



## The Reference Intervals of Serum Protein Electrophoresis in Thai Populations

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### Abstract

Serum protein electrophoresis (SPEP) is an essential analytical technique for assessing the distribution of proteins in serum, and some laboratories rely on reference intervals (RIs) provided by reagent manufacturers. However, these RIs may not adequately reflect population-specific biological variations. Therefore, the establishment of population-based RIs for Thai individuals was performed in the present study to improve diagnostic accuracy for several disorders that are associated with protein abnormalities, such as plasma cell dyscrasias, hepatic diseases, and renal disorders. A total of 409 individuals aged 17-66 years were recruited for SPEP using both capillary-based and gel-based electrophoresis. In the capillary electrophoresis, RIs for albumin, alpha-1, alpha-2, beta-1, beta-2, and gamma globulins were 53.3-65.6% (4.13-5.37 g/dL), 2.4-4.1% (0.19-0.33 g/dL), 5.9-10.6% (0.46-0.86 g/dL), 4.7-7.4% (0.37-0.60 g/dL), 3.6-6.9% (0.27-0.56 g/dL), and 12.8-22.7% (0.94-1.91 g/dL), respectively. In the gel electrophoresis, RIs for albumin, alpha-1, alpha-2, beta-1, beta-2, and gamma globulins were 47.0-61.1% (3.70-4.93 g/dL), 1.9-4.0% (0.15-0.32 g/dL), 6.2-12.0% (0.48-0.98 g/dL), 7.1-11.3% (0.54-0.93 g/dL), 2.8-7.6% (0.22-0.62 g/dL), and 13.9-25.1% (1.02-2.11 g/dL), respectively. Comparative analysis demonstrated statistically significant differences between the RIs that were derived from capillary-based and gel-based electrophoresis for all protein fractions, with the exception of beta-2 globulin. Hence, we proposed the RIs of SPEP for implementation in Thai population.

**Keywords:** Reference intervals, Serum protein electrophoresis (SPEP), Capillary electrophoresis, Gel electrophoresis, Thai blood donor

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## การศึกษาช่วงค่าอ้างอิงของ Serum Protein Electrophoresis ในประชากรไทย

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และ อัมภาศ สัพพนิชกุล<sup>1, 3, 4</sup><sup>1</sup>ฝ่ายวิชาจุลชีววิทยา โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย กรุงเทพมหานคร<sup>2</sup>ฝ่ายผลิตน้ำยาแอนติซีรัมและผลิตภัณฑ์เซลล์ ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย กรุงเทพมหานคร<sup>3</sup>ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร<sup>4</sup>ฝ่ายอายุรศาสตร์ โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย กรุงเทพมหานคร

## บทคัดย่อ

การตรวจวิเคราะห์โปรตีนในซีรัมด้วยวิธี serum protein electrophoresis (SPEP) เป็นการตรวจที่ใช้กันอย่างแพร่หลายในการประเมินความผิดปกติของโปรตีนในสิ่งส่งตรวจ อย่างไรก็ตามห้องปฏิบัติการหลายแห่งยังคงใช้ช่วงค่าอ้างอิงจากเอกสารกำกับน้ำยา ซึ่งอาจไม่สอดคล้องกับลักษณะของประชากรท้องถิ่น การกำหนดช่วงค่าอ้างอิง (reference intervals, RIs) ที่เหมาะสมสำหรับประชากรไทยจะช่วยเพิ่มความแม่นยำในการวินิจฉัยโรคที่เกี่ยวข้องกับความผิดปกติของโปรตีน เช่น ภาวะไขกระดูกสร้างโปรตีนผิดปกติ (myeloma) โรคตับ และโรคไต ชัดเจนยิ่งขึ้น ลดการวินิจฉัยผิดพลาด และสนับสนุนการติดตามการรักษาได้อย่างแม่นยำ วัตถุประสงค์ของงานวิจัยนี้คือเพื่อกำหนดช่วงค่าอ้างอิงของการตรวจวิเคราะห์ระดับ SPEP โดยศึกษาในกลุ่มตัวอย่างผู้บริจาคโลหิตชาวไทย จำนวน 409 ราย อายุระหว่าง 17-66 ปี และทำการตรวจวิเคราะห์ระดับ SPEP โดยใช้หลักการ capillary electrophoresis และ gel electrophoresis ข้อมูลที่ได้วิเคราะห์โดยใช้ซอฟต์แวร์ statistical package for social sciences (SPSS 23.0, SPSS Inc., IL, USA) ผลการศึกษาได้ช่วงค่าอ้างอิง SPEP ด้วยหลักการ capillary electrophoresis สำหรับ albumin, alpha-1 globulin, alpha-2 globulin, beta-1 globulin, beta-2 globulin และ gamma globulin คือ 53.3-65.6% (4.13-5.37 g/dL), 2.4-4.1% (0.19-0.33 g/dL), 5.9-10.6% (0.46-0.86 g/dL), 4.7-7.4% (0.37-0.60 g/dL), 3.6-6.9% (0.27-0.56 g/dL) และ 12.8-22.7% (0.94-1.91 g/dL) ตามลำดับ ขณะที่ค่าช่วงอ้างอิงของ SPEP ด้วยหลักการ gel electrophoresis คือ 47.0-61.1% (3.70-4.93 g/dL), 1.9-4.0% (0.15-0.32 g/dL), 6.2-12.0% (0.48-0.98 g/dL), 7.1-11.3% (0.54-0.93 g/dL), 2.8-7.6% (0.22-0.62 g/dL) และ 13.9-25.1% (1.02-2.11 g/dL) ตามลำดับ พบว่า RIs ที่ตรวจด้วยหลักการ capillary electrophoresis และ gel electrophoresis มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ยกเว้นสำหรับ beta-2 globulin ช่วงค่าอ้างอิงดังกล่าวอาจจะสามารถนำไปใช้เป็นข้อมูลอ้างอิงสำหรับประชากรไทยได้อย่างเหมาะสม

**คำสำคัญ:** ช่วงค่าอ้างอิง Serum protein electrophoresis (SPEP), Capillary electrophoresis, Gel electrophoresis, ผู้บริจาคโลหิตชาวไทย

