*PLEASE READ!!*

**HELENA LABORATORIES**

**PROCEDURE DOWNLOAD END USER AGREEMENT**

In response to customer requests, Helena is pleased to provide the text for procedural package inserts in a digital format editable for your use. The text for the procedure you requested begins on page three of this document. Helena procedures contain the content outlined in the NCCLS (GP2-A#) format, except in the order sequence required by FDA regulations. As the NCCLS format is a guideline, you may retain these procedures as developed by the manufacturer (adding your title/authorization page) or manipulate the text file to produce your own document, matching the NCCLS section order exactly, if preferred.

We also provide the procedure in an Adobe Acrobat PDF format for download at www.helena.com as a “MASTER” file copy. Below are the specifications and requirements for using these digital files. Following the specifications is the procedure major heading sequence as given in the FDA style. Where there is a difference in order, or other notation in the outline, this will be indicated in braces { }.

WHAT YOU NEED TO KNOW:

1) These files represent the most current revision level to date. Your current product inventory could contain a previous revision level of this procedure.

2) The Microsoft Word document provides the text only from the master procedure, in a single-column format.

- It may not contain any illustrations, graphics or captions that may be part of the master procedure included in the kit.

- The master procedure may have contained special formatting characters, such as subscripts, superscripts, degree symbols, mean symbols and Greek characters such as alpha, beta, gamma, etc. These symbols may or may not display properly on your desktop.

- The master procedures may also contain columns of tabbed data. Tab settings may or may not be displayed properly on your desktop.

3) The Adobe Acrobat PDF file provides a snapshot of the master procedure in a printable 8.5 x 11” format. It is provided to serve as a reference for accuracy.

4) By downloading this procedure, your institution is assuming responsibility for modification and usage.

**HELENA LABORATORIES**

**PROCEDURE DOWNLOAD END USER AGREEMENT**

HELENA LABORATORIES LABELING – Style/Format Outline

1) PRODUCT {Test} NAME

2) INTENDED USE and TEST TYPE (qualitative or qualitative)

3) SUMMARY AND EXPLANATION

4) PRINCIPLES OF THE PROCEDURE

{NCCLS lists SAMPLE COLLECTION/HANDLING next}

5) REAGENTS (name/concentration; warnings/precautions; preparation; storage; environment; Purification/treatment; indications of instability)

6) INSTRUMENTS required – Refer to Operator Manual (... for equipment for; use or function; Installation; Principles of operation; performance; Operating Instructions; Calibration\* {\*is next in order for NCCLS – also listed in “PROCEDURE”}’ precautions/limitations/hazards; Service and maintenance information

7) SAMPLE COLLECTION/HANDLING

8) PROCEDURE

{NCCLS lists QUALITY CONTROL (QC) next}

9) RESULTS (calculations, as applicable; etc.)

10) LIMITATIONS/NOTES/INTERFERENCES

11) EXPECTED VALUES

12) PERFORMANCE CHARACTERISTCS

13) BIBLIOGRAPHY (of pertinent references)

14) NAME AND PLACE OF BUSINESS OF MANUFACTURER

15) DATE OF ISSUANCE OF LABELING (instructions)

For Sales, Technical and Order Information, and Service Assistance,   
call Helena Laboratories toll free at 1-800-231-5663.

Form 364

Helena Laboratories

1/2006 (Rev 3)

**SPIFE® Nexus**

**Split Beta SPE-20, 40, 60 Procedure**

Cat. No. **2420, 2421, 2422**

**INTENDED USE**

The SPIFE Nexus Split Beta SPE method is intended to quantitatively determine the presence of normal and abnormal serum proteins and qualitatively determine the presence of urine proteins by agarose electrophoresis using the SPIFE Nexus System.

For *In Vitro* Diagnostic Use.

