

SPIFE® Ultra ImmunoFix-15 Procedure

Helena  Laboratories

Cat. No. 3444, 3444T

INTENDED USE

SPIFE Ultra ImmunoFix-15 is intended for the qualitative identification of monoclonal gammopathies in serum, cerebrospinal fluid (CSF) or urine using protein electrophoresis and immunofixation on the SPIFE 3000 system.

SUMMARY

Immunofixation electrophoresis (IFE) is a two stage procedure using agarose gel high resolution electrophoresis in the first stage and immunoprecipitation in the second. There are numerous applications for IFE in research, forensic medicine, genetic studies and clinical laboratory procedures. The greatest demand for IFE is in the clinical laboratory where it is primarily used for the detection of monoclonal gammopathies. A monoclonal gammopathy is a primary disease state in which a single clone of plasma cells produces elevated levels of an immunoglobulin of a single class and type. Such immuno-globulins are referred to as monoclonal proteins, M-proteins or paraproteins. Their presence may be of a benign nature or of uncertain significance. In some cases they are indicative of a malignancy such as multiple myeloma or Waldenstrom's macroglobulinemia. Differentiation must be made between polyclonal and monoclonal gammopathies because polyclonal gammopathies are only a secondary disease state due to clinical disorders such as chronic liver diseases, collagen disorders, rheumatoid arthritis and chronic infections.

Alfonso first described immunofixation in the literature in 1964.¹ Alper and Johnson published a more practical procedure in 1969 as a result of their work devoted to the detection of genetic polymorphisms of ceruloplasmin and Gc-globulin and the conversion of C3 during activation.² They later extended their studies to genetic polymorphisms of complement components and the identification of alpha₁ antitrypsin.^{3, 4} Immunofixation has been used as a procedure for the study of immunoglobulins since 1976.^{5, 6}

The SPIFE IFE methods offer many advantages. These include ease of interpretation, excellent resolution, reagent conservation and rapid turnaround.

In addition, the SPIFE Ultra IFE-15 method offers a larger sample surface area (enabling fifteen specimens to be run at the same time) and shortened electrophoresis time.

PRINCIPLE

Proteins are first resolved by electrophoresis. In the second stage, the soluble antigen and antibody are allowed to react. The resultant antigen-antibody complex(es) may become insoluble (as long as the antibody is in slight excess or near equivalency) and precipitate. The precipitation rate depends on the proportions of the reactants, temperature, salt concentration and the pH of the solution. The unreacted proteins are removed by a washing step and the antigen-antibody complex (which might be visible as a white cloudy band in the unstained gel against a dark background) is visualized by staining. The bands in the protein separation are compared with the precipitin bands obtained with immunofixation.

REAGENTS

1. SPIFE Ultra IFE-15 Gel

Ingredients: Each gel contains agarose in tris-barbital/MOPS buffer with a stabilizer and a preservative added.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. - TOXIC -CAUTION: DO NOT INGEST. 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 horizontally at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. DO NOT REFRIGERATE OR FREEZE.

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 gel blocks.

2. Acid Violet 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 liter of 10% acetic acid and mix thoroughly. Fill the SPIFE stain vat.

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 for 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.

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. Tris-Buffered Saline

Ingredients: The powder contains a Tris base with Tris HCl and sodium chloride.

WARNING: FOR IN-VITRO DIAGNOSTIC USE.

Preparation for Use: Dissolve the powder in 8 L of deionized water and mix thoroughly.

Storage and Stability: Store the dry powder at 15 to 30°C until the expiration date indicated on the label. The buffer solution should be stored at 15 to 30°C.

Signs of Deterioration: The buffer solution should be discarded if it shows signs of bacterial contamination.

5. SPIFE IFE Protein Fixative

Ingredients: The fixative contains 10% sulfosalicylic acid, 10% acetic acid, and 0.125% glutaraldehyde.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. CORROSIVE - NEVER PIPETTE BY MOUTH. DO NOT INGEST.

