

# QuickGel® Touch Split Beta SPE Procedure

Cat. No. 3350

The QuickGel Split Beta SPE method is intended for the separation of serum, cerebrospinal fluid (CSF) or urine proteins by agarose gel electrophoresis using the SPIFE Touch system.

## SUMMARY

Serum contains over one hundred individual proteins, each with a specific set of functions and subject to specific variation in concentration under different pathologic conditions.<sup>1</sup> Since the introduction of moving-boundary electrophoresis by Tiselius<sup>2</sup> 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, alpha<sub>1</sub>, alpha<sub>2</sub>, beta and gamma proteins. Each of these classical electrophoretic zones, with the exception of albumin, normally contains two or more components. The relative proportions of these fractions have proven to be useful aids in the diagnosis and prognosis of certain disease states.<sup>3,5</sup>

## 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. In the QuickGel Serum Protein procedures, proteins are separated according to their respective electrical charges on agarose gel using both the electrophoretic and electroosmotic forces present in the system. The proteins are then stained with a visible stain.

## COMPONENTS

### 1. QuickGel 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.**

**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. Acid Blue Stain

**Ingredients:** When dissolved as directed, the stain contains 0.5% (w/v) acid blue stain.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** Dissolve the dry stain (entire contents of vial) in 1 L of 5% acetic acid. Mix thoroughly for 30 minutes.

**Storage and Stability:** The dry stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The diluted stain is stable six months when stored at 15 to 30°C.

**Signs of Deterioration:** The diluted stain should be a homogeneous mixture free of precipitate. Discard if precipitate forms.

### 3. Citric Acid Destain

**Ingredients:** After dissolution, the 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 the Destain vat. Add the entire package of Destain. Mix well 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. Acid Violet Stain (Optional Urine Stain)

**Ingredients:** The stain is comprised of Acid Violet stain.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** Dissolve the dry stain in 1 L of 10% acetic acid and mix thoroughly.

**Storage and Stability:** The dry stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The stain solution is stable six months when stored at 15 to 30°C in a closed container.

**Signs of Deterioration:** The diluted stain should be a homogeneous mixture free of precipitate.

## INSTRUMENTS

A SPIFE Touch must be used to electrophorese, stain, destain and dry the gels. The gels may be scanned on the QuickScan Touch/2000 (Cat. No. 1690/1660) or a separate densitometer. Refer to the appropriate Operator's Manual for detailed instructions.

## SPECIMEN COLLECTION AND HANDLING

**Specimen:** Fresh serum, CSF 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 of serum is necessary, samples may be stored covered at 15 to 30°C for 4 days, 2 to 8°C for 2 weeks, or -20°C for 6 months.<sup>6</sup> Urine or CSF samples may be stored covered at 2 to 8°C for up to 72 hours or at -20°C for 1 month.

**Urine Sample Preparation:** Urine samples may be run diluted, neat or concentrated. Shake samples to homogenize. Centrifuge desired volume at 2000 x g for 5 minutes. Remove supernatant and concentrate as follows:

Total Protein (mg/dL)	Conc. Factor
<50	100x
50-100	50x
100-300	25x
300-600	10x
>600	5x

**CSF Sample Preparation:** CSF samples may be used after proper concentration (10-50X).

### Interfering Factors:

- Hemolysis may cause false elevation in the alpha<sub>2</sub> and beta fractions.
- Inaccurate results may be obtained on specimens left uncovered, due to evaporation.

## PROCEDURE

**Materials provided:** The following materials needed for the procedure are contained in the QuickGel Split Beta SPE Kit (Cat. No. 3350). Individual items are not available.

QuickGel Split Beta SPE Gels (10)  
Acid Blue Stain (1 vial)  
QuickGel Blotter C (10)  
Citric Acid Destain (1 pkg)  
Blade Applicator Kit (10)

### Material provided but not contained in the kit:

ITEM	CAT. NO.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
Applicator Blade Weights	3387
Gel Block Remover	1115
SPE Normal Control	3424
SPE Abnormal Control	3425
REP Prep	3100
Disposable Sample Cups (Shallow Wells)	3369
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrode	1111
SPIFE QuickGel Holder	3358
SPIFE QuickGel Chamber Alignment Guide	86541003
QuickGel Accessory Kit (Templates)	3426
Acid Violet Stain	552351

### Materials needed but not provided:

5% acetic acid  
0.85% saline

## STEP-BY-STEP METHOD

### I. Chamber Preparation

- The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
- Remove the contact sheet and clean the chamber floor according to instructions in the Operator's Manual.
- Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
- Using an indelible marker, outline the square open area onto the copper floor. Allow marking to dry, and apply another contact sheet.