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## 1. Introduction

Proteins within the immune system are essential components involved in the production of antibodies and imminent downstream functioning of various immune cells. They play crucial roles in detecting and eliminating foreign agents, inclusive of bacteria and viruses. Key proteins within this system comprise immunoglobulins, cytokines, and complement proteins. The intake of high-quality dietary proteins from sources inclusive of meat, eggs, fish, tofu, legumes, and milk is essential toward developing a robust immune system which enhances the body's capability to remove infections. Immunoglobulins (Ig) or commonly referred to as antibodies are produced by B lymphocytes and are crucial proteins within the immune system. They are designed to specifically bind to foreign molecules known as antigens and initiate processes that eliminate the antigens, a mechanism known as humoral immunity.<sup>(1)</sup> In terms of humoral immune response (HIR), activated B lymphocytes proliferate and differentiate into plasma cells, which produce and secrete immunoglobulins into the bloodstream and various other body fluids.<sup>(2)</sup> Immunoglobulins, which are also found within the serum component of whole blood, function as antibodies which specifically bind to antigens, trigger various effector functions, and enable antigen elimination. Serum enriched with high concentration of antibodies capable of pathogen- or toxin-binding are referred to as antiserum. When proteins are separated by electrophoresis, most immunoglobulins are found in the gamma ( $\gamma$ ) globulin region, although some may also be present in the beta ( $\beta$ ) and alpha ( $\alpha$ ) regions. In healthy individuals, immunoglobulins account for approximately 20% of total serum proteins. However, during infection or in response to antigenic stimulation, immunoglobulin levels increase. Serum protein electrophoresis (SPEP) maintains its role among the most important diagnostic and monitoring methods for multiple myeloma due to operational convenience and speed in quantifying the level of myeloma cell-derived M protein. Quantity of M protein correlates with the amount and/or activity of myeloma cells, except in cases of non-secretory myeloma. SPEP separates and quantifies proteins based on their electrical charge, size, and shape. Serum proteins are broadly classified into two main groups, albumin and globulins. Globulins can be further subdivided into several fractions, which typically appear on SPEP as alpha-1, alpha-2, beta-1, beta-2, and gamma globulins. Most immunoglobulins are found in the gamma globulin fraction. Typically, M protein produced by myeloma cells is detected in the gamma globulin region of the electrophoretic graph. However, in the case of IgA, the protein may occasionally migrate to the beta or even the alpha-2 region. In such cases, supplementary tests become diagnostically valuable as opposed to SPEP as a stand-alone method. Monoclonal immunoglobulins appear on the graph as a sharp, narrow-based spike due to the uniformity of the protein molecules, which show identical structure and electrical charge. As a result, monoclonal immunoglobulins collectively migrate and form a distinct peak—commonly referred to as the “M spike”—in the gamma region and the respective spike amplitude reflects myeloma cell activities. Pathologists quantify the concentration of M protein through calculating the area under the curve (AUC) of the spike subtracted by background immunoglobulin levels. Serum albumin, which is synthesized exclusively by hepatocytes in the liver, typically accounts for approximately 55% of total plasma proteins. In multiple myeloma, increased production of specific cytokines interferes with the liver's ability to synthesize albumin, thus decreasing serum albumin levels. According to the Revised International Staging System (R-ISS), serum albumin and serum beta-2 microglobulin ( $\beta$ 2-microglobulin,  $\beta$ 2M) are two key blood proteins used to predict disease prognosis at diagnosis. Additionally, SPEP is commonly performed in conjunction with immunofixation electrophoresis (IFE) to identify the specific immunoglobulin type and with the Serum Free Light Chain (SFLC) assays to improve diagnostic accuracy. In Thailand, standardized

clinical reference ranges for SPEP, including capillary-based and gel-based electrophoresis<sup>(1,2)</sup>, have yet to be established, and predominantly rely on manufacturer-provided reference intervals (RIs), which may not accurately reflect biological characteristics of the local population. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines,<sup>(3)</sup> each country is required to establish RIs for its own population to ensure accurate and reliable laboratory test interpretation and diagnosis. As such, RIs for SPEP may vary across different populations, and such data are essential for research, clinical diagnosis, and therapeutic monitoring in diverse demographic groups.<sup>(4)</sup> Establishing normal reference ranges for serum SPEP represents a key approach in immunological assessment. Although SPEP levels may vary geographically, these differences are generally not clinically significant; however, detailed knowledge of ethnic and population-specific variations is critical for precise clinical interpretation.<sup>(5)</sup> In the present study, blood samples from Thai blood donors were analyzed using both capillary electrophoresis and gel electrophoresis. The results were expected to enable clinicians to make accurate diagnoses in the Thai population and provide laboratories with robust, population-specific reference data to support precise interpretation of SPEP results.

## 2. Materials and Methods

### 2.1 Sample Collection and Measurement

A total of 409 serum samples were collected from Thai blood donors. The sample size was determined using Taro Yamane's formula, based on the average number of blood donors over the preceding three years, as follows.

$$n = \frac{N}{1 + N(e)^2}$$

$$n = \frac{500000}{1 + 500000(0.05)^2} \quad n = \sim 399.99 \text{ samples}$$

N represents the average number of blood donors over the past three years (500,000)

e denotes the acceptable margin of error (5%)

Accordingly, a total of 409 samples were obtained by considering a 3% rejection rate from already collected samples.

The sample selection criteria were defined to minimize pre-analytical variability, excluding hemolyzed, lipemic, and icteric serum. All donor samples had undergone infectious disease screening at the Laboratory Screening Division, National Blood Centre, Thai Red Cross Society. Samples with positive infectious disease screening results were excluded from the study. This study was approved by the Human Research Ethics Committee of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand (project approval number: NBC 14/2568), in accordance with ethical standards for research involving human subjects. The automated capillary electrophoresis analyzer (Capillarys 2 Flex Piercing, EVRY Cedex, France), the automated gel electrophoresis analyzer (SPIFE Nexus, Texas, USA) were used in accordance with the manufacturers' instructions. For the capillary electrophoresis, the protein separation was achieved by applying a high-voltage electric field ranging from 10,000 to 30,000 volts across capillary tubes with inner diameters of 25-100  $\mu\text{m}$ , which were filled with an electrolyte solution. The ends of each capillary tube were immersed in reservoirs containing electrolyte solution. Upon application of the electric field, ions within the serum migrated from the anode to the cathode under the influence of electroosmotic flow

(EOF). The separated protein zones then passed through a detection window, where signals generated by the detector were transmitted to a data processor for analysis. The resulting output, known as an electropherogram, provided quantitative and qualitative assessment of the major serum protein fractions. Additionally, the RIs for SPEP by capillary electrophoresis have been established from a cohort of 225 healthy adult males and females in France<sup>(5)</sup> in Table 1, which serve as baseline values for interpreting electrophoretic patterns in clinical practice. For gel electrophoresis, the protein separation was achieved through electrophoresis on agarose gel based on the electrical charge of each protein molecule, utilizing both electrophoretic force and electroendosmotic flow. Negatively charged proteins migrated toward the anode, whereas positively charged proteins migrated toward the cathode. Albumin, having the highest mobility, migrated the fastest and formed a band near the anode, whereas gamma globulins migrated the slowest, forming a band near the cathode. Following electrophoretic separation, the proteins were stained with a visible dye to allow densitometric analysis for quantification and characterization. The RIs for SPEP by gel electrophoresis have been established from a cohort of 47 healthy adult males and females in the United States in Table 1, serving as baseline values for clinical interpretation.