Preparation for Use: The fixative is ready for use as packaged.

Storage and Stability: The fixative should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

Signs of Deterioration: The fixative should be a clear solution.

6. Antisera to Human IgG, IgA, IgM, Kappa Light Chain and Lambda Light Chain

Ingredients: Antisera vials in the kit contain monospecific antisera to human immunoglobulin heavy chains, IgG, IgM, IgA and to human light chains, Kappa and Lambda. The antisera have been prepared in goat. Each vial of antiserum contains a stabilizer and sodium azide as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. To prevent the formation of toxic vapors, do not mix with acidic solutions. When discarding, always flush sink with copious amounts of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

Preparation for Use: The antisera are ready for use as packaged.

Storage and Stability: The antisera should be stored at 2 to 8°C and are stable until the expiration date indicated on the vial.

Signs of Deterioration: Extremely cloudy antisera may be indicative of bacterial contamination.

INSTRUMENT

A SPIFE 3000 instrument must be used to electrophorese, stain, destain and then dry the gels. The gels may be scanned on a densitometer such as the QuickScan 2000 (Cat. No. 1660). Refer to the Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh serum, CSF or urine is the specimen of choice.

Interfering Factors:

1. Evaporation of uncovered specimens may cause inaccurate results.
2. Plasma should not be used because the fibrinogen may adhere to the gel matrix resulting in a band in all patterns across the gel.

Storage and Stability: If storage is necessary, samples may be stored covered at 2 to 8°C for up to 72 hours.

PROCEDURE

Materials Provided: The following materials needed for the procedure are contained in the SPIFE Ultra IFE-15 Kit (Cat. No. 3444 or 3444T). Individual items are not available.

SPIFE IFE Gels (10)	Fixative
Acid Violet Stain (1 vial)	IgG
Tris-Buffered Saline (1 pkg)	IgA
Citric Acid Destain (1 pkg)	IgM
SPIFE Blotter C (20)	Kappa
SPIFE Blotter J (10)	Lambda
SPIFE IFE-15 Blotter Combs (10)	
Applicator Blade Assembly (50)	

or
 SPIFE Urine IFE Templates (50)
 Blotter A-Plus (50)

Materials provided by Helena Laboratories but not contained in the kits above:

	Cat. No.
SPIFE 3000 Analyzer	1088
Quick Scan 2000	1660
REP Prep	3100
Gel Block Remover	1115
SPIFE IFE Multi-Channel Pipettor	1122
Pipette Tips for SPIFE IFE Multi-Channel Pipettor	3355
Tip Spacers	3356
SPIFE Ultra IFE-15 Antisera Template	3448
SPIFE IFE Antisera Tray	3394
SPIFE IFE-9/15 Disposable Cups	3363
SPIFE 3000 IFE-15 Disposable Cup Tray	3362
SPIFE Urine IFE-15 Alignment Guide	3407

Materials and Supplies Needed but not Supplied

10% acetic acid
 0.85% saline

STEP-BY-STEP METHOD

I. Sample Preparation (SPIFE 3000)

A. Serum

The patient serum samples are diluted 1:3 (1 part serum with 2 parts 0.85% saline) for serum protein lanes and diluted 1:5 (1 part serum with 4 parts 0.85% saline) for immunofix lanes. However, due to desired sensitivity variations, serum samples may also be diluted as follows:

IgG	= 1:5 to 1:10
IgA	= undiluted to 1:5
IgM	= undiluted to 1:5
K	= 1:5 to 1:10
L	= undiluted to 1:5

The more concentrated samples are more likely to prozone while the more diluted samples may not exhibit desired sensitivity.

B. Urine

Urine samples may be run diluted, neat or concentrated. To achieve higher sensitivity, samples may be concentrated as follows. 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

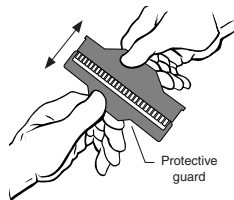
C. CSF

Concentrate CSF to an IgG level of 100-200 mg/dL for typing oligoclonal bands in CSF. Use concentrated specimen for all patterns.