### II. Sample Blade Application Method

- Remove one Disposable Applicator Blade from the packaging. If testing more than 10 samples, remove two Applicator Blades from the packaging.
- Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly. If using two Applicator Blades, place them into the vertical slots numbered 6 and 12. When testing serum with urine or CSF samples, serum application is made after the third urine or CSF application. Therefore the blade for serum application is not added until after the third urine/CSF application.

**NOTE: The Applicator Blade will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blades into the slots.**

If testing serum only, follow the instructions marked "• Serum (Blade Application)". If testing urine/CSF only, follow instructions marked "• Urine/CSF (Blade Application)". If testing serum with urine or CSF, follow instructions marked "• Serum and Urine/CSF (Blade Application)".

- Place an Applicator Blade Weight on top of each Applicator Blade. When placing the weight on the blades, position the weight with the thick side to the right.
- Slide the Disposable Sample Cups into the top row numbered 1 to 10 of the appropriate cup tray. If testing more than 10 samples, place cups into both rows.
- Pipette 15 µL of control or serum or 20 µL of urine or CSF into Disposable Sample Cups (Cat. No. 3369 for both) 1 to 5 and 6 to 10. If testing more than 10 samples, pipette sample into cups 11 to 15 and 16 to 20. Cover until ready to use.
- Carefully cut open the end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
- Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
- Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
- Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
- Clean the QuickGel Electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
- Place a QuickGel Electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact of the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
- Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select the test, press **SETUP** and proceed to Step IV. Once parameters have been verified, proceed to Step IV.A if running serum only or urine/CSF only or IV.B if running serum and urine/CSF.

### III. Template Application Method

Template application may be used for testing CSF or urine specimens which have insufficient volumes for blade application.

- Carefully open one end of the pouch and remove one gel from the protective packaging. Reseal the pouch with tape to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
- Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter then remove the blotter.
- Dispense about 1 mL of REP Prep onto the left side of the marked area of the chamber.
- Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side. Use lint-free tissue to wipe around the edges of the gel backing to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
- Depending on the number of samples tested, place one or two templates across the gel aligning the slits with the arrows on the gel backing.
- Apply fingertip pressure to each template, making sure there are no bubbles under it. **NOTE:** If wearing rubber gloves to perform this step, place a QuickGel Blotter A over the template and then apply fingertip pressure to the template. Remove the blotter. Powder from the gloves can produce gel artifacts.
- Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
- Place a carbon electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact of the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
- Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test, press **SETUP** and proceed to Step IV. Once parameters have been verified, proceed to Step IV.C.

### IV. Parameters

Using the instructions provided in the appropriate Operator's Manual, set up the parameters as follows for the SPIFE Touch:

\* Due to variation in environmental conditions, a Dry time of 10 minutes is recommended, but a range of 10 to 15 minutes is acceptable.

\*\* An Apply Sample time of 3 or 30 seconds is acceptable.

#### SEPARATOR UNIT

##### • Serum (Blade Application)

**Load Sample** Prompt: None  
Time: 0:01  
Temperature: 21°C  
Speed: 1

**Apply Sample** Prompt: None  
Time: 0:30\*\*  
Temperature: 21°C  
Speed: 1  
Location: 1

**Electrophoresis** Prompt: None  
Time: 8:00  
Temperature: 21°C  
Voltage: 350 V  
mA: 60 mA

**Dry** Prompt: Remove Gel Blocks  
Time: 10:00\*  
Temperature: 54°C

**End**

##### • Urine/CSF (Blade Application)

**Load Sample 1** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample 1** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Load Sample 2** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample 2** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Load Sample 3** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample 3** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Electrophoresis** Prompt: None  
Time: 8:00  
Temperature: 21°C  
Voltage: 350 V  
mA: 60 mA

**Dry** Prompt: Remove Gel Blocks  
Time: 10:00\*  
Temperature: 54°C

**End**

##### • Serum and Urine/CSF (Blade Application)

**Load Sample** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Load Sample 2** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample 2** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Load Sample 3** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1

