's costs, yet it affects 95% of the remaining costs. Laboratory findings have a crucial role in diagnosis, monitoring and screening of different pathologies through influencing up to 70% (on average around 60-80%) of clinical decisions. Hence, the interpretation of laboratory result must rely on the background of well-established reference interval that discriminates between "health" and "disease" (27).

Therefore, in order to reliably interpret laboratory results of serum protein fraction, establishment of reference interval of serum protein electrophoresis may support a better understanding of serum protein related abnormalities like multiple myeloma.

## 1.3 Significance of the study:

Identification of problems associated with serum protein fractions need a locally established reference interval. To the best of the investigators knowledge, no research has been done on establishment of reference interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia. Therefore, findings of the present study are of great importance, since it can assist in the correct interpretation of serum protein fraction for diagnosis, treatment and management of abnormalities associated with it.

This study will also be useful for patients, clinicians, community, and other researchers, policy makers at large for prevention, diagnosis, result interpretation, early control and management of abnormalities associated with serum protein fraction like multiple myeloma in Ethiopia in general and in the study area in particular.

Since no other study has been conducted in the study area, the findings of the present study will serve as baseline information and reference for the future studies.

# 2. Literature Review

## 2.1 Reference interval studies

A study conducted in Belgium on method comparison and the establishment of reference interval for the five serum protein fractions (albumin, a 1 globulin, a 2-globulin, b -globulin, and g -globulin.) as per CLSI guideline C28-A3. A total of 161 of 200 healthy adults of ages 18-65 years fulfilled the eligibility criteria. Of them, 82 were men and 79 women. The established RI revealed statistically significant sex-related differences for all fractions except the γ-globulin fraction (18).

The five serum protein fractions' reference range determined by capillary zone electrophoresis from 129 (49 men and 80 women) healthy Japanese volunteers, aged 7 to 90 years showed no statistically significant gender based differences for α1-globulin, α2-globulin, β-globulin fractions. However, the study showed statistically significant age based differences for albumin and γ-globulin fractions in men aged 20 to 49y in which the concentrations of albumin fractions was higher in men than women of the same age group and in ages 30 to 49y. The concentration of γ-globulin was higher in men than women of the same age group (28).

The reference interval was obtained from a study done on 50 samples of adult blood donors using the Capillarys system in Belgium by Bossuyt et al (3). The analysis revealed a 2.5th and 97.5th percentile of 55.6% and 66%, respectively, for the albumin fraction, 2% and 6.9% for the α1-globulin fraction, 5.9% and 11.4% for the α2-globulin fraction, 9.4% and 14.4% for the β-globulin fraction, and 8% and 18.8% for the γ-globulin fraction.

In study conducted in USA by Jollif et al (19) using capillary zone electrophoresis (CZE) system the established RIs were: Albumin 52-67%, Alpha-1 globulin 3.8-8.3%, Alpha-2 globulin 5.8-12.4%, and gamma globulin 9.3-20.4%.

## 2.2 Factors Influencing serum protein electrophoresis

Several factors are implicated in affecting serum protein electrophoresis. Major factors affecting the results are reviewed as follows.

### 2.2.1 Adsorption of protein to the capillary wall

A major problem for the analysis of proteins by capillary electrophoresis method is the interaction between positively charged proteins and negatively charged silanol groups on the capillary surface. The influence will be of concern especially if the proteins are separated at pH values lower than their isoelectric points (pI)-(the pH at which the net charge is neutral). As a result, adsorptions at the capillary wall frequently happen. This can result in peak broadening and asymmetrical peak shapes, reduced efficiency, low recovery of analysis, irreversible protein adsorption, a drifting of Electro-Osmotic Force and non-reproducible migration times (29, 30).

### 2.2.2 Mechanisms for Minimizing/eliminating adsorption of protein to the capillary wall

Different mechanisms have been devised to minimize the issue of protein predilection to the capillary wall. In serum protein electrophoresis, the choice of pH buffer influences the charges of analytes. pH condition close to and less than pI of proteins are able to increase amount of possible binding sites; thus, proteins have a stronger tendency to be adsorbed to the capillary wall. Therefore, extreme pHs or pH values higher than the protein pI are favorable to overcome the adsorption problem. The use of extreme basic or acidic pHs give the same sign of the capillary wall and the proteins. They repel each other and the adsorption can be minimized (30).

Adding alkali salts of high concentration, zwitterions or other additives to the buffer solution can be used to suppress the electrostatic interaction between the capillary wall and the proteins. The high concentrations of positively charged ions compete with positive charges of the protein to interact with the negative silanol groups of the capillary wall. Zwitterions function as ion pairing with the proteins, thus protein-wall and protein-protein interactions can be reduced. The use of buffer additives is also useful for masking the activity of silanol groups. However, in the addition of ionic salt, the applied field strength should be controlled to avoid high current that may possibly lead to denaturation and precipitation of proteins (29, 30).

The use of coated capillaries in serum protein electrophoresis is recommended to reduce the wall interactions of protein molecules by deactivating the silanol groups. Coating of the capillary wall can provide separation efficiency, better protein recovery and reproducibility of Electro-Osmotic Force (EOF) and migration time of analytes. In recent days, significant adsorption of proteins to capillary wall is still observed in using coated capillaries (31, 32). Improvement of separation efficiency can be made by controlling temperature, ion strength, pH, chemical and structural properties of the capillary surface, rinsing procedure when using coated capillaries (33, 34). In addition to this, the stability of a protein is also one of the determinants related to the adsorption behavior and can even be one of the driving forces for protein adsorption. Therefore, the possible way to reduce protein adsorption in this case is by increasing the stability of proteins (35). In a recent study, sugar excipients such as trehalose, mannitol, sucrose and sorbitol have shown a decrease of protein adsorption by stabilizing the native state of the protein in the solution (36-38).