II. Sample Application

A. Serum or Urine Blade Application

- Slide the Disposable Cup strips into the IFE-15 Cup Tray.
- Pipette 17 µL of diluted serum or 20 µL of urine into the Disposable Cups.
- Place the Cup Tray into the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
- Remove five disposable Applicator Blade Assemblies from the packaging. Remove the protective guard from the blades by gently bending the protective piece back and forth until it breaks free.
- Place the five Applicator Blades into the vertical slots numbered 1, 5, 8, 12, and 15 in the Applicator Assembly.



Please note that the blade assembly will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.

- Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
- Remove the gel from the protective packaging and discard overlay.
- Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. 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 no bubbles remain under the gel.

9. Place a SPIFE Blotter C on the gel with the longer edge parallel with gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and remove the blotter.

- Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.

- Place a carbon electrode on the outside edge of each gel block outside the magnetic posts. Improper contact between the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
- Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **SERUM/URINE IFE** option appears on the displays. Proceed to Step III.

B. Urine or CSF Template Application

- Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
- Remove the gel from the protective packaging and discard overlay. Carefully place the gel on the SPIFE Urine IFE-15 Alignment Guide. Place a SPIFE Blotter C on the gel with the longer edge parallel with the gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and remove the blotter.
- Place the Urine IFE Templates on the gel aligning the application slits with the appropriate set of pins on the sides of the Alignment Guide. The templates have been marked with a hole in one corner. Place the marked corner in the lower left position. Apply slight fingertip pressure to the template, making sure there are no air bubbles under it. Up to five templates can be placed on a gel at one time. **NOTE:** If wearing rubber gloves to perform this step, place a Blotter A-Plus over the template and the apply fingertip pressure to the template. Powder from the gloves can produce gel artifacts. Remove the blotter.
- Carefully remove the gel from the guide, and place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to the electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
- 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 outside the magnetic posts. Close the chamber lid.
- Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **URINE IFE** option appears on the displays.

III. Electrophoresis

Using the instructions provided in the SPIFE 3000 Operator's Manual, set up parameters as follows:

Due to variation in environmental conditions,

* **A Blot 1 time of 3 minutes is recommended, but a Blot time range of 2 to 5 minutes is acceptable.**

** **An electrophoresis time of 6 minutes is recommended, but it may range 5:30 to 6:30 minutes.**

*** **An antisera absorption time of 2 minutes is recommended, but 1 to 3 minutes is acceptable.**

● Serum (Blade Application)

Electrophoresis Unit					
1) No prompt					
Load Sample 1	00:30	21°C	SPD6		
2) No prompt					
Apply sample 1	00:30	21°C	SPD1	LOC 2	
3) No prompt					
Electrophoresis 1	**6:00	21°C	650 V	160 mA	
4) Remove gel blks, apply antisera (continue)					
Absorb 1	***2:00	21°C			
5) Remove excess antisera (continue)					
Blot 1	*3:00	21°C			
6) Remove template, install blot (continue)					
Blot 2	5:00	40°C			
7) Remove blotter, (continue)					
Dry 1	15:00	50°C			
8) No prompt					
END OF TEST					

● Urine or Urine and Serum (Blade Application)

Electrophoresis Unit

NOTE: Serum and urine samples may be run on the same gel on different rows by pipetting 20 µL urine and 17 µL diluted serum into the cups. Change Step "5) No prompt" to "5) To Continue, (continue)".

Place applicator blades into the slots that correspond to the urine sample. After the second urine application, the machine will beep and stop. Add an applicator blade into the remaining slot for serum samples. Press **TEST SELECT/CONTINUE**, the machine will apply and continue.