Rx Only

**SUMMARY**

Serum contains over one hundred individual proteins, with specific functions and various concentrations under different pathologic conditions.1 Since introduction of moving-boundary electrophoresis by Tiselius2 and the subsequent use of zone electrophoresis, serum proteins have been fractionated on the basis of their electrical charge at a particular pH into five classical fractions: albumin, alpha1, alpha2, beta and gamma proteins. Each of these classical electrophoretic zones, with the exception of albumin, normally contains two or more components. Knowing the relative proportions of these fractions has proven useful in the diagnosis and prognosis of certain disease states.3,4,5

**PRINCIPLE**

Proteins are large molecules composed of covalently linked amino acids. Depending on electron distributions resulting from covalent or ionic bonding of structural subgroups, proteins can be either polar or nonpolar at a given pH. Proteins are separated according to their respective electrical charges on agarose gel using both the electrophoretic and electroendosmotic forces present in the system. The proteins are then stained with a visible stain.

**REAGENTS**

**1. SPIFE Split Beta SPE Gel   
Ingredients:** Each gel contains agarose in a tris-barbital/MOPS buffer with calcium lactate, a stabilizer, and a preservative.  **WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.** The gel contains barbital which, in sufficient quantity, can be toxic.

**Preparation for Use:** The gels are ready for use as packaged.  **Storage and Stability:** The gels should be stored at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored horizontally in the protective packaging in which they are shipped. **DO NOT REFRIGERATE OR FREEZE THE GELS.** Avoid storage close to a window or heat source, and avoid temperature variation during storage. **Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of the gel blocks.

**2. SPIFE Nexus Blue   
Ingredients:** The stain contains 0.5% (w/v) acid blue stain, 5% acetic acid, and surfactant.  **WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** The stain is ready for use as packaged. **Storage and Stability:** The stain solution is stable for one year when stored at 15 to 30°C in a closed container.  **Signs of Deterioration:** The prepared stain should be a homogeneous mixture free of precipitate. Discard if precipitate forms.

**3. Citric Acid Destain   
Ingredients:** After dissolution, destain contains 0.3% (w/v) citric acid.  **WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST - IRRITANT.   
Preparation for Use:** Pour 11 L of deionized water into Destain vat. Add full package of destain and mix until completely dissolved.  **Storage and Stability:** Store the destain at 15 to 30°C. It is stable until the expiration date on the package. **Signs of Deterioration:** Discard if solution becomes cloudy.

**4. SPIFE Nexus Violet (Optional Urine Stain)   
Ingredients:** Contains 0.2% (w/v) acid violet stain and 10% acetic acid.   
**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** The stain is ready for use as packaged. **Storage and Stability:** The stain solution is stable for one year when stored at 15 to 30°C in a closed container.  **Signs of Deterioration:** The stain should be a homogeneous mixture free of precipitate.

**INSTRUMENT**

A SPIFE Nexus analyzer must be used to apply samples, electrophorese, stain, destain, dry and then scan the gels. The gels may also be scanned on a separate densitometer such as the QuickScan Touch Plus (Cat. No. 1640). Refer to the Operator's Manuals for detailed instructions.

**SPECIMEN COLLECTION AND HANDLING**

**Specimen:** Fresh serum or urine is the specimen of choice. Use of plasma will cause a fibrinogen band to appear as a distinct narrow band between the beta and gamma fractions.

**Storage and Stability:** If storage is necessary, store serum samples covered at 15 to 30°C for 4 days, 2 to 8°C for 2 weeks or -20°C for 6 months.6 Urine samples may be stored covered at 2 to 8°C for up to 72 hours or at -20°C for 1 month. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

**Interfering Factors:**

1. Hemolysis may cause false elevation in the alpha2 and beta fractions.

2. Uncovered specimens may yield inaccurate results due to evaporation.

**PROCEDURE**

**Materials provided:** The following materials needed for the procedure are contained in the SPIFE Nexus Split Beta SPE Kit. Individual items are not available.