### 2.2.3 Effect of Buffer solution on serum protein electrophoresis

During serum protein capillary electrophoresis, the role of buffer is very crucial. In analogy to chromatography, the buffer in CE assumes the role of the mobile phase and the stationary phase. It is the semiconducting nature of the buffer that allows the free-zone electrophoretic migration of analyte ions in an electric field. Electo-Osmotic Force, which is an integral part of CE, is mainly driven by the residual charges on the inner wall of the capillary, and may be controlled by carefully selecting constituents of buffer solution. In CE, solute migration velocity, separation, column efficiency, and peak shape are sensitive to changes in buffer characteristics. In particular, the pH is of prime importance. The buffer capacity (which is a quantitative measure of buffering ability) must be high enough such that the local pH and conductivity will not change as a result of sample introduction and migration across the capillary (39).

### 2.2.4 Effect of temperature on Serum protein Capillary Electrophoresis

During quantitative determination of serum protein fractions by capillary electrophoresis sufficient temperature control is of prime importance. This is primarily because of thermal change of the pH of the buffers; small changes in pH can have noticeable effect on pattern shape. Good temperature control can be achieved through Peltier effect, heat being either absorbed or

emitted between the junctions, which give excellent cooling results and important for reproducibility of migration times(9).

### 2.2.5 Sample related factors

The charge, size and shape of serum protein fractions being separated influence its own migration rate. The rate of migration can be increased by increasing a net charge which is pH-dependent. Rate of migration is affected by increased in size of molecule (inversely proportional) and difference in shape of the sample (9).

Hemolyzed specimens can cause interference on spectral and electrophoretic pattern, which resembles monoclonal protein during serum protein electrophoresis. Free hemoglobin or hemoglobin-haptoglobin complexes due to hemolysis can produce distinguishable electrophoretic pattern that migrate in the α2-β region. This can lead to misidentification of monoclonal protein. Visual inspection of the specimen is crucial to observe the characteristics of α2-β region migrating band either due to hemoglobin or hemoglobin-haptoglobin (40).

As reviewed above serum electrophoresis is affected by several factors. Measurement of the protein fractions be it during RIs determinations or analyzing patients' samples, therefore, has to be handled by strictly adhering to standard operating procedures. In Ethiopia, few health facilities have serum electrophoresis facility. Since determination of RI is resource demanding many laboratories in resource constrained settings, including in Ethiopia, depend on RIs established elsewhere and provided by the instrument and kit manufacturers. This is a gap that this study is trying to fill by establishing RI using community samples from adult population of Addis Ababa.

# 3. Objectives:

## 3.1 General objective

To establish reference interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia

## 3.2 Specific objectives

- To establish reference interval for serum albumin electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia
- To establish reference interval for serum globulin fractions (Alpha, Beta, Gamma) globulin electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia
- To establish reference interval for albumin to globulin ratio of apparently healthy adults in Addis Ababa, Ethiopia

# 4. Materials and methods

## 4.1 Study area

The study was conducted in four selected sub cities (Akaki, Kirkos, Arada, Yeka) of Addis Ababa, Ethiopia. Addis Ababa, is the capital city of Ethiopia established in 1887, and serves as the headquarters of several international organizations such as the African Union (AU) and the United Nations Economic Commissions for Africa (UNECA). The city is located between 8055' and 9005' North Latitude and between 38040' and 38050' East Longitude. The capital city's average elevation is 2,500 meters above sea level, and hence has a fairly favorable climate and moderate weather conditions. It is the largest city in the country by population, with a total population of more than 3 million. The area of the capital city is about 527 square kilometers of Ethiopia. There is an estimated population density of about five thousand per available square kilometer. As per 2007 census, the city of Addis Ababa has a higher population of female residents than male residents. Almost one-quarter of all people in Ethiopia that live in urban areas live in the capital city (41, 42).

## 4.2 Study design and period:

A cross-sectional study design was conducted to establish reference interval for serum protein electrophoresis of apparently healthy adults from April-October 2019 in Addis Ababa, Ethiopia.

## 4.3 Population

### 4.3.1 Source population

All adult individuals who live in Addis Ababa, Ethiopia were the source population.

### 4.3.2 Study Population

Adult individuals who live in four selected sub-cities (Akaki, Kirkos, Arada, Yeka) of Addis Ababa, Ethiopia and willing to give blood samples as per the eligibility criteria were the study population.

## 4.4 Inclusion and exclusion criteria

This study is part of the national reference interval study; thus, both the inclusion and exclusion criteria followed the national reference study protocol; accordingly, the following eligibility criteria were applied in participant selection.

### 4.4.1. Inclusion criteria

- Apparently healthy individuals aged 18 years and above who were willing to participate in the study and lived in the study area for at least 5 years.

### 4.4.2. Exclusion criteria

- Samples that show hemolysis, icterus, lipemia or that displayed an abnormality were excluded. Outlier exclusion was as described in CLSI Guideline EP28A3C (22).

## 4.5 Study variables

### 4.5.1 Dependent variables

- RI for Serum protein fractions (Albumin, Alpha Globulin, Beta Globulin, Gamma Globulin and Albumin to Globulin ratio)

### 4.5.2 Independent variables

- Age, Sex

## 4.6 Participant Selection, Sample size calculation and Sampling method

### 4.6.1 Participants Selection and Sample size calculation

As per CLSI Guideline EP28A3C recommendation, the best means to establish a reference interval is to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by non-parametric means for each partition (e.g. sex, age range) with a power of 90%. One of the IFCC's primary recommendations, posteriori sampling approach, was employed as a direct means to determine reference interval. Thus, to obtain a minimum recommended sample size (120 men and 120 women) for two partitions a total of 297 samples was obtained by considering 20% rejection rate (18) from already collected samples.

### 4.6.2 Sampling Method

As this study is part of the national RI study, two types of sampling technique were employed to obtain the required sample size. First, for the establishment of reference interval of Clinical chemistry parameters and other hematological parameters a total of 1030 participants were recruited by using Probability Proportional to Size (PPS) sampling method where the size depends on the number of households of Woredas (former Kebeles) in a city/town. Accordingly, all the woredas in the town were considered/selected to be the participants of the study. Since Addis Ababa is very large city, four sub-cities were selected based on PPS, namely Arada, Kirkos, Akaki and Yeka sub-cities; thus all woredas under the selected sub-cities was included. To recruit 1030 participants, the number of households was determined by dividing the total household in the selected towns (sub-cities for A.A) by the estimated number of individuals per household which is 4 for urban. Individuals in every $K^{th}$ household (as shown in **table 1**) were approached at their households through well trained sample collectors. Given the average number of individuals in each household of 4, the next households were used to recruit the remaining age groups that are not found in the selected household. Once volunteering participants fulfilling the eligibility criteria was identified by the well trained sample collectors, they were invited to go to nearby health facilities to facilitate biological sample collection. Finally, for the current study, convenient sampling technique was employed to obtain 288 required sample sizes. That is one of the partitions which include individuals aged 18-65 years included in the current study.