1) No prompt				
Load sample 1	00:25	21°C	SPD6	

2) No prompt					
Apply sample 1	00:25	21°C	SPD6	LOC2	
3) No prompt					
Load sample 2	00:25	21°C	SPD6		
4) No prompt					
Apply sample 2	00:25	21°C	SPD6	LOC2	
5) No prompt					
Load sample 3	00:25	21°C	SPD6		
6) No prompt					
Apply sample 3	00:25	21°C	SPD6	LOC2	
7) No prompt					
Absorb 1	2:00	21°C			
8) No prompt					
Electrophoresis 1	**6:00	21°C	650 V	160 mA	
9) Remove gel blks, apply Antisera (continue)					
Absorb 2	***2:00	21°C			
10) Remove excess Antisera (continue)					
Blot 1	*3:00	21°C			
11) Remove template, install blot (continue)					
Blot 2	5:00	40°C			
12) Remove blotter, (continue)					
Dry 1	15:00	50°C			
13) No prompt					
END OF TEST					

● Urine or CSF (Template Application)

Electrophoresis Unit

- Apply sample to template, (continue)
 - Absorb 1 5:00 21°C
- Blot and remove template, (continue)
 - Electrophoresis 1 **6:00 21°C 650V 160 mA
- Remove gel blocks, apply antisera, (continue)
 - Absorb 2 ***2:00 21°C
- Remove excess antisera, (continue)
 - Blot 1 *3:00 21°C
- Remove template, install blot (continue)
 - Blot 2 5:00 40°C
- Remove blotter, (continue)
 - Dry 1 15:00 50°C
- No prompt
- END OF TEST

● Serum, CSF or Urine (Both Application Methods)

Stainer Unit

- Plate Out, Holder In, Press (Continue)
 - Wash 1 00:03 REC=ON Valve=1
- Plate In, Gel Holder In, Press (Continue)
 - Wash 2 10:00 REC=ON Valve=1
- No prompt
 - Stain 1 4:00 REC=OFF Valve=5
- No prompt
 - Destain 1 1:00 REC=ON Valve=2
- No prompt
 - Destain 2 1:00 REC=ON Valve=2
- No prompt
 - Dry 1 8:00 63°C
- No prompt
 - Destain 3 1:00 REC=ON Valve=2
- No prompt
 - Dry 2 5:00 63°C
- No prompt
- END OF TEST

A. Serum or Urine Blade Application

- With the appropriate display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin.
- The SPIFE 3000 will apply samples onto the gel and electrophorese, then beep when completed. Dispose of blades and cups as biohazardous waste. Proceed to Step IV.

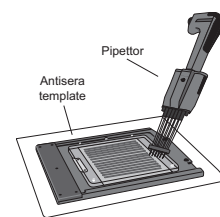
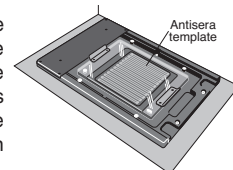
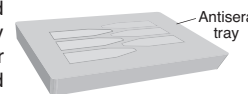
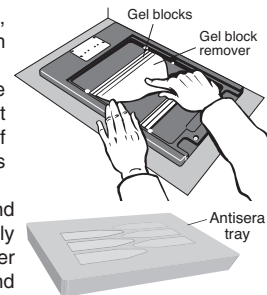
B. Urine or CSF Template Application

- With **URINE IFE** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. Open the chamber lid.
- Place 3 μ L of each sample onto the slits in the template (one protein and five immunofixation) for each patient. Apply the samples as quickly as possible.
- Close the chamber lid, and press the **TEST SELECT/CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 5 minutes.
- After sample application is complete, open the chamber lid and gently blot the template with a Blotter A-Plus.
- Carefully remove the blotter(s) and template(s) and discard as biohazardous waste.
- Close the chamber lid, and press the **TEST SELECT/CONTINUE**

button to start electrophoresis. SPIFE will beep when electrophoresis is complete.