**Table 1** Reference intervals obtained from the package inserts provided by the manufacturers.<sup>(5)</sup>

| Protein Zone<br>Electrophoresis | Capillary electrophoresis |                        | Gel electrophoresis |                        |
|---------------------------------|---------------------------|------------------------|---------------------|------------------------|
|                                 | Reference range (%)       | Reference range (g/dl) | Reference range (%) | Reference range (g/dl) |
| Albumin                         | 55.8-66.1                 | 4.02-4.76              | 45-61.6             | NA                     |
| Alpha-1 globulin                | 2.9-4.9                   | 0.21-0.35              | 1.9-4.3             | NA                     |
| Alpha-2 globulin                | 7.1-11.8                  | 0.51-0.85              | 6.8-13.7            | NA                     |
| Beta-1 globulin                 | 4.7-7.2                   | 0.34-0.52              | 1.7-11.4            | NA                     |
| Beta-2 globulin                 | 3.2-6.5                   | 0.23-0.47              | 4.6-8.3             | NA                     |
| Gamma globulin                  | 11.1-18.8                 | 0.80-1.35              | 8.9-25.8            | NA                     |

NA, not available.

## 2.2 Quality Control and Data Analysis

Internal quality control (IQC) of SPEP was performed by analyzing normal control serum daily to assess the reliability and performance of the reagents used in the assays. This practice also served to monitor potential analytical interferences. Specific precautions were observed, as certain sample conditions could affect the accuracy of the results. For example, hemolyzed serum may lead to the appearance of a double alpha-2 globulin zone and lipemic serum can cause spurious elevations in protein fractions, while icteric serum may result in artificially increased albumin measurements. Implementing these IQC measures ensured consistency, accuracy, and reproducibility of SPEP results throughout the study. Data were recorded in Microsoft Excel and subsequently imported into the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., IL, USA) for analysis. Descriptive statistics, including mean, median, standard deviation, 95% confidence intervals, and range, were calculated for each protein fraction. Differences between groups were evaluated using the Mann-Whitney and Kruskal-Wallis tests. A  $p$ -value < 0.05 was considered statistically significant. RIs were established using a non-parametric method in accordance with the CLSI EP28-A3c guideline.<sup>(3)</sup> The lower and upper limits, representing the central 95% distribution with 90% confidence intervals, were calculated. The results were expressed as percentages and absolute concentrations and presented in tables.

### 3. Result

#### 3.1 Characteristics of the Study Participants

The study initially recruited a total of 414 blood donors from the National Blood Centre, Thai Red Cross Society. Upon screening, five participants were identified as exhibiting a monoclonal protein pattern in the gamma-globulin zone and were therefore excluded from the study. Consequently, the final study population comprised 409 donors, aged between 17 and 66 years, with a mean age of 40 years. The cohort included 184 males and 225 females. For analytical purposes, participants were stratified into five age groups and six regional groups, with regional classification based on the population distribution across regions in Table 2. All serum samples tested negative for infectious disease markers according to the standard screening protocols of the National Blood Centre, Thai Red Cross Society. These demographic and health screening data provided a representative baseline for the establishment of RIs for SPEP in the Thai adult population.

**Table 2** Socio-demographic characteristics of 409 volunteer blood donors included in this study.

| Variables         |                     | Frequency | Percent (%) |
|-------------------|---------------------|-----------|-------------|
| Sex               | Male                | 184       | 45.0        |
|                   | Female              | 225       | 55.0        |
| Age group (years) | 17-24               | 67        | 16.4        |
|                   | 25-34               | 97        | 23.7        |
|                   | 35-44               | 97        | 23.7        |
|                   | 45-54               | 78        | 19.1        |
|                   | 55-66               | 70        | 17.1        |
| Region            | Central region      | 195       | 47.7        |
|                   | Northern region     | 50        | 12.2        |
|                   | Southern region     | 55        | 13.4        |
|                   | Northeastern region | 48        | 11.7        |
|                   | Eastern region      | 35        | 8.6         |
|                   | Western region      | 26        | 6.4         |
| Total             |                     | 409       | 100         |

#### 3.2 RIs of SPEP by Capillary Electrophoresis

The RIs of serum protein fractions obtained by capillary electrophoresis are presented as mean, median, 95% confidence interval (CI), and range in Table 3. The study also evaluated the relationships between sample subgroups and the established RIs using capillary electrophoresis, as illustrated in Fig. 1. These analyses provided a comprehensive overview of the distribution and variability of serum protein fractions in the Thai adult population and served as essential baseline data for clinical interpretation.