**Table 1: Selected sites with household information**

| **Selected Sites** | **No. Households** | **Individuals per household** | **No. Household to be included in the study*** | **$K^{th}$** |
|---|---|---|---|---|
| Akaki | 47021 | 3.8 | 55 | 861 |
| Kirkos | 54398 | 4.0 | 52 | 1049 |
| Arada | 49564 | 4.1 | 50 | 995 |
| Yeka | 90195 | 3.8 | 109 | 829 |

Note: Source for total population and number of households is from CSA 2007;

*total number of household sampled was computed from the total sample size needed (i.e. 1030) by the estimated individuals per household for each study site

## 4.7 Measurement and Data collection

### 4.7.1 Data collection procedure

The required data for the current study was collected by using data collection format which contains code, age, sex, residence, and laboratory result of the six serum protein fractions plus albumin to globulin ratio. The original data was collected by mobilizing eligible individuals from the community to a nearby health facility by Health extension workers. Serum samples were stored at -80$^{O}$C at EPHI until analysis.

### 4.7.2 Laboratory analysis

The collected serum samples were analyzed using Capillarys 2 Flex Piercing analyzer (Sebia, France) at EPHI clinical chemistry reference laboratory as shown in **figure 1**. The analyzer was approved by Food and Drug Administration (FDA) for clinical use (3). The separation of proteins occurs in free solution in narrow fused silica capillary that is exposed to a high voltage at a PH of 10 buffers at which serum protein fractions exhibit negative charge. After application of voltage, the two forces that act in opposite directions on serum proteins are the force of Electric field and electro-osmotic force (EOF). At PH of 10, the internal surface of the fused-silica capillaries is negatively charged due to ionization of the silanol groups.

Cations in the electrolyte near the capillary wall migrate toward the negative electrode (cathode), pulling electrolyte solution with them which constitutes electro-osmotic force (EOF) which surpasses the force of electric field and carries all serum proteins towards cathode. Albumin is more acidic than gamma globulins which makes albumin to resist EOF resulting in delay of albumin to reach cathode. There is direct qualitative and quantitative determinations of Serum protein fractions at 200 nm, for detail principle and procedure of the test refer annex I (43).

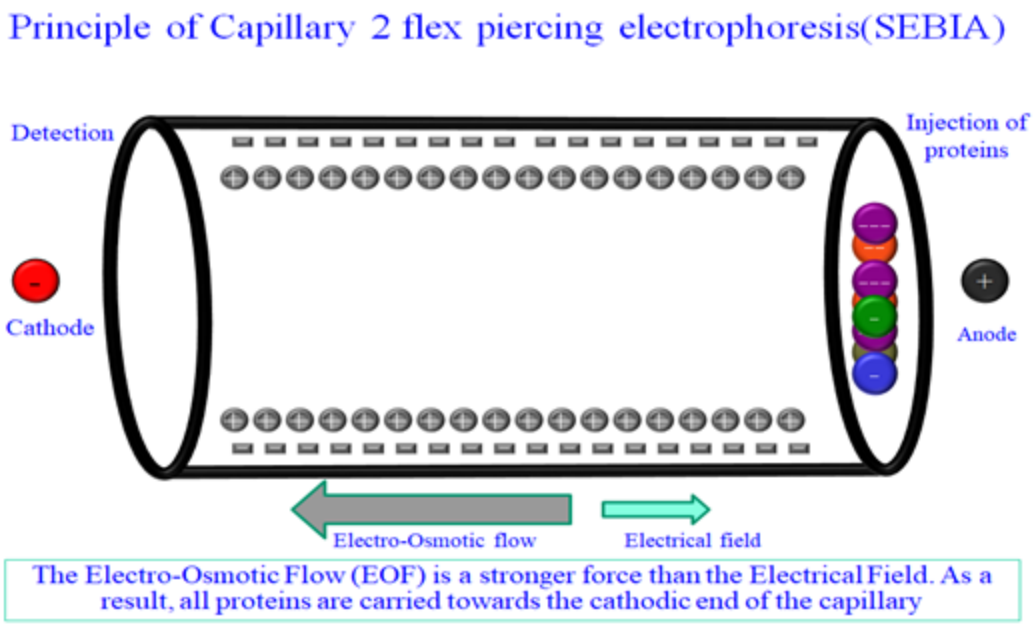


**Figure 1: Principle of Capillary 2 flex piercing Electrophoresis (SEBIA)**

## 4.8 Data Quality Assurance:

### 4.8.1 Data collection tool

Data regarding demographic characteristics of study participants were collected by using a data collection format from previously gathered data of national reference interval study.

### 4.8.2. Pre-analytical

Blood sample quality was ensured during collection and processing by following the standard operating procedures. In the study serum sample stored at -80$^{°C}$ at Ethiopian Public Health Institute was used. Samples with incomplete information, Turbid and hemolyzed was rejected.

### 4.8.3. Analytical

Analytical quality assurance was done in the EPHI national clinical chemistry reference laboratory which is accredited by the Ethiopian national accreditation office [ENAO] to

perform tests in accordance with the requirement of ISO 15189: 2012, Medical laboratory requirements for quality and competence [Accreditation No.: M0025]. Well trained and experienced laboratory professionals performed the analysis and Standard operating procedures [SOPs] were strictly followed for respective measured parameters.

The performance of fully automated clinical chemistry analyzers (Capillary flex 2 piercing) was checked by running quality control samples and results was evaluated using control data provided by manufacturer.

### 4.8.4. Post-analytical

The results were printed out after checking appropriateness of all the test results and the data was carefully entered into Microsoft Excel worksheet and was saved for statistical analysis.

