SPIFE® Touch Ultra ImmunoFix Procedure

Cat. No. 3445, 3446

INTENDED USE

The SPIFE Touch Ultra ImmunoFix method is intended for the qualitative identification of monoclonal gammopathies in serum, cerebrospinal fluid (CSF) or urine using protein electrophoresis and immunofixation on the SPIFE Touch 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 immunoglobulins 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 as polyclonal gammopathies are only a secondary disease state due to clinical disorders such as chronic liver diseases, collagen disorders, rheumatoid arthritis and chronic infections.

The process of immunofixation was first described in 1964 by Alfonso[1], followed by a