**Test Size Cat. No.**

60 Samples 2420

40 Samples 2421

20 Samples 2422

**Cat. No. 2420, 2421, 2422**

SPIFE Split Beta SPE Gels (10)

SPIFE Nexus Blue (1 vial)

SPIFE Blotter C (10)

Citric Acid Destain (1 pkg)

Serrated Blade Applicator Kit, 20 Sample (10/20/30)

**Materials provided but not contained in the kit:**

**Item Cat. No.**

SPIFE Nexus Analyzer 1650

QuickScan Touch Plus 1640

SPIFE Gel Block Remover 1115

SPE Normal Control 3424

SPE Abnormal Control 3425

SPIFE Dispo Sample Cups, Deep Well 3360

SPIFE 20,40,60 Dispo Cup Tray 3370

SPIFE Nexus Cassette 2580

SPIFE Nexus Applicator Templates 2570

SPIFE Nexus Applicator Blade Weights 2572

SPIFE Nexus Dispo Stain Cups 2575

Pos ID Barcode Labels for Touch and

SPIFE Nexus Systems 1696

Rep Prep 3100

SPIFE Nexus Reagent Roller 2583

SPIFE Nexus Ready Run Kit 2582

SPIFE Nexus Carbon Electrode Insert 2576

SPIFE Nexus A22 Short Electrode Insert 2577

SPIFE Nexus Violet 552683

**Materials needed but not provided:**

0.85% saline

**STEP-BY-STEP METHOD**

**I. Sample Preparation   
Serum**: No specimen preparation is necessary for serum. Serum samples will be automatically pipetted into sample cups at a volume of 80 µL per sample.   
**Urine:** Due to differences in the running parameters, urine specimens cannot be processed on the SPIFE Nexus in combination with serum samples unless urine specimens are applied using template application. The Split Beta SPE procedure in combination with template urine application is available on the Helena website (https://www.helena.com/procedures.htm).   
Urine samples should be concentrated if a higher sensitivity is desired. A total protein range of 2,000-3,000 mg/dL is generally sufficient for optimum sensitivity. The sensitivity is approximately 7 mg/dL for a single band. Shake samples to homogenize. Centrifuge desired volume at 2000 x g for 5 minutes. Remove supernatant and concentrate to lab specifications per laboratory protocol.   
Urine samples or concentrations will be automatically pipetted into sample cups at a volume of 80 µL per sample. For urine specimen volumes measuring less than 500 µL, contact Technical Service for instructions on manual loading.

**II. SPIFE Nexus Preparation**

A. Fill designated bottles with 0.85% saline, deionized water, and destain.

B. Turn on the SPIFE Nexus. Click on the SPIFE Nexus icon to initialize.

C. If this is the first test of the day, prime the instrument according to the instructions in the SPIFE Nexus Operator’s Manual.

D. Load the correct number of uncapped patient sample test tubes into test tube racks and place racks within the tube transport area.

E. Open the main door of the instrument and prepare the items onboard the instrument.

1. Ensure that the following items are in their respective onboard storage locations: **Platen Cover** with the Electrode Insert and **Dryer Cover** with the red sticker toward the back of the instrument.

2. **Sample Cup Tray**

a. Prepare the sample cup tray with the appropriate disposable deep well sample cups. Slide the Disposable Sample Cups into the cup tray. Use only the top row for 20 or fewer samples, top and middle rows for up to 40 samples, and all three rows for up to 60 samples.

b. Place the cup tray onto the sample tray platform.

3. **Stain/Reagent Dispenser**

a. Fill three Stain Cups each with 700 µL of SPIFE Nexus Blue stain and place a Stain Cup in each slot of the Stain/Reagent Dispenser. **NOTE: As an optional urine stain, SPIFE Nexus Violet may be substituted for SPIFE Nexus Blue.**

b. Place a clean Reagent Roller bar between the hooks on the Stain/Reagent Dispenser.