## 4.9 Data analysis and interpretation

The data was entered into Microsoft Excel, exported to SPSS version 23 and med-cal software for analysis. Before data analysis was performed all continuous variables was assessed whether they are normality distributed or not visually using histogram. Data was also explored and was tested by Kolmogorov-Smirnov (K-S) and Shapiro-Wilk test of normality and Mann-Whitney test to check Partitions. For $p \geq 0.05$, the data was considered as normally distributed. Non-parametric method of reference range establishment was performed as per CLSI guideline EP28A3C (22). Using a non-parametric method, the lower and upper limits, including 95% distribution with 90% confidence intervals, were calculated. The results was expressed as percentages, absolute concentration and presented by tables accordingly.

## 4.10 Operational definitions

The definitions were as per the CLSI Guideline EP28A3C (22)

**Reference Interval**: the interval between, and including, two reference limits; Note: It is designated as the interval of values from the lower reference limit to the upper reference limit (EP28A3C) (22).

**Establishing (or determining) a reference interval**-the process used in creating a reference interval de novo, encompassing all of the steps from selection of reference individuals, through exact details of the analytical methods, and concluding with data collection and analysis (EP28A3C)(22).

**Reference individual**- a person selected for testing on the basis of well-defined criteria (EP28A3C) (22).

**Reference limit**- a value derived from the reference distribution and used for descriptive purposes; the descriptive of the reference values and may be distinguished from various other types of decision limits (EP28A3C) (22).

**Reference distribution**: the distribution of reference values (EP28A3C) (22).

**Serum Proteins Fractions**: Albumin, Alpha globulin, Beta globulin and Gamma globulin

**Electrophoresis**: Separation Technique (migration of charged particles in an electrical field) (**1**).

**Adult**: In this study refers to individuals aged 18 to 65 years

## 4.11 Ethical considerations

Ethical clearance and permission was obtained from Department of Medical Laboratory Science research and ethical review committee (DRERC), College of Health Science, Addis Ababa University. Permission was also obtained from Addis Ababa Health Bureau and was sought from the respective health institutions before the start of the data collection process.

The study participants was informed about the purpose of the study and the importance of their participation in the study by providing blood samples that may help Establishment of

Reference Interval for serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia.

The study participants was also informed that they can stop participating at any time if they want to do so. Then after assuring the confidentiality of the information obtained and completion of the informed consent from the study participants sample collection was preceded with strict privacy. Confidentiality of participant's data was maintained throughout the study by locking and limiting accessibility of the study information.

## 4.12 Dissemination of the result

Upon completion of the research, the finding of the study was submitted to Department of Medical Laboratory Science, Addis Ababa University and Ethiopian Public Health Institute. Oral presentation of the thesis will be made for both institutions. In addition the finding will also be presented on annual conferences. Up on completion of thesis defence, the findings of the study will be published on one of the peer reviewed journals.

# 5. Result

## 5.1 Socio-demographic characteristics of study participants

Out of 356 study participants recruited for the serum protein electrophoresis RI establishment, 297 apparently healthy adults (124 males and 173 females) were involved in the final analysis to establish the RI for serum protein electrophoresis from Addis Ababa, Ethiopia. The median age of the study participants was 30 years with interquartile range of 27-39 years. The distribution of study participants in terms of age and sex is depicted in **table 2**. Before doing the actual test, the collected serum samples were screened for highly sensitive C-reactive protein (hsCRP), lipemia and hemolysis. Among these, 34(9.55%), 13(3.65%), 11(3.09%), 1(0.28%) were rejected because of elevated hsCRP, both elevated hsCRP and lipemia, lipemia and hemolysis respectively.

**Table 2: Socio-demographic characteristics of study participants in Addis Ababa, Ethiopia 2020 (N = 297; Male = 124, and Female = 173).**

| Variables | | Frequency | % |
|---|---|---|---|
| Sex | Male | 124 | 41.75 |
| | Female | 173 | 58.25 |
| Age group | 18-24 | 40 | 13.49 |
| | 25-34 | 148 | 49.83 |
| | 35-44 | 60 | 20.20 |
| | 45-60 | 49 | 16.50 |
| Total | | 297 | 100 |

## 5.2 Normality test for laboratory data distribution

Primarily, continuous variables were tested for normality with Kolmogorov-Smirnov and Shapiro-Wilk test. The test showed normal distribution for Albumin (%), Alpha-1(%), Beta-1 (%), Gamma (%) and Albumin to Globulin Ratio. However, Albumin (g/dl), Alpha-1(g/dl), Alpha-2 (%), Alpha-2 (g/dl), Beta-1 (g/dl), Beta-2 (%), Beta-2 (g/dl), Gamma (g/dl) were failed to be normally distributed even after they were logarithmically transformed.

### 5.3 Reference intervals of serum protein electrophoresis

As shown in **table 3**, except for the Gamma and beta globulin fraction most of the serum protein fractions show significant difference between males and females. The established reference intervals with 90% CI for the six serum protein fractions including albumin to globulin ratio is summarized in **table 4**. They were: Albumin 53.83-64.59%, 52.24-63.55%; Alpha-1 globulin 3.04-5.40%, 3.44-5.60%; Alpha-2 globulin 8.0-12.67%, 8.44-12.87%; Beta-1 globulins 5.01-7.38%, 5.14-7.86%; Albumin to globulin ratio 1.16-1.81 and 1.09-1.74 for male and female, respectively. The combined RIs for Beta-2 globulin and Gamma globulin were 2.54-4.90% and 12.40-21.66% respectively.