**Table 3** Reference intervals of serum protein electrophoresis (SPEP) by capillary electrophoresis.

| Analyte          | Sex      | N   | Unit | Mean | SD   | Median | 95% CI     | Range     |
|------------------|----------|-----|------|------|------|--------|------------|-----------|
| Albumin          | Male     | 184 | %    | 60.7 | 2.9  | 60.9   | 60.3, 61.1 | 55.0-66.4 |
|                  |          |     | g/dL | 4.84 | 0.33 | 4.80   | 4.79, 4.88 | 4.19-5.48 |
|                  | Female   | 225 | %    | 58.4 | 2.9  | 58.5   | 58.0, 58.8 | 52.6-64.2 |
|                  |          |     | g/dL | 4.68 | 0.28 | 4.69   | 4.64, 4.71 | 4.12-5.23 |
|                  | Combined | 409 | %    | 59.4 | 3.1  | 59.5   | 59.1, 59.7 | 53.3-65.6 |
|                  |          |     | g/dL | 4.75 | 0.31 | 4.73   | 4.72, 4.78 | 4.13-5.37 |
| Alpha-1 globulin | Male     | 184 | %    | 3.2  | 0.4  | 3.1    | 3.1, 3.2   | 2.4-3.9   |
|                  |          |     | g/dL | 0.25 | 0.03 | 0.25   | 0.25, 0.26 | 0.19-0.31 |
|                  | Female   | 225 | %    | 3.4  | 0.4  | 3.3    | 3.3, 3.4   | 2.5-4.2   |
|                  |          |     | g/dL | 0.27 | 0.04 | 0.27   | 0.26, 0.27 | 0.20-0.34 |
|                  | Combined | 409 | %    | 3.3  | 0.4  | 3.2    | 3.2, 3.3   | 2.4-4.1   |
|                  |          |     | g/dL | 0.26 | 0.04 | 0.26   | 0.26, 0.26 | 0.19-0.33 |
| Alpha-2 globulin | Male     | 184 | %    | 8.0  | 1.1  | 7.8    | 7.8, 8.1   | 5.7-10.2  |
|                  |          |     | g/dL | 0.63 | 0.10 | 0.62   | 0.62, 0.65 | 0.44-0.83 |
|                  | Female   | 225 | %    | 8.5  | 1.1  | 8.5    | 8.4, 8.7   | 6.3-10.8  |
|                  |          |     | g/dL | 0.68 | 0.10 | 0.68   | 0.67, 0.70 | 0.49-0.88 |
|                  | Combined | 409 | %    | 8.3  | 1.2  | 8.3    | 8.1, 8.4   | 5.9-10.6  |
|                  |          |     | g/dL | 0.66 | 0.10 | 0.66   | 0.65, 0.67 | 0.46-0.86 |
| Beta-1 globulin  | Male     | 184 | %    | 6.0  | 0.6  | 5.9    | 5.9, 6.1   | 4.7-7.2   |
|                  |          |     | g/dL | 0.47 | 0.06 | 0.47   | 0.47, 0.48 | 0.37-0.58 |
|                  | Female   | 225 | %    | 6.1  | 0.7  | 6.1    | 6.0, 6.2   | 4.8-7.5   |
|                  |          |     | g/dL | 0.49 | 0.06 | 0.48   | 0.48, 0.50 | 0.37-0.61 |
|                  | Combined | 409 | %    | 6.1  | 0.7  | 6.0    | 6.0, 6.1   | 4.7-7.4   |
|                  |          |     | g/dL | 0.48 | 0.06 | 0.48   | 0.48, 0.49 | 0.37-0.60 |
| Beta-2 globulin  | Male     | 184 | %    | 5.2  | 0.8  | 5.1    | 5.1, 5.3   | 3.6-6.8   |
|                  |          |     | g/dL | 0.41 | 0.07 | 0.40   | 0.40, 0.42 | 0.27-0.56 |
|                  | Female   | 225 | %    | 5.2  | 0.9  | 5.1    | 5.1, 5.3   | 3.6-6.9   |
|                  |          |     | g/dL | 0.42 | 0.07 | 0.42   | 0.41, 0.43 | 0.27-0.57 |
|                  | Combined | 409 | %    | 5.2  | 0.8  | 5.1    | 5.1, 5.3   | 3.6-6.9   |
|                  |          |     | g/dL | 0.48 | 0.07 | 0.48   | 0.48, 0.49 | 0.27-0.56 |
| Gamma globulin   | Male     | 184 | %    | 17.0 | 2.4  | 17.1   | 16.7, 17.4 | 12.4-21.7 |
|                  |          |     | g/dL | 1.36 | 0.23 | 1.36   | 1.33, 1.40 | 0.91-1.81 |
|                  | Female   | 225 | %    | 18.3 | 2.5  | 18.1   | 18.0, 18.7 | 13.5-23.2 |
|                  |          |     | g/dL | 1.47 | 0.25 | 1.45   | 1.44, 1.51 | 0.99-1.96 |
|                  | Combined | 409 | %    | 17.8 | 2.5  | 17.6   | 17.5, 18.0 | 12.9-22.7 |
|                  |          |     | g/dL | 1.42 | 0.25 | 1.41   | 1.40, 1.45 | 0.94-1.91 |

SD, standard deviation; CI, confidence interval.