**Apply Sample 3** Prompt: None  
Time: 0:30  
Temperature: 21°C  
Speed: 1  
Location: 1

**Load Sample 4** Prompt: To Continue  
Time: 0:01  
Temperature: 21°C  
Speed: 1

**Apply Sample 4** Prompt: None  
Time: 0:30\*\*  
Temperature: 21°C  
Speed: 1  
Location: 1

**Electrophoresis** Prompt: None  
Time: 8:00  
Temperature: 21°C  
Voltage: 350 V  
mA: 60 mA

**Dry** Prompt: Remove Gel Blocks  
Time: 10:00\*  
Temperature: 54°C

**End**

• **Urine/CSF (Template Application)**

**Pause** Prompt: None  
Time: 10:00  
Temperature: 21°C

**Electrophoresis** Prompt: None  
Time: 8:00  
Temperature: 21°C  
Voltage: 350 V  
mA: 60 mA

**Dry** Prompt: Remove Gel Blocks  
Time: 10:00\*  
Temperature: 54°C

**End**

**STAINER UNIT**

• **Serum, CSF and Urine (Both application methods)**

**NOTE: If testing urines with Acid Violet Stain, change "Valve: 3" to "Valve: 5" in Stain Section.**

**Stain** Prompt: None  
Time: 4:00  
Recirculation: Off  
Valve: 3  
Fill, Drain

**Destain 1** Prompt: None  
Time: 1:00  
Recirculation: On  
Valve: 2  
Fill, Drain

**Destain 2** Prompt: None  
Time: 1:00  
Recirculation: On  
Valve: 2  
Fill, Drain

**Destain 3** Prompt: None  
Time: 1:00  
Recirculation: On  
Valve: 2  
Fill, Drain

**Dry** Prompt: None  
Time: 10:00\*  
Temperature: 63°C

**End**

**A. Serum or Urine/CSF (Blade Application)**

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples, electrophorese and beep when completed. Proceed to Step V.

**B. Serum and Urine/CSF (Blade Application)**

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an option to proceed. **NOTE:** Serum and CSF or urine samples are run on the same gel on different rows. Place the Applicator Blade into the slots that correspond to CSF or urine samples. After the third CSF/urine application, the instrument will beep and stop. Open the chamber lid, add an Applicator Blade into the remaining slot for the serum samples and remove the urine/CSF blade. Close the chamber lid and press **CONTINUE**. The instrument will apply and continue. Proceed to Step V.

**C. Serum and Urine/CSF (Template Application)**

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. Open the chamber lid and apply urine and/or CSF by placing 3 µL of each sample onto one of the ten available slits on the Sample Template.
3. Close the chamber lid and press the **CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 10 minutes.
4. Open the chamber lid and gently blot the template with a QuickGel Blotter A and carefully remove the blotter and template. Dispose of templates as biohazardous waste.
5. Close the chamber lid and press the **CONTINUE** button to start electrophoresis. SPIFE will beep when electrophoresis is complete.

**V. Visualization**

1. After electrophoresis is complete, open the chamber lid and use the Gel Block Remover to remove the gel blocks. Dispose of blades and cups as biohazardous waste. Replace the electrodes on each end of the gel to prevent curling during drying.
2. Close the chamber lid and press the **CONTINUE** button to dry the gel.
3. After the gel has been dried, open the chamber lid and carefully remove the gel from the electrophoresis chamber.
4. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side **down**, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
5. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
6. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
7. When the process is completed, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot. Scan the bands in a densitometer.

**Evaluation of the Protein Bands**

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

**Quantitative Evaluation:** Scan the samples on the QuickGel in the QuickScan Touch/2000, agarose side up, on the acid blue setting. A slit size of 5 is recommended.

**Stability of End Product:** The completed, dried, QuickGel 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 CSFs for qualitative comparison. Refer to the package insert provided with the control for assay values.

**REFERENCE VALUES**

The reference range presented was established with the QuickGel Split Beta SPE System on 50 normal specimens using the SPIFE Touch Analyzer. Each laboratory should perform its own normal range study. These values are presented as a guideline.

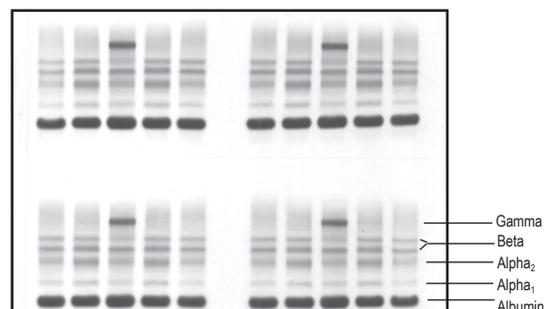
<u>Protein Fraction</u>	<u>% of Total Protein</u> <u><math>\bar{x} \pm 2 S.D.</math></u>
Albumin	47.6 - 61.9
Alpha <sub>1</sub>	1.4 - 4.6
Alpha <sub>2</sub>	7.3 - 13.9
Beta	10.9 - 19.1
Gamma	9.5 - 24.8