**Table 3: The Mean, Median with IQR of serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia, 2020 (N = 297; Male = 124, and Female = 173).**

| Analytes | Unit | Sex | N | Range | Mean | 95%CI of mean | Median | $25^{th}$-$75^{th}$ Percentile (IQR) | p-value* |
|---|---|---|---|---|---|---|---|---|---|
| Albumin | % | Male | 124 | 53.5-65.9 | 59.28 | 58.80,59.76 | 59.10 | 57.60-61.28 | =0.0003 |
| | g/dl | | | 3.9-5.9 | 4.83 | 4.77,4.89 | 4.80 | 4.60-5.0 | <0.0001 |
| | | Female | 173 | 50.0-65.0 | 58.06 | 57.67,58.46 | 58.20 | 56.25-59.80 | |
| | | | | 4.0-5.5 | 4.59 | 4.55,4.64 | 4.60 | 4.40-4.80 | |
| | | Combined | 297 | 50.0-65.9 | 58.57 | 58.26,58.88 | 58.50 | 56.8,60.4 | |
| | | | | 3.9-5.9 | 4.69 | 4.66,4.73 | 4.70 | 4.50-4.90 | |
| Alpha-1 | % | Male | 124 | 2.6-5.8 | 4.23 | 4.13,4.33 | 4.10 | 3.82-4.68 | <0.0001 |
| | g/dl | | | 0.2-0.5 | 0.35 | 0.34,0.36 | 0.30 | 0.30-0.40 | =0.4735 |
| | | Female | 173 | 3.0-6.0 | 4.53 | 4.46,4.62 | 4.50 | 4.2-4.9 | |
| | | | | 0.2-0.5 | 0.36 | 0.35,0.37 | 0.40 | 0.30-0.40 | |
| | | Combined | 297 | 2.6-6.0 | 4.41 | 4.34,4.47 | 4.40 | 4.0,4.80 | |
| | | | | 0.2-0.5 | 0.36 | 0.35,0.36 | 0.40 | 0.30-0.40 | |
| Alpha-2 | % | Male | 124 | 7-13.6 | 9.80 | 9.58,10.01 | 9.50 | 9.0-10.60 | <0.0001 |
| | g/dl | | | 0.6-1.2 | 0.80 | 0.77,0.82 | 0.80 | 0.70-0.90 | =0.0006 |
| | | Female | 173 | 8-13 | 10.57 | 10.40,10.74 | 10.50 | 9.75-11.40 | |
| | | | | 0.6-1.2 | 0.84 | 0.82,0.86 | 0.80 | 0.75-0.90 | |
| | | Combined | 297 | 7.0-13.6 | 10.25 | 10.11,10.40 | 10.20 | 9.30,11.10 | |
| | | | | 0.6-1.2 | 0.82 | 0.81,0.84 | 0.80 | 0.70,0.90 | |
| Beta-1 | % | Male | 124 | 4.7-7.7 | 6.14 | 6.04,6.25 | 6.10 | 5.70-6.58 | =0.0015 |
| | g/dl | | | 0.4-0.7 | 0.50 | 0.49,0.51 | 0.50 | 0.50-0.50 | 0.8572 |
| | | Female | 173 | 4.8-7.9 | 6.38 | 6.29,6.48 | 6.40 | 6.0-6.70 | |
| | | | | 0.4-0.7 | 0.50 | 0.49,0.51 | 0.50 | 0.50-0.50 | |
| | | Combined | 297 | 4.7-7.9 | 6.28 | 6.21,6.36 | 6.30 | 5.90,6.70 | |
| | | | | 0.4-0.7 | 0.50 | 0.49,0.51 | 0.50 | 0.50-0.50 | |
| Beta-2 | % | Male | 124 | 2.4-5.3 | 3.62 | 3.51,3.72 | 3.60 | 3.20-4.0 | =0.1372 |
| | g/dl | | | 0.2-0.5 | 0.29 | 0.28,0.31 | 0.30 | 0.30-0.30 | =0.0319 |
| | | Female | 173 | 2.4-5.7 | 3.52 | 3.43,3.61 | 3.40 | 3.10-3.80 | |
| | | | | 0.2-0.5 | 0.28 | 0.27,0.29 | 0.30 | 0.20-0.30 | |
| | | Combined | 297 | 2.4-5.7 | 3.56 | 3.49,3.63 | 3.50 | 3.20,3.90 | |
| | | | | 0.2-0.5 | 0.29 | 0.28,0.29 | 0.30 | 0.20-0.30 | |
| Gamma | % | Male | 124 | 12-22 | 16.96 | 16.57,17.34 | 16.90 | 15.55-18.58 | =0.7145 |
| | g/dl | | | 0.80-2.1 | 1.38 | 1.34,1.43 | 1.40 | 1.20-1.50 | =0.1411 |
| | | Female | 173 | 11.2-23 | 16.93 | 16.60,17.26 | 16.80 | 15.30-18.30 | |
| | | | | 0.8-2.1 | 1.35 | 1.31,1.38 | 1.30 | 1.20-1.50 | |
| | | Combined | 297 | 11-23 | 16.94 | 16.69,17.19 | 16.90 | 15.45,18.30 | |
| | | | | 0.8-2.1 | 1.36 | 1.34,1.39 | 1.40 | 1.20-1.50 | |
| A/G ratio | | Male | 124 | 1.15-1.93 | 1.46 | 1.44,1.49 | 1.44 | 1.36-1.58 | =0.0005 |
| | | Female | 173 | 1.0-1.86 | 1.39 | 1.37,1.42 | 1.39 | 1.28-1.49 | |
| | | Combined | 297 | 1.0-1.93 | 1.42 | 1.41,1.44 | 1.41 | 1.31,1.53 | |

*****P-value: Mann-Whitney U test for male versus female; %: percent; g: gram; dl: deciliter; CI: confidence interval; RI: Reference interval; A/G ratio: Albumin to globulin ratio.