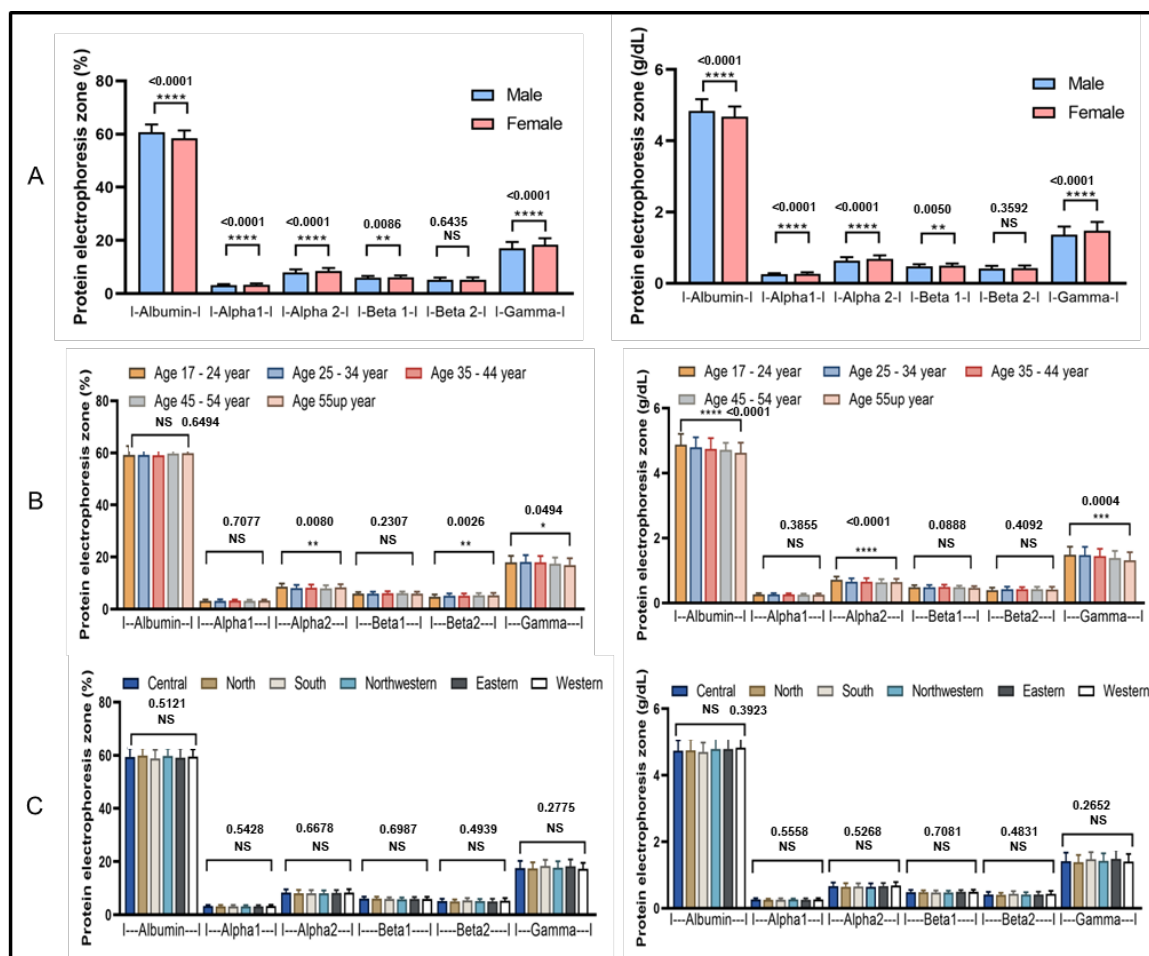


Fig. 1 Correlation of SPEP reference values using Capillary electrophoresis between sample groups: A) gender; B) age range; C) region. NS, non-significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$

### 3.3 RIs of SPEP by Gel Electrophoresis

The RIs of serum protein fractions obtained by gel electrophoresis were presented as mean, median, 95% CI, and range in Table 4. Additionally, the relationships between sample subgroups and the established RIs using gel electrophoresis are illustrated in Fig. 2. These results provided a detailed overview of the distribution and variability of serum protein fractions in the Thai adult population and offer essential baseline data for clinical interpretation.



**Table 4** Reference intervals for serum protein electrophoresis (SPEP) by gel electrophoresis.

| Analyte          | Sex      | N   | Unit | Mean | SD   | Median | 95% CI     | Range     |
|------------------|----------|-----|------|------|------|--------|------------|-----------|
| Albumin          | Male     | 184 | %    | 55.2 | 3.4  | 55.3   | 54.7, 55.7 | 48.6-61.9 |
|                  |          |     | g/dL | 4.40 | 0.33 | 4.37   | 4.35, 4.45 | 3.75-5.05 |
|                  | Female   | 225 | %    | 53.2 | 3.7  | 52.9   | 52.7, 53.7 | 45.9-60.6 |
|                  |          |     | g/dL | 4.26 | 0.29 | 4.22   | 4.22, 4.30 | 3.69-4.83 |
|                  | Combined | 409 | %    | 54.0 | 3.6  | 54.0   | 53.7, 54.4 | 47.0-61.1 |
|                  |          |     | g/dL | 4.32 | 0.31 | 4.27   | 4.29, 4.35 | 3.70-4.93 |
| Alpha-1 globulin | Male     | 184 | %    | 2.8  | 0.5  | 2.9    | 2.8, 2.9   | 1.9-3.8   |
|                  |          |     | g/dL | 0.23 | 0.04 | 0.23   | 0.22, 0.23 | 0.15-0.30 |
|                  | Female   | 225 | %    | 3.0  | 0.6  | 3.0    | 2.9, 3.1   | 1.9-4.1   |
|                  |          |     | g/dL | 0.24 | 0.05 | 0.24   | 0.23, 0.25 | 0.15-0.33 |
|                  | Combined | 409 | %    | 2.9  | 0.5  | 2.9    | 2.9, 3.0   | 1.9-4.0   |
|                  |          |     | g/dL | 0.23 | 0.04 | 0.23   | 0.23, 0.24 | 0.15-0.32 |
| Alpha-2 globulin | Male     | 184 | %    | 8.6  | 1.4  | 8.5    | 8.4, 8.8   | 5.8-11.4  |
|                  |          |     | g/dL | 0.69 | 0.12 | 0.68   | 0.67, 0.70 | 0.45-0.93 |
|                  | Female   | 225 | %    | 9.2  | 1.3  | 9.0    | 9.0, 9.4   | 6.6-11.8  |
|                  |          |     | g/dL | 0.74 | 0.12 | 0.73   | 0.72, 0.75 | 0.51-0.97 |
|                  | Combined | 409 | %    | 9.1  | 1.5  | 8.9    | 8.9, 9.2   | 6.2-12.0  |
|                  |          |     | g/dL | 0.73 | 0.13 | 0.71   | 0.72, 0.74 | 0.48-0.98 |
| Beta-1 globulin  | Male     | 184 | %    | 9.2  | 1.1  | 9.2    | 9.1, 9.4   | 7.0-11.4  |
|                  |          |     | g/dL | 0.74 | 0.10 | 0.72   | 0.72, 0.75 | 0.53-0.94 |
|                  | Female   | 225 | %    | 9.1  | 1.1  | 9.1    | 9.0, 9.3   | 6.9-11.3  |
|                  |          |     | g/dL | 0.73 | 0.10 | 0.73   | 0.72, 0.74 | 0.53-0.93 |
|                  | Combined | 409 | %    | 9.2  | 1.1  | 9.1    | 9.1, 9.3   | 7.1-11.3  |
|                  |          |     | g/dL | 0.73 | 0.10 | 0.72   | 0.73, 0.74 | 0.54-0.93 |
| Beta-2 globulin  | Male     | 184 | %    | 5.2  | 1.3  | 5.0    | 5.0, 5.4   | 2.6-7.8   |
|                  |          |     | g/dL | 0.41 | 0.11 | 0.40   | 0.40, 0.43 | 0.20-0.63 |
|                  | Female   | 225 | %    | 5.2  | 1.2  | 5.0    | 5.1, 5.4   | 2.9-7.5   |
|                  |          |     | g/dL | 0.42 | 0.10 | 0.41   | 0.41, 0.43 | 0.23-0.61 |
|                  | Combined | 409 | %    | 5.2  | 1.2  | 5.0    | 5.1, 5.4   | 2.8-7.6   |
|                  |          |     | g/dL | 0.42 | 0.10 | 0.40   | 0.41, 0.43 | 0.22-0.62 |
| Gamma globulin   | Male     | 184 | %    | 18.9 | 2.6  | 18.8   | 18.5, 19.3 | 13.9-23.9 |
|                  |          |     | g/dL | 1.51 | 0.25 | 1.52   | 1.47, 1.55 | 1.02-2.00 |
|                  | Female   | 225 | %    | 20.2 | 2.8  | 20.3   | 19.9, 20.6 | 14.7-25.8 |
|                  |          |     | g/dL | 1.63 | 0.28 | 1.63   | 1.59, 1.67 | 1.08-2.18 |
|                  | Combined | 409 | %    | 19.5 | 2.9  | 19.5   | 19.2, 19.8 | 13.9-25.1 |
|                  |          |     | g/dL | 1.57 | 0.28 | 1.57   | 1.54, 1.59 | 1.02-2.11 |

SD, standard deviation; CI, confidence interval.