4. **Consumables Tray**

a. Slide the Consumables Tray forward from its home position.

b. Prepare the Applicator Holder

(1) Place a Split Beta (60) Applicator Template on top of the Applicator Holder. Place Applicator Blades in the designated slots corresponding to the sample cups loaded within the sample tray. **NOTE: The Applicator Blades will only fit into the slots in the Applicator Holder one way; do not try to force the Applicator Blades into the slots.**

(2) Place the Applicator Blade Weights on top of the Applicator Blades with the thick side facing the front of the instrument.

c. Slide the Consumables Tray into position in the back of the instrument. **Note: Do not store extra components or consumables in the Consumables Tray during a test.**

5.  **Gel Cassette**

a. Place the bottom half of the Gel Cassette on the electrophoresis platen with the two pins lined up on the left side.

b. Dispense 2 mL of REP Prep on the platen.

c. Remove the gel from the protective packaging and discard the overlay.

d. Using a SPIFE Blotter C, gently blot the entire gel. Discard the blotter.

e. Place the left edge of the gel into the bottom of the cassette fitting the round hole over the upper pin and the obround hole over the lower pin. Gently lay the gel down over the REP Prep making sure no bubbles remain under the gel.

f. Place the top half of the Gel Cassette over the gel. Make sure the 2D barcode is located in the upper right corner of the cassette.

g. Place a Positive ID Barcode Label on the upper right hand side of the gel backing. Select the barcode that starts with the letter “G”.

F. Close the main door of the instrument.

**III. Automated Gel Electrophoresis**

A. Click the Start button on the menu bar. Select the **SPIFE Split Beta Serum Proteins 60 (Acid Blue)**, **SPIFE Urine Proteins 60 (Acid Blue)** or **SPIFE Urine Proteins 60 (Acid Violet)** test name from the drop down menu. Ensure the toggles for all Run Processes are set to “Yes” and click the Start Run button. The analyzer will load samples when appropriate, apply samples, electrophorese, stain, destain, dry and scan the gel. **For details of Automated Gel Electrophoresis parameters, contact Technical Services.**

B. After scanning, the Gel Cassette with the finished gel will be located in the scanner port on the front side of the instrument. If gel storage is required, remove and discard the two gel blocks.

C. After every test: discard the used blotters, Applicator Blades, Stain Cups and sample cups as biohazardous waste. Clean any residual stain from the electrophoresis platen, Gel Cassette and the Reagent Roller bar. For daily, weekly, and monthly maintenance, reference the SPIFE Nexus Operator’s Manual.

**Evaluation of the Protein Bands**

**Quantitative Evaluation of Serum:** The SPIFE Nexus Split Beta SPE Gel will be automatically scanned. An aperture size of 5 with the acid blue setting is recommended. Refer to the QuickScan Touch Plus Operator’s Manual for scanning parameters.

**Qualitative Evaluation of Urine:** The urine samples run on the SPIFE Nexus Split Beta SPE Gel can only be visually inspected for the presence of the bands.

**Stability of End Product:** The completed, dried SPIFE Split Beta SPE Gel is stable for an indefinite period of time.

**Quality Control:** SPE Normal Control (Cat. No. 3424) and SPE Abnormal Control (Cat. No. 3425) may be used to verify all phases of the procedure and should be used on each gel run. If desired, a control or patient sample may be diluted 1:7 with 0.85% saline (1 part sample + 6 parts saline) and run with urines for qualitative comparison. Refer to the package insert provided with the control for assay values.

**REFERENCE VALUES**

The reference ranges presented were established with the Split Beta SPE System on 38 normal specimens using the SPIFE Nexus. These values are presented as a guideline.

**% of Total**

**Protein Fraction Mean ± 2 S.D.**

Albumin 46.2 - 63.0

Alpha1 1.7 - 4.4

Alpha2 6.8 - 13.7

Beta 12.5 - 18.5

Gamma 10.6 - 22.7

Each laboratory should perform its own normal range study.

**Variations of Expected Values5**

Studies show that values are the same for both males and nonpregnant females (Some differences are seen in pregnant females at term and in women on oral contraceptives).