IV. Immunofixation

- When electrophoresis is complete, open the chamber. Remove the carbon electrodes.
- Using the Gel Block Remover, remove and discard both gel blocks. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture.
- Pour the contents of the Fixative vial and each antisera vial into the appropriately labeled wells of the Antisera Tray. Cover the tray when not in use. Store tray and antisera at 2 to 8°C.
- Place six (6) tips onto the SPIFE IFE-15 Pipettor. Note that one side of the Tip Spacer is concave around the holes. Holding the pipettor with the tips up, slide the concave side of the spacer down over the tips so that the tips squeeze together.
- Open the chamber lid. Holding the template by the handles, gently place the Antisera Template onto the surface of the gel such that the round alignment hole is positioned on the pin to the left and the obround hole fits over the alignment pin on the right. No further pressure is needed.
- Pipette 250 μ L of Fixative and Antisera from the Antisera Tray and carefully dispense them into the oval slots at the right end of each antisera channel in the template.
- Close the chamber lid and press the **TEST SELECT/CONTINUE** button to continue with antisera absorption. After the incubation period, the SPIFE will beep.
- When antisera absorption is complete, open the chamber lid. Place one Blotter Comb into the slots on the right end of the antisera channels such that the tips of the combs touch the gel. Close the chamber lid, and press the **TEST SELECT/CONTINUE** button. After the preliminary blot of *3 minutes, the instrument will beep.
- Remove the Blotter Comb and the Antisera Template. Gently blot the gel surface with a Blotter C, then remove the blotter. Place a Blotter J on the surface of the gel. Place the Antisera Template on top of the Blotter J. Close the chamber lid and press the **TEST SELECT/CONTINUE** button. The final blot will be timed for 5 minutes.
- When the beeper sounds, open the chamber lid and remove the Antisera Template and the blotter. Lay one electrode across each end of the gel to prevent curling during the drying step. Close the chamber lid and press the **TEST SELECT/CONTINUE** button. The gel will be predried in the electrophoresis chamber. **NOTE:** Do not allow antisera to dry in the template. The Antisera Template should be cleaned with a mild biocidal detergent. The template may also be scrubbed with a soft brush to remove any antisera residue. Rinse with deionized water.
- After the gel has been predried, carefully remove the gel from the electrophoresis chamber.



V. Washing, Staining, and Destaining

- With **SERUM/URINE IFE** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin.
- Press the **TEST SELECT/CONTINUE** button. This will initiate the chamber prerinse cycle.
- After the chamber has been rinsed, remove the gel holder from the stainer chamber. Attach the gel to the holder by placing the round hole on the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.
- Place the gel holder with attached gel into the stainer chamber. The gel should face backwards in the stainer.
- Press the **TEST SELECT/CONTINUE** button to begin the staining process. The instrument will wash, stain, destain, and dry the gel.
- When the gel has completed the process, the instrument will beep. Remove the gel holder from the stainer to view the bands.

Stability of the End Product: The completed, stained and dried immunofixation gel is stable for an indefinite period of time.

Quality Control: IFE controls may be required by federal, state and local regulations.

INTERPRETATION OF RESULTS

The majority of monoclonal proteins migrate in the cathodic (gamma) region of the protein pattern. But, due to their abnormality, they may migrate anywhere within the globulin region on protein electrophoresis.

The monoclonal protein band on the immunofixation pattern will occupy the same migration position and shape as the monoclonal band on the reference protein electrophoresis pattern. The abnormal protein is identified by the corresponding antiserum used. Because of the increased sensitivity of the procedure, it is not uncommon to see a fixed band that is not visible in the serum protein procedure.

When low concentrations of M-protein are present, the immunofixation band may appear on the stained background of the polyclonal immunoglobulin. A stained background may also appear when the M-protein is present along with a large polyclonal increase.

For an in-depth discussion of IFE interpretation, call Helena Laboratories toll free and request the free publication "ImmunoFixation for the Identification of Monoclonal Gammopathies" Form R5.