**Table 4: The Reference Interval with 90% CI of reference limits of serum protein electrophoresis of apparently healthy adults in Addis Ababa, Ethiopia, 2020 (N = 297; Male = 124, and Female = 173).**

| Analytes | Unit | Sex | N | Range | $2.5^{th}$-$97.5^{th}$ Percentile, RI | 90% CI (lower reference limit) | 90% CI (Upper reference limit) |
|---|---|---|---|---|---|---|---|
| Albumin | % | Male | 124 | 53.5-65.9 | 53.83-64.59 | 53.5-54.5 | 63.5-65.9 |
| | g/dl | | | 3.9-5.9 | 4.31-5.89 | 3.90-4.40 | 5.30-5.90 |
| | | Female | 173 | 50.0-65.0 | 52.24-63.55 | 50.0-53.6 | 62.0-65.0 |
| | | | | 4.0-5.5 | 4.10-5.20 | 4.0-4.20 | 5.10-5.50 |
| | | Combined | 297 | 50.0-65.9 | 53.18-64.10 | 52.10-53.80 | 63.30-64.90 |
| | | | | 3.9-5.9 | 4.1-5.36 | 4.0-4.20 | 5.20-5.90 |
| Alpha-1 | % | Male | 124 | 2.6-5.8 | 3.04-5.40 | 2.60-3.50 | 5.20-5.80 |
| | g/dl | | | 0.2-0.5 | 0.21-0.50 | 0.20-0.30 | 0.50-0.50 |
| | | Female | 173 | 3.0-6.0 | 3.44-5.60 | 3.0-3.70 | 5.5-6.0 |
| | | | | 0.2-0.5 | 0.30-0.50 | 0.20-0.30 | 0.50-0.50 |
| | | Combined | 297 | 2.6-6.0 | 3.4-5.6 | 3.0-3.50 | 5.40-5.80 |
| | | | | 0.2-0.5 | 0.30-0.50 | 0.2-0.3 | 0.5-0.5 |
| Alpha-2 | % | Male | 124 | 7-13.6 | 8.0-12.67 | 7.0-8.10 | 12.10-13.60 |
| | g/dl | | | 0.6-1.2 | 0.60-1.1 | 0.60-0.70 | 1.10-1.20 |
| | | Female | 173 | 8-13 | 8.44-12.87 | 7.90-8.60 | 12.50-13.30 |
| | | | | 0.6-1.2 | 0.64-1.10 | 0.60-0.70 | 1.0-1.20 |
| | | Combined | 297 | 7.0-13.6 | 8.04-12.76 | 7.90-8.30 | 12.5-13.3 |
| | | | | 0.6-1.2 | 0.60-1.10 | 0.6-0.7 | 1.10-1.10 |
| Beta-1 | % | Male | 124 | 4.7-7.7 | 5.01-7.38 | 4.80-5.20 | 7.30-7.90 |
| | g/dl | | | 0.4-0.7 | 0.40-0.69 | 0.40-0.40 | 0.60-0.70 |
| | | Female | 173 | 4.8-7.9 | 5.14-7.86 | 4.80-5.30 | 7.30-7.90 |
| | | | | 0.4-0.7 | 0.40-0.66 | 0.36-0.40 | 0.60-0.70 |
| | | Combined | 297 | 4.7-7.9 | 5.10-7.56 | 4.80-5.20 | 7.30-7.90 |
| | | | | 0.4-0.7 | 0.40-0.66 | 0.40-0.40 | 0.60-0.70 |
| Beta-2 | % | Male | 124 | 2.4-5.3 | 2.70-4.89 | 2.40-2.90 | 4.70-5.30 |
| | g/dl | | | 0.2-0.5 | 0.20-0.49 | 0.20-0.20 | 0.40-0.50 |
| | | Female | 173 | 2.4-5.7 | 2.50-4.96 | 2.40-2.60 | 4.80-5.70 |
| | | | | 0.2-0.5 | 0.20-0.40 | 0.20-0.20 | 0.40-0.50 |
| | | Combined | 297 | 2.4-5.7 | 2.54-4.90 | 2.40-2.70 | 4.80-5.40 |
| | | | | 0.2-0.5 | 0.20-0.40 | 0.20-0.20 | 0.40-0.50 |
| Gamma | % | Male | 124 | 12-22 | 12.05-21.43 | 11.70-13.10 | 20.80-21.70 |
| | g/dl | | | 0.80-2.1 | 0.91-1.90 | 0.80-1.0 | 1.80-2.10 |
| | | Female | 173 | 11.2-23 | 12.58-22.26 | 11.20-13.70 | 21.0-23.0 |
| | | | | 0.8-2.1 | 0.90-1.86 | 0.80-1.0 | 1.80-2.10 |
| | | Combined | 297 | 11-23 | 12.40-21.66 | 11.70-13.10 | 20.90-22.80 |
| | | | | 0.8-2.1 | 0.90-1.90 | 0.80-1.0 | 1.80-2.0 |
| A/G ratio | | Male | 124 | 1.15-1.93 | 1.16-1.81 | 1.15-1.2 | 1.73-1.93 |
| | | Female | 173 | 1.0-1.86 | 1.09-1.74 | 1.0-1.16 | 1.63-1.86 |
| | | Combined | 297 | 1.0-1.93 | 1.14-1.77 | 1.09-1.16 | 1.72-1.85 |

RI: Reference interval; %: percent; g: gram; dl: deciliter; CI: confidence interval; A/G ratio: Albumin to globulin ratio.

The current established RIs of Apparently Healthy Adults in Addis Ababa, Ethiopia were compared with manufacturer provided RIs and with already published RIs (Table 5). The lower limit of combined RIs established in the current study is higher than manufacturer provided RIs except for albumin and beta-2 globulin. The Upper reference limit of alpha-1 globulin is lower than study conducted in Belgium (18) but higher for Alpha-2 globulin. Comparison of the published RIs obtained with Paragon and with Capillarys is summarized in **table 5.**

**Table 5: Comparison of serum protein electrophoresis RIs of apparently healthy adults in Addis Ababa, Ethiopia with manufacturer ranges and published RIs.**