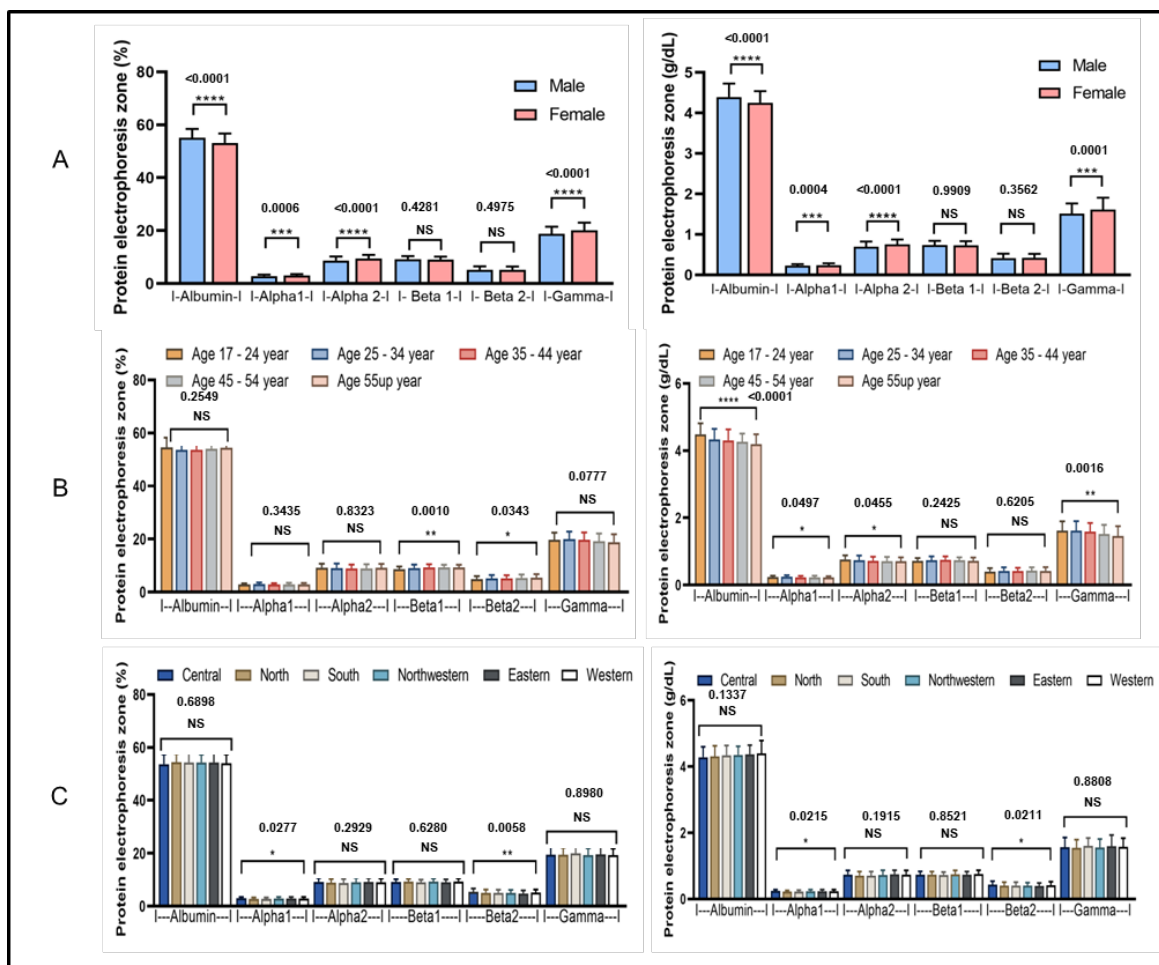


Fig. 2 Correlation of SPEP reference values using Gel electrophoresis between sample groups: A) gender; B) age range; C) region. NS, non-significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$

### 3.4 The comparison of RIs between Capillary and Gel Electrophoresis

The comparison of RIs between capillary electrophoresis and gel electrophoresis was analyzed for individual protein fractions, including albumin, alpha-1 globulin, alpha-2 globulin, beta-1 globulin, beta-2 globulin, and gamma globulin in Fig. 3.

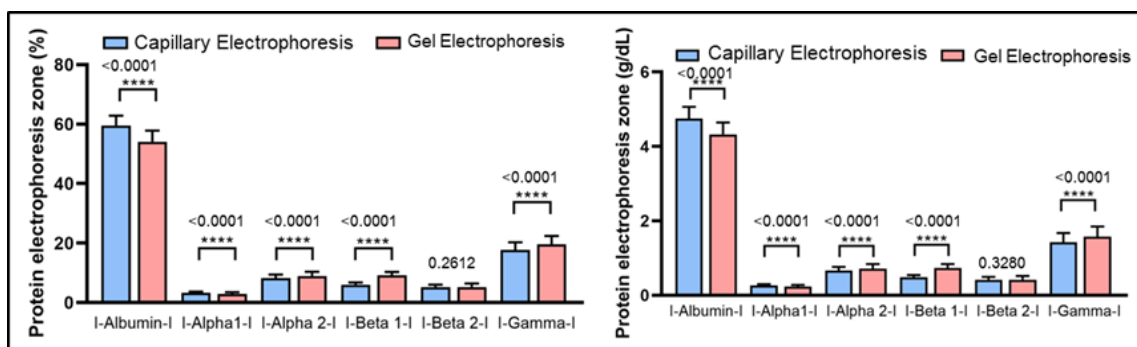


Fig. 3 The comparison of Reference Intervals (RIs) measured by capillary electrophoresis and gel electrophoresis.