**Variations of Expected Values<sup>5</sup>**

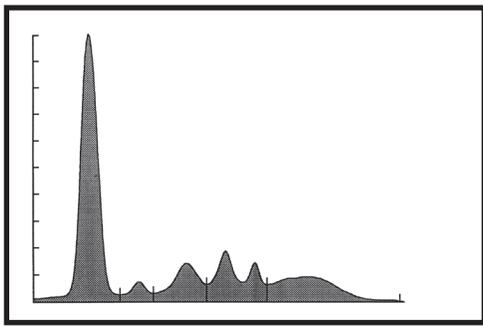
Studies show that values are the same for both males and nonpregnant females. (Some differences are seen in pregnant females at term and in women on oral contraceptives.) Age has some effect on normal levels. Cord blood has decreased total protein, albumin, alpha<sub>2</sub>, and beta fractions, slightly increased alpha<sub>1</sub>, 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 increases after the age of 40.

**RESULTS**

Figure 1 illustrates the electrophoretic mobilities of the albumin, alpha<sub>1</sub>, alpha<sub>2</sub>, beta and gamma protein bands on QuickGel 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 alpha<sub>1</sub>, followed by alpha<sub>2</sub>, beta and gamma globulins.



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



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

**Calculations of the Unknown**

The Helena QuickScan Touch/2000 will automatically calculate and print the relative percent and the absolute value of each band when the total protein is entered. Refer to the Operator's Manual provided with the instrument.

**INTERPRETATION OF RESULTS<sup>5</sup>**

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, pregnancy and genetic deficiencies.

**Further Testing Required**

The serum protein electropherogram or densitometric tracing should be evaluated for abnormalities. If abnormalities are observed, appropriate follow-up studies should be initiated. These may include immunoelectrophoresis, immunofixation, quantitation of immunoglobulins, bone marrow examination and other appropriate tests.

**LIMITATIONS**

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

**PERFORMANCE CHARACTERISTICS**

**Within Run:** A normal patient sample, a normal control and an abnormal control were run alternately on a single gel with the following results:

Normal Control n = 7

Protein Fraction	Mean %	SD	CV
Albumin	53.8	1.0	1.9%
Alpha <sub>1</sub>	3.9	0.2	5.4%
Alpha <sub>2</sub>	9.6	0.3	3.3%
Beta	16.5	0.3	1.6%
Gamma	16.1	0.6	3.9%

Abnormal Control n = 7

Protein Fraction	Mean %	SD	CV
Albumin	47.9	0.7	1.6%
Alpha <sub>1</sub>	3.4	0.1	4.1%
Alpha <sub>2</sub>	8.8	0.3	3.3%
Beta	12.6	0.3	2.4%
Gamma	27.2	0.3	1.2%

Normal Patient n = 6

Protein Fraction	Mean %	SD	CV
Albumin	51.8	0.4	0.8%
Alpha <sub>1</sub>	3.2	0.2	5.0%
Alpha <sub>2</sub>	11.6	0.2	1.7%
Beta	16.3	0.2	1.1%
Gamma	17.1	0.4	2.4%

**Between-Run:** A normal patient sample, a normal control and an abnormal control were run in replicate on 9 gels with the following results:

Normal Control n = 63

Protein Fraction	Mean %	SD	CV
Albumin	55.3	1.2	2.1%
Alpha <sub>1</sub>	3.8	0.3	7.9%
Alpha <sub>2</sub>	9.6	0.3	3.5%
Beta	16.0	0.5	3.1%
Gamma	15.4	0.7	4.5%

Abnormal Control n = 63

Protein Fraction	Mean %	SD	CV
Albumin	48.7	0.8	1.7%
Alpha <sub>1</sub>	3.4	0.2	4.6%
Alpha <sub>2</sub>	8.8	0.3	2.9%
Beta	12.2	0.4	3.4%
Gamma	26.9	0.4	1.4%

Normal Patient n=53

Protein Fraction	Mean %	SD	CV
Albumin	52.5	1.0	1.9%
Alpha <sub>1</sub>	3.0	0.2	6.1%
Alpha <sub>2</sub>	11.5	0.3	2.8%
Beta	16.1	0.4	2.6%
Gamma	16.8	0.5	2.9%

**CORRELATION**

Normal (n = 28) and abnormal (n = 28) serum samples were analyzed running the QuickGel Split Beta SPE Kit on the QuickGel Chamber and on the SPIFE Touch.

n = 30  
 $Y = 0.9949X + 0.104$   
 $R = 0.9996$   
 X = QuickGel Split Beta SPE on the SPIFE 3K  
 Y = QuickGel Split Beta SPE on SPIFE Touch

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