| References | Instrument(Software Version) | | N | Age (Year) | Albumin | Alpha-1 | Alpha-2 | Beta-1 | Beta-2 | Gamma | A/G ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Current Study) | Capillarys (5.2.1) | M | 124 | 18-65 | 53.83-64.59 | 3.04-5.40 | 8.00-12.67 | 5.01-7.38 | 2.70-4.89 | 12.05-21.43 | 1.16-1.81 |
| | | F | 173 | 18-65 | 52.24-63.55 | 3.44-5.60 | 8.44-12.87 | 5.14-7.86 | 2.50-4.96 | 12.58-22.26 | 1.09-1.74 |
| | | C | 297 | 18-65 | 53.18-64.10 | 3.40-5.60 | 8.04-12.76 | 5.10-7.56 | 2.54-4.90 | 12.40-21.66 | 1.14-1.77 |
| Sebia, France (**45**) | Capillarys (5.2.1) | | 246 | NA | 55.8-66.1 | 2.9-4.9 | 7.1-11.8 | 4.7-7.2 | 3.2-6.5 | 11.1-18.8 | NA |
| USA (**19**) | Paragon | | NA | NA | 52-67 | 3.8-8.3 | 5.8-12.4 | NA | NA | 9.3-20.4 | NA |
| Belgium(**18**) | Paragon(1.08) | M | 82 | 18-65 | 60-70 | 3.9-6.4 | 4.9-9.3 | NA | NA | 7.9-18.3 | NA |
| | | F | 79 | 18-65 | 56-70 | 3.6-7.4 | 5.6-9.7 | NA | NA | 7.9-18.3 | NA |
| Switzerland (**20**) | Paragon | | NA | NA | 57-70 | 3.8-7.8 | 4.4-10.0 | NA | NA | 9.0-17.4 | NA |
| Japan (**47**) | Paragon(1.16) | | 63 | NA | 56-69 | 3.9-6.3 | 4.8-8.4 | NA | NA | 9.9-21.5 | NA |
| Italy (**21**) | Paragon | | 167 | NA | 54-68 | 3.7-8.8 | 5.5-11.7 | NA | NA | 10-20.3 | NA |
| | | | | NA | 53-70 | 4.4-9.3 | 6.4-10.3 | NA | NA | 11-16.8 | NA |
| Italy (**48**) | Paragon(1.6) | | NA | NA | 52.3-68.9 | 4.8-9.8 | 8.9-12.8 | NA | NA | 10.4-16.2 | NA |
| Belgium(**3**) | Capillarys(1.41) | | 50 | NA | 56-66 | 2.0-6.9 | 5.9-11.4 | NA | NA | 8.5-12.9 | NA |
| France (**49**) | Capillarys(4.14) | M | 60 | NA | 50.5-62.7 | 4.8-8.3 | 7.1-12.2 | NA | NA | 10.2-18.5 | NA |
| | | F | 60 | | | | | | | | |

M: Male; F: Female; C: combined; A/G ratio Albumin to globulin ratio; NA: Not Available

## 6. Discussion

Reference intervals are useful to interpret laboratory results to screen, diagnose and monitor different disease states in patient management as well as research undertakings including in clinical trials. Due to lack of locally established reference intervals, the clinical laboratory reference intervals currently being in use in many African countries including Ethiopia are provided by companies based on mainly western populations (23). There is also paucity of data on reference intervals for serum protein fractions in the literature.

The marked difference in the existing established reference intervals among population groups may lead to either unnecessary additional investigations or failure to detect underlying disease or mismanagement of patients (24).

Since little or no reference interval data is available in poor setting countries like Ethiopia, the current study established reference interval for serum protein fractions which serve as baseline data for the diagnosis, interpretation and monitoring of problems associated with serum proteins fractions in clinical laboratories.

The reference intervals provided by manufacturer of the reagents France (43) and other published RIs in Belgium (3), USA (19), Switzerland (20), Japan (45), Italy (46) and France (47) revealed a combined reference interval for both male and female for all serum protein fractions. But, study conducted by Lim et al (44) recommended establishment of gender specific reference interval which plays a crucial role in laboratory result interpretation of serum protein fractions. As a result, current established reference interval provides gender specific reference interval for serum protein fractions.

In the current study, Non-parametric test (Mann-Whitney U test) reveals statistically significant gender specific differences for Albumin and Alpha-2 globulin (expressed both in percentage and absolute concentration); Alpha-1, Beta-1 and albumin to globulin ratio. However, the difference of Beta-2 and Gamma globulin were not statistically significant. The Study is in agreement with a study conducted by Bossuyt et al Belgium (18), which showed insignificant gender specific differences in gamma globulin RIs.

The upper percentile of RIs established for albumin in current study is lower than either RIs provided by manufacturer of reagent France (43) or already published RIs in Belgium(3,18),

USA (19), Switzerland (20), Italy (21,46), Japan (45) but higher than RIs established by Lissoir et al in France (47) .The lower percentile for albumin is lower than already published RIs in Belgium (3, 18), Switzerland (20), France (43) and Japan (45) but higher than that of USA (19), Italy (46) and France (47). The discrepancies may be attributed to genetic makeup and feeding styles of study participants involved in RIs establishment. The Lower percentile is concordant with RIs established by Petrini et al in Italy (21).

In the established RIs, females have higher upper limit ($97.5^{th}$ percentile) for all globulin fractions (Alpha-1 globulin, alpha-2 globulin, beta-1 globulin, beta-2 globulin and gamma globulin) than males. The study is in agreement with findings of Bossuyt et al in Belgium (18) for alpha and alpha 2 but differs in gamma globulin which is the same for male and females. The variation may be resulted from difference in antibody production during exposure to different disease states.

The current established reference interval of serum protein electrophoresis fractions (albumin, alpha globulin, beta globulin and gamma globulin RIs) is different from previously published reference interval study conducted in Belgium on 161 apparently healthy adults aged 18-65 years by Bossuyt et al Belgium (18).

Formerly, albumin to globulin ratio (A/G ratio) was mainly used for the diagnosis of liver function and immunological diseases. In recent times, it has been reported as a novel inflammatory indicator for prognosis in various cancers such as colorectal cancer, lung cancer, esophageal cancer, and breast cancer (48).The study established RIs for albumin to globulin ratio which is not provided by manufacturer of reagents France (43) or study conducted by Bossuyt et al Belgium (18) or other published RIs in Belgium (3,18), USA (19), Switzerland (20), Italy (21,46), Japan (45) and France (47).

Except for beta-2 globulin, the current established RIs reveals higher upper percentile in all globulin fractions (alpha-1 globulin, alpha-2 globulin, beta-1 globulin, beta-2 globulin and gamma globulin) compared to manufacturer of reagents France (43). The variations may be attributed to racial differences (genetic predispositions) (44).

# 7. Strength and Limitation

## 7.1. Strength

The present study establishes gender specific reference intervals for serum protein fractions, for the first time in the country and provides baseline data for future studies and hence has a prime importance in interpretation of laboratory test results of serum protein fractions. The analysis was done in the National reference laboratory which participates in external quality assurance as well as accredited by ENAO.