Age has some effect on normal levels. Cord blood has decreased total protein, albumin, alpha2 and beta fractions, slightly increased alpha1 and normal or increased gamma fractions (largely of maternal origin). The gamma globulins drop rapidly until about three months of age, while the other fractions have reached adult levels by this time. Adult levels of the gamma globulins are not reached until 16 years of age. The albumin decreases and beta globulin increases after the age of 40.

**RESULTS**

Figure 1 illustrates the electrophoretic mobilities of the albumin, alpha1, alpha2, beta and gamma protein bands on a SPIFE Split Beta SPE Gel. The fastest moving band, and normally the most prominent, is the albumin band found closest to the anodic edge of the gel. The faint band next to this is alpha1, followed by alpha2 globulin, split beta and gamma globulins.

****

**Figure 1:** A SPIFE Split Beta SPE-60 Gel showing relative position of the bands.

****

**Figure 2:** A scan of a SPIFE Split Beta SPE pattern.

**Calculations of the Unknown**

The SPIFE Nexus scanner will automatically calculate and print the relative percent and the absolute value of each band when the total protein is entered. Refer to the SPIFE Nexus and QuickScan Touch Plus Operator’s Manuals provided with the instrument.

**INTERPRETATION OF RESULTS5,6**

Results on normal individuals will cover age and sex-related variations and day-to-day biologic variations. Disease states in which abnormal patterns are observed include inflammatory response, rheumatic disease, liver diseases, protein-loss disorders, plasma cell dyscrasias, infectious disorders, renal disorders, pregnancy, and genetic deficiencies. Patients with high levels of IgG4 can produce a relatively restricted band cathodic in the beta gamma region or beta gamma bridging.7 Proteins migrating in the alpha2 and beta region may show slight variation in migration under a variety of circumstances.8

**Further Testing Required**

The serum or urine protein electropherogram, or densitometric tracing, should be evaluated for abnormalities. If abnormalities are observed, appropriate follow-up studies should be initiated.9 Not all clinically significant monoclonal gammopathies will display a distinct band detectable by protein electrophoresis.10 Further studies may be indicated based on clinical context. These may include immunofixation, quantitation of immunoglobulins, bone marrow examination and other appropriate tests.

**LIMITATIONS**

1. Since all electrophoretic procedures are nonlinear, it is critical to fill the wells with the recommended volume of serum or urine to obtain optimal resolution and reproducible results. Noncompliance with the recommended procedure may affect the results.

2. Therapeutic monoclonal antibodies may be used in the treatment of multiple myeloma as well as various other malignancies or medical conditions. If present in sufficient concentration, these agents may be indistinguishable from a pathologic monoclonal protein on serum protein electrophoresis.11,12

3. Use of plasma will cause a fibrinogen band to appear as a distinct narrow band between the beta and gamma fractions. A fibrinogen band may also be present in patients on heparin therapy.