#### 4. Discussion

Serum protein electrophoresis (SPEP) can be performed using either capillary electrophoresis or gel electrophoresis, both of which separate proteins based on their charge and size but employ different separation media. Capillary electrophoresis utilizes a narrow-bore capillary tube as the separation medium, whereas gel electrophoresis relies on an agarose gel matrix to achieve protein separation. The present study demonstrated that the RIs of SPEP obtained from capillary electrophoresis or gel electrophoresis differed significantly for most protein fractions, except for beta-2 globulin. This indicates that SPEP results generated by one method cannot be directly substituted for those obtained by the other. Consequently, clinical laboratories must select the analytical method based on their operational requirements and available resources. Capillary electrophoresis offers advantages of simplicity, speed, and reduced handling procedures,<sup>(6, 7)</sup> as it does not require staining or gel cleanup steps, thereby minimizing labor and exposure to chemicals. However, this method is associated with higher costs. Meanwhile, gel electrophoresis is less expensive but requires longer analysis time, making it suitable for the cost reduction. These findings highlight the possible importance of method specific RIs for SPEP in clinical practice. Accurate RIs are essential for disease diagnosis, therapeutic monitoring, and research applications. Given the limited data on population specific RIs in Thailand, this study provides valuable baseline data for serum protein fractions in Thai blood donors, addressing a crucial gap in current laboratory and clinical practice. However, the age range of the sample group in this study was based on the eligibility criteria for blood donors (17-66 years), which constitutes a limitation of this research. In the future, the researcher hopes to collect samples from other age groups in order to better represent the entire Thai population.

According to the reagent manufacturer's documentation, RIs for serum proteins determined by capillary electrophoresis were previously established in a cohort of 225 healthy adults (male and female) in France.<sup>(5)</sup> The RIs for albumin, alpha-1, alpha-2, beta-1, and beta-2 globulin were largely comparable between the French and Thai populations. However, the gamma globulin fraction in the Thai population exhibited a broader RI range compared to the French cohort. This discrepancy may be attributed to differences in sample size and ethnic background, highlighting the importance of population specific RIs for accurate interpretation of laboratory results. Previous studies on reference intervals (RIs) in various populations, including Belgium,<sup>(8)</sup> the United States,<sup>(9)</sup> Switzerland,<sup>(10)</sup> Japan,<sup>(11)</sup> Italy,<sup>(12)</sup> and France,<sup>(13)</sup> have established capillary electrophoresis-based RIs in both male and female cohorts. These studies evaluated all five major serum protein fractions—albumin, alpha-1, alpha-2, beta, and gamma globulin—with protein band intensities expressed as percentages, as summarized in Table 5. Our data indicate that albumin, alpha-1, and alpha-2 globulin exhibited comparable RIs across these populations. However, direct comparison of beta-1 and beta-2 globulin was not possible, as previous studies did not distinguish between these subfractions. In contrast, the gamma globulin fraction in the Thai population showed a wider RI range, likely attributable to differences in sample size and ethnic background in Table 5. A study conducted in Ethiopia<sup>(14)</sup> using capillary electrophoresis included 297 healthy adults (male and female) aged 18-60 years, reporting both percentage band intensity and absolute concentration (g/dL) for each protein fraction in Table 6. Comparisons with the Thai population, albumin, beta-1, and gamma globulin RIs were largely similar, whereas alpha-1 and alpha-2 globulin exhibited lower RIs, and beta-2 globulin showed higher RIs than in the Thai cohort. Such discrepancies are likely influenced by genetic factors and dietary habits of the study populations, which are important determinants in establishing population specific RIs in Table 6.



**Table 5** Reference Intervals (%) for the five serum protein fractions by capillary electrophoresis in different laboratories.

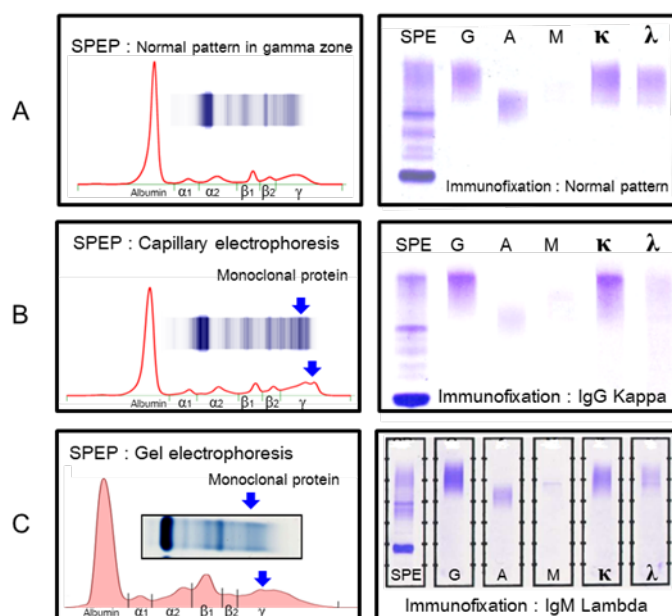
| References                  | Sex    | N   | Unit | Albumin   | Alpha-1 globulin | Alpha-2 globulin | Beta globulin | Gamma globulin |
|-----------------------------|--------|-----|------|-----------|------------------|------------------|---------------|----------------|
| Belgium <sup>(8)</sup>      | Male   | 82  | %    | 60.0-70.0 | 3.9-6.4          | 4.9-9.3          | 8.4-12.7      | 7.9-18.3       |
|                             | Female | 79  | %    | 56.0-70.0 | 3.6-7.4          | 5.6-9.7          | 8.0-12.7      | 7.9-18.3       |
| USA <sup>(9)</sup>          | NA     | NA  | %    | 52.0-67.0 | 3.8-8.3          | 5.8-12.4         | 9.8-13.0      | 9.3-20.4       |
| Switzerland <sup>(10)</sup> | NA     | NA  | %    | 57.0-70.0 | 3.8-7.8          | 4.4-10.0         | 7.8-12.8      | 9.0-17.4       |
| Japan <sup>(11)</sup>       | NA     | 63  | %    | 56.0-69.0 | 3.9-6.3          | 4.8-8.4          | 7.9-11.9      | 9.9-21.5       |
| Italy <sup>(12)</sup>       | NA     | 167 | %    | 54.0-68.0 | 3.7-8.8          | 5.5-11.7         | 8.5-13.8      | 10.0-20.3      |
| France <sup>(13)</sup>      | Male   | 60  | %    | 56.0-66.0 | 2.0-6.9          | 5.9-11.4         | 9.4-14.4      | 8.0-18.8       |
|                             | Female | 60  |      |           |                  |                  |               |                |

**Table 6** Comparison of reference intervals for serum protein electrophoresis in adults from Ethiopia (Bikila *et al.*, 2022) and Thailand.