## 7.2 Limitation of the study

There was no published similar study in Ethiopia to compare with the present study

# 8. Conclusion and Recommendation

## 8.1 Conclusion

The present study established RI for albumin, alpha-1 globulin, alpha-2 globulin, beta-1 globulin, beta-2 globulin, gamma globulin and albumin to globulin ratio. The established RI revealed gender specific differences in reference interval except for Beta-2 globulin and Gamma globulin.

## 8.2 Recommendation

Since the current study revealed the difference in gender-specific reference intervals for serum protein fractions, the use of locally established gender specific reference interval is recommended for patients, clinicians, community, and other researchers, policy makers to avoid misinterpretation of laboratory results. Moreover, similar studies need to expand in Ethiopia, since there was no any published RIs data about Serum protein electrophoresis.

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## Annex I: Standard operating procedure (SOP) for Capillary Electrophoresis

### A. Sample Collection

Blood samples were collected from the anti-cubital vein of the arm by using syringes after proper antisepsis with alcohol and sterile cotton swabs. Then the blood from each participant was transferred to serum separator tube and allowed to stand for 30 minutes. Serum was separated by centrifugation at 4000 rpm. All the medical equipment used for blood collections were safe and sterile.

**Procedure for serum separation**

1. 5 ml whole blood was drawn into serum separator tube containing no anticoagulant.
2. It was kept in upright position at room temperature for 30-45 min to allow clotting.
3. It was centrifuged for 5 min at manufacturer's recommended speed 4000 rpm.
4. The serum was carefully aspirated at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. A clean pipette for each tube was used.
5. Serum was inspected for turbidity and hemolysis.
6. Aliquot into cryo-vials and stored at –80 °C. The cryo-vials were labeled with participant's identification number.

### B. Serum Protein Capillary Electrophoresis

**Clinical Utility (**43**):**

The *capillarys 2 flex-piercing* protein (e) 6 kits is designed for the separation of human serum proteins in alkaline buffer (pH 9.9) by capillary Electrophoresis with the *capillarys 2 flex-piercing* System into six major fractions.

The *capillarys 2 flex-piercing* performs all sequences automatically to obtain a protein profile for qualitative or quantitative analysis. The proteins, separated in silica capillaries, are directly detected at an absorbance of 200 nm. The electro-phoregram(s) can be interpreted visually to screen for any pattern abnormalities. Direct detection provides accurate relative quantification of individual protein fractions.

**Principle of the test (**43**)**

The *capillarys 2 flex-piercing* System uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electro-osmotic flow.

The *capillarys 2 flex-piercing* System has 8 capillaries functioning in parallel allowing 8 simultaneous analyses. A sample dilution with buffer is prepared and injected by aspiration at the anodic end of the capillary. A high voltage protein separation is then performed and direct detection of the proteins is made at 200 nm at the cathodic end of the capillary. The capillaries are immediately washed with a Wash Solution and prepared for the next analysis with buffer.

Proteins are detected in the following order: gamma globulins, beta-2 globulins, beta-1 globulins, alpha-2 globulins, alpha-1 globulins and albumin with each zone containing one or more proteins.

**Reagents and materials supplied in the** *capillarys 2 flex-piercing* **protein (e) 6 kits**

A. **Buffer (ready to use):** contains buffer solution pH 9.9 ± 0.5, additives, nonhazardous at concentrations used, necessary for optimum performance of capillary electrophoresis in protein analysis.
B. **Wash Solution:** Useful for washing the capillaries after protein electrophoretic separation.
C. **Dilution Segments:** important for sample dilution on the automated instrument.
D. **Filters:** Useful for filtration of analysis buffer, working wash solution and distilled water (used for capillaries rinsing).

Reagents required but not supplied

A. **Distilled or deionized water:** Useful for capillaries rinsing in automated system *capillarys 2 flex-piercing,* SEBIA, for capillary electrophoresis.
B. **Capi-clean:** contains proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances. It is useful for sample probe cleaning in automated system *capillarys 2 flex-piercing,* SEBIA, for capillary electrophoresis, during the Capiclean cleaning sequence.

C. **Sodium hypochlorite solution (for sample probe cleaning):** Useful for the sample probe cleaning in the *capillarys 2 flex-piercing* System (weekly maintenance in order to eliminate adsorbed proteins from the probe).

D. *capillarys 2 flex-piercing* wash solution: Useful for additional washing of the capillaries of *capillarys 2 flex-piercing* when the number of tests by serie is below 40.

**Procedure:**

The *capillarys 2 flex-piercing* system is a multi-parameter instrument for serum proteins analysis on 8 parallel capillaries in the following sequence:

1. Bar code reading of sample tubes (for up to 8 tubes) and samples-racks
2. Sample dilution from primary tubes into dilution segments
3. Capillary washing
4. Injection of diluted samples
5. Migration under constant voltage, controlled by Peltier effect for about 4 minutes
6. Detection of Serum Protein by directly scanning at 200 nm and an electrophoretic profile appears on the screen of the system.

**The manual steps include:**

1. Placement of sample tubes in sample-racks
2. Placement of racks on the *capillarys 2 flex-piercing* instrument
3. Removal of sample-racks after analysis.

**Note: Quality control serum is included with each sequence of protein analysis.**

## Annex II: Laboratory Data Extraction form

| Residence | Code | Age | Sex | Result of Serum Protein Fractions | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Percentages (%) | | | | | | | Absolute Concentrations(g/dl) | | | | | | |
| | | | | ALB | α1 | α2 | β1 | β2 | Γ | A/G | ALB | α1 | α2 | β1 | β2 | γ | A/G |
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**Note: ALB=Albumin, α1=alpha-1 globulin, α2=alpha-2 globulin, β1=beta-1 globulin, β2=beta- 2 globulin, γ =globulin, A/G=Albumin to globulin ratio**

## Declaration

I, the undersigned, declare that this MSc. 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.

**MSc. candidate:** **Demiraw Bikila (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

**Advisor:** **Mistire Wolde (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

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

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.