LIMITATIONS

1. Antigen excess will occur if there is not a slight antibody excess or antigen/antibody equivalency at the site of precipitation. Antigen excess in IFE is usually due to a very high level of immunoglobulin in the patient sample. The dissolution of immunoprecipitation is manifested by a loss of protein at the point of highest antigen concentration, resulting in staining in the margins and leaving the central area with little demonstrable protein stain. In this case it may be necessary to adjust the protein content of the sample by dilution. Electrophoresing excessive amounts of antigen decreases resolution and requires higher concentrations of antibody. For optimum separation and sufficient intensity for visual detection, care must be taken in adjusting antibody content, sample concentration, time and voltage. The SPIFE ImmunoFix method has been optimally developed to minimize the antigen excess phenomenon.
2. Monoclonal proteins may occasionally adhere to the gel matrix, especially IgM. These bands will appear in all five antisera reaction areas of the gel. However, where the band reacts with the specific antisera for its heavy chain and light chain, there will be a marked increase in size and staining activity, allowing the band to be identified.

Further Testing Required:

Specimens containing a band on serum protein electrophoresis suggestive of a monoclonal protein, but which do not react with IgG, IgA or IgM antisera, may require further testing as follows:

1. Serum samples which have a precipitin band with Kappa or Lambda Light Chain Antisera but none corresponding with IgG, IgA or IgM antisera may have a free light chain or they may have an IgD or IgE monoclonal protein. Such sera should be tested with ImmunoFix IgD and IgE antisera.
2. A CRP band may be detected in patients with acute inflammatory response.^{7,8} CRP appears as a narrow band on the most cathodic end of the high resolution agarose protein electrophoresis pattern. Elevated alpha₂ antitrypsin and haptoglobin (acute phase proteins) are supportive evidence for the presence of a CRP band. Patients with a CRP band will have a positive CRP by latex agglutination or an elevated quantitative CRP.

3. Cerebrospinal fluid may contain a non-immunoglobulin band, referred to as gamma-trace, which migrates in the gamma region. Because gamma-trace is non-immunoglobulin in nature, it will not react with antisera against human immunoglobulins. Gamma-trace is often detected in normal cerebrospinal fluid.^{9,10}

PERFORMANCE CHARACTERISTICS

Twenty-eight abnormal and eight normal serum specimens and nine urine specimens were tested using the SPIFE Ultra ImmunoFix-15 Procedure and the SPIFE ImmunoFix-9 Procedure. The test results showed good agreement between methods.

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SPIFE Ultra ImmunoFix-15 System

SPIFE Ultra ImmunoFix-15 Kits	Cat. No. 3444
SPIFE IFE Gels (10)	Fixative
Acid Violet Stain (1 vial)	IgG
Tris-Buffered Saline (1 pkg)	IgA
Citric Acid Destain (1 pkg)	IgM
Applicator Blade Assembly - (50)	Kappa
SPIFE Blotter C (20)	Lambda
SPIFE Blotter J (10)	
SPIFE IFE-15 Blotter Combs (10)	

SPIFE Ultra ImmunoFix-15 Kits	Cat. No. 3444T
SPIFE IFE Gels (10)	Fixative
Acid Violet Stain (1 vial)	IgG
Tris-Buffered Saline (1 pkg)	IgA
Citric Acid Destain (1 pkg)	IgM
SPIFE Urine IFE Templates (50)	Kappa
SPIFE Blotter C (20)	Lambda
SPIFE Blotter J (10)	
SPIFE IFE-15 Blotter Combs (10)	
Blotter A-Plus (50)	

Other Supplies and Equipment

The following items, needed for performance of the SPIFE IFE-15 procedure, must be ordered individually.

	Cat. No.
SPIFE 3000 Analyzer	1088
Quick Scan 2000	1660
REP Prep	3100
Gel Block Remover	1115
SPIFE IFE Multi-Channel Pipettor	1122
Pipette Tips for SPIFE IFE Multi-Channel Pipettor	3355
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SPIFE 3000 IFE-15 Disposable Cup Tray	3362
SPIFE Urine IFE-15 Alignment Guide	3407

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