| References               | Instrument       | Sex      | N   | Unit | Albumin     | Alpha-1 globulin | Alpha-2 globulin | Beta-1 globulin | Beta-2 globulin | Gamma globulin |
|--------------------------|------------------|----------|-----|------|-------------|------------------|------------------|-----------------|-----------------|----------------|
| Ethiopia <sup>(14)</sup> | Capillary, Sebia | Male     | 124 | %    | 53.83-64.59 | 3.04-5.40        | 8.0-12.67        | 5.01-7.38       | 2.70-4.89       | 12.05-21.43    |
|                          |                  |          |     | g/dL | 4.31-5.89   | 0.21-0.50        | 0.60-1.1         | 0.40-0.69       | 0.20-0.49       | 0.91-1.90      |
|                          |                  | Female   | 173 | %    | 52.24-63.55 | 3.44-5.60        | 8.44-12.87       | 5.14-7.86       | 2.50-4.96       | 12.58-22.26    |
|                          |                  |          |     | g/dL | 4.10-5.20   | 0.30-0.50        | 0.64-1.10        | 0.40-0.66       | 0.20-0.40       | 0.90-1.86      |
|                          |                  | Combined | 297 | %    | 53.18-64.10 | 3.4-5.6          | 8.04-12.76       | 5.10-7.56       | 2.54-4.90       | 12.40-21.66    |
|                          |                  |          |     | g/dL | 4.10-5.36   | 0.30-0.50        | 0.60-1.10        | 0.40-0.66       | 0.20-0.40       | 0.90-1.90      |
| Thailand                 | Capillary, Sebia | Male     | 184 | %    | 55.0-66.4   | 2.4-3.9          | 5.7-10.2         | 4.7-7.2         | 3.6-6.8         | 12.4-21.7      |
|                          |                  |          |     | g/dL | 4.19-5.48   | 0.19-0.31        | 0.44-0.83        | 0.37-0.58       | 0.27-0.56       | 0.91-1.81      |
|                          |                  | Female   | 225 | %    | 52.6-64.2   | 1.9-3.8          | 6.3-10.8         | 4.8-7.5         | 3.6-6.9         | 13.5-23.2      |
|                          |                  |          |     | g/dL | 4.12-5.23   | 0.15-0.30        | 0.49-0.88        | 0.37-0.61       | 0.27-0.57       | 0.99-1.96      |
|                          |                  | Combined | 409 | %    | 53.3-65.6   | 2.4-4.1          | 5.9-10.6         | 4.7-7.4         | 3.6-6.9         | 12.9-22.7      |
|                          |                  |          |     | g/dL | 4.13-5.37   | 0.19-0.33        | 0.46-0.86        | 0.37-0.60       | 0.27-0.56       | 0.94-1.91      |
| Thailand                 | Gel, Helena      | Male     | 184 | %    | 48.6-61.9   | 1.9-3.8          | 5.8-11.4         | 7.0-11.4        | 2.6-7.8         | 13.9-23.9      |
|                          |                  |          |     | g/dL | 3.75-5.05   | 0.15-0.30        | 0.45-0.93        | 0.53-0.94       | 0.20-0.63       | 1.02-2.00      |
|                          |                  | Female   | 225 | %    | 45.9-60.6   | 1.9-4.1          | 6.6-11.8         | 6.9-11.3        | 2.9-7.5         | 14.7-25.8      |
|                          |                  |          |     | g/dL | 3.69-4.83   | 0.15-0.33        | 0.51-0.97        | 0.53-0.93       | 0.23-0.61       | 1.08-2.18      |
|                          |                  | Combined | 409 | %    | 47.0-61.1   | 1.9-4.0          | 6.2-12.0         | 7.1-11.3        | 2.8-7.6         | 13.9-25.1      |
|                          |                  |          |     | g/dL | 3.70-4.93   | 0.15-0.32        | 0.48-0.98        | 0.54-0.93       | 0.22-0.62       | 1.02-2.11      |

To date, no studies have established RIs in humans using gel electrophoresis that include all six protein fractions, possibly be due to the longer analysis time than capillary electrophoresis. Here, several differences in RIs between gel-based and capillary-based electrophoresis across sex, age, and geographic region were demonstrated. Specifically, capillary electrophoresis demonstrated sex-related differences for all protein fractions except for beta-2 globulin, and age-related differences in albumin (g/dL), alpha-2 globulin (%), and gamma globulin (%), while no significant differences were observed across geographic regions. In contrast, gel electrophoresis exhibited sex-related differences for all fractions except beta-1 and beta-2 globulin, age-related differences in albumin (g/dL), alpha-2 globulin (g/dL), beta-1 globulin (%), and gamma globulin (g/dL), and region-related differences in alpha-1 globulin and beta-2 globulin. These findings indicated that both the analytical method and biological factors substantially influence serum protein RIs, emphasizing the importance of method- and population-specific RIs for accurate clinical interpretation and laboratory standardization.

In this study, monoclonal proteins were detected in five out of 414 blood donors, accounting for approximately 1% of the cohort. Subsequent analysis using the more specific immunofixation electrophoresis (IFE) identified the following paraproteins: IgG Kappa in two donors, IgG Lambda in one donor, IgM Kappa in one donor, and IgM Lambda in one donor in Fig. 4. Notably, the presence of monoclonal proteins in otherwise individuals may represent monoclonal gammopathy of undetermined significance (MGUS). This condition is typically asymptomatic, but in certain cases, it may progress to more severe hematologic disorders, including specific types of blood malignancies. The detection of such proteins underscores the importance of sensitive and specific laboratory methods, such as IFE, for identifying subclinical or early-stage abnormalities in otherwise healthy populations.<sup>(15-19)</sup>



**Fig. 4** The results of serum protein electrophoresis (SPEP) and immunofixation (IFE) analysis of blood donors found to have abnormal proteins: A) Normal control serum; B) The results of SPEP found a monoclonal in the gamma globulin zone, and the IFE method detected an IgG kappa monoclonal protein. C) The results of SPEP found a monoclonal in the gamma globulin zone, and the IFE method detected an IgM lambda monoclonal protein.

## 5. Conclusion

Hence, RIs for serum proteins are influenced by multiple factors, including sex, age, geographic region, and the analytical method employed, as different techniques exhibit varying sensitivities to specific protein fractions. These findings provide reliable population-specific reference data for the Thai adult population and can be applied in clinical laboratories for accurate diagnosis, monitoring of immunological diseases, and research involving serum proteins, thereby enhancing the precision and validity of laboratory assessments.

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