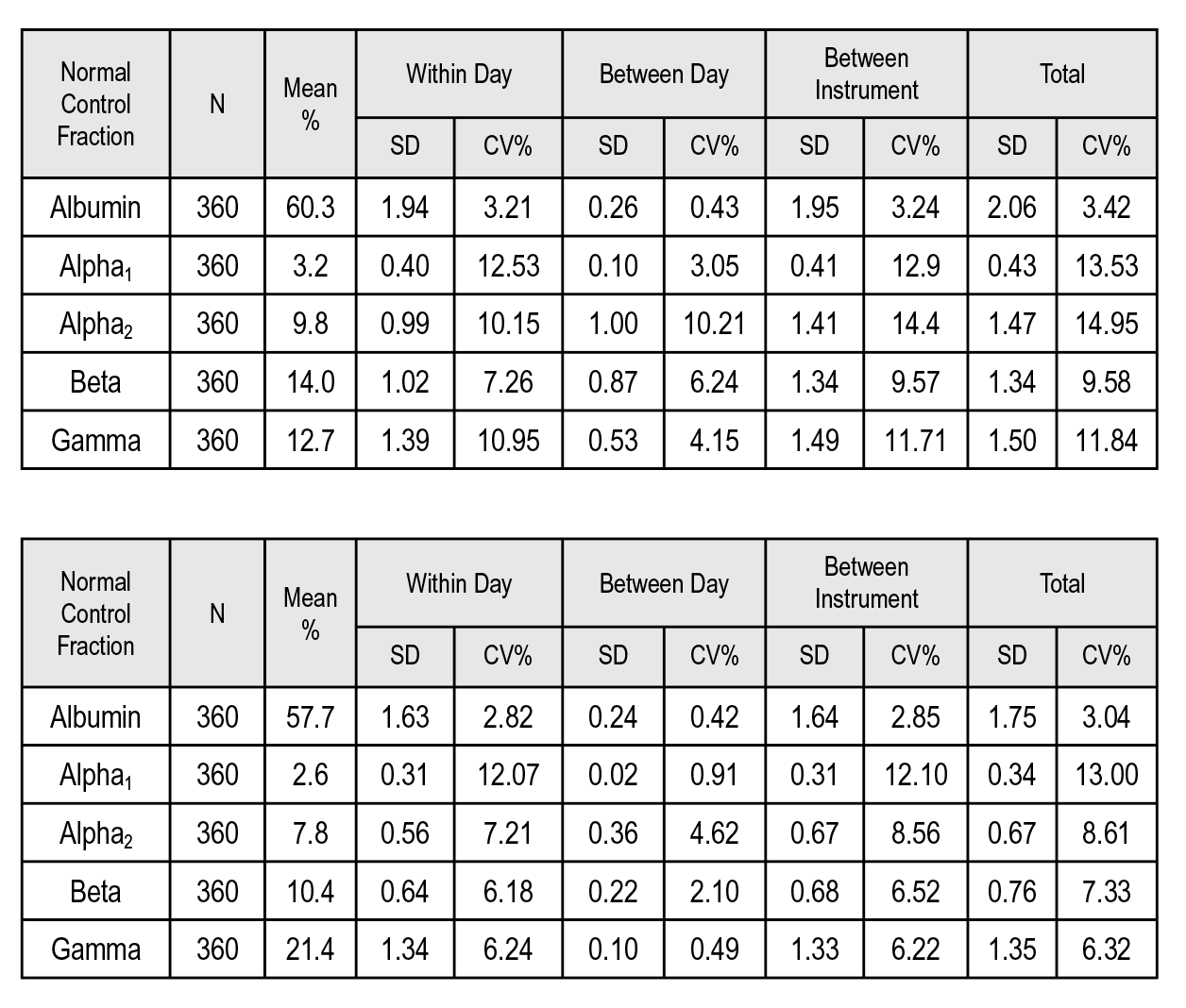
4. Hemolyzed samples should be avoided as the changes in hemoglobin-haptoglobin may affect the alpha2 and beta migration.13,14

5. The mobility of beta lipoprotein (low density lipoprotein) can vary considerably and may migrate under normal conditions anywhere between the alpha2 and beta region. Beta lipoproteins can be recognized by their characteristic appearance as a thin, irregular line, regardless of its migration location.

6. An artifact may be present at the point of application, particularly with the use of frozen samples, older samples, or samples containing debris.13 An application artifact may appear as fine clear line (negative space) that may be visible to a faint degree across the entire gel in the beta region. This can on occasion cause the edge of a normal blush to appear slightly blunted.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

Reproducibility was assessed over a 5 day period. Normal and abnormal serum controls were tested on two gels per day on each of three SPIFE Nexus instruments. Three hundred sixty determinations per protein fraction in total were collected for the normal and abnormal serum protein controls respectively.



**SENSITIVITY**

A pathological serum sample with a monoclonal protein was serially diluted and the dilutions electrophoresed on the SPIFE Split Beta SPE gels on the SPIFE Nexus. After visual inspection of the gel, the lowest detectable concentration of a monoclonal protein was between approximately 0.14 and 0.28 g/L (14 and 28 mg/dL).

Serial dilutions of a pathological urine sample containing a monoclonal were analyzed on the SPIFE Nexus using blade application on the SPIFE Split Beta SPE gels. The sensitivity was determined to be approximately 0.07 g/L (7 mg/dL) for a single band when using three blade applications of neat urine.

**NOTE:** The migration position of the monoclonal protein and the presence of a polyclonal background in the gamma zone may affect the detection limit.

**CORRELATION**

Normal and abnormal serum samples were analyzed using the SPIFE Touch Split Beta SPE system and the SPIFE Nexus Split Beta SPE system. Deming regression with 95% confidence intervals and Pearson correlation coefficient are presented below.

n = 30

Slope: 1.046 (1.026 to 1.066)

Intercept: -0.93 (-1.48 to -0.38)

R = 0.99

**BIBLIOGRAPHY**

1. Alper CA. Plasma protein measurements as a diagnostic aid. *New England Journal of Medicine.* 1974;291(6):287-290.

2. Tiselius A. A New Apparatus for Electrophoretic Analysis of Colloidal Mixtures. *Transactions of the Faraday Society.* 1937;33:524.

3. Ritzmann SE, Daniels JC. Diagnostic Proteinology: Separation and Characterization of Proteins, Qualitative and Quantitative Assays. In: *Laboratory Medicine.* Vol 1. Hagerstown (MD): Harper and Row, Inc.; 1979.

4. Tietz NW, ed. In: *Textbook of Clinical Chemistry.* Philadelphia (PA): WB Saunders Company; 1986:579-582.

5. Ritzmann SE, ed. *Protein Abnormalities, Volume 1. Physiology of Immunoglobulins: Diagnostic and Clinical Aspects.* New York (NY): Allen R. Liss; 1982.

6. Keren DF. *Protein Electrophoresis in Clinical Diagnosis.* London: Edward Arnold; 2003.

7. Chen LYC, Mattman A, Seidman MA, Carruthers MN. IgG4-related disease: What a hematologist needs to know. *Haematologica*. 2019;104(3):444-455.

8. Hrkal Z, Kuzelová K, Muller-Eberhard U, Stern R. Hyaluronan-binding properties of human serum hemopexin. *FEBS Letters*. 1996;383(1-2):72-74.

9. Lakshminarayanan R, Li Y, Janatpour K, Beckett L, Jialal I. Detection by immunofixation of M proteins in hypogammaglobulinemic patients with normal serum protein electrophoresis results. *American Journal of Clinical Pathology.* 2007;127(5):746-751.

10. Kyle RA, Gertz MA, Witzig TE, et al. Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clinic Proceedings.* 2003;78(1):21-33.

11. Mills JR, Murray DL. Identification of friend or Foe: The Laboratory Challenge of Differentiating M-proteins from Monoclonal Antibody Therapies. *The Journal of Applied Laboratory Medicine.* 2017;1(4):421-431.

12. Keren DF. Therapeutic Complications: A Caveat for M-protein detection. *The Journal of Applied Laboratory Medicine.* 2017;1(4):342-345.

13. McCudden CR, Jacobs JFM, Keren D, Caillon H, Dejoie T, Andersen K. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. *Clinical Biochemistry.* 2018;51:72-79.

14. Strobel SL. The incidence and significance of pseudoparaproteins in a community hospital. *Ann Clin Lab Sci.* 2000;30(3):289-294.

For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.

Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement or refund of any amount not to exceed the purchase price attributable to the goods as to which such claim is made. These alternatives shall be buyer’s exclusive remedies.

In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damages.

The foregoing warranties are in lieu of all warranties expressed or implied including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

© 2023 Helena Laboratories, Corp.

Pro. 274

9/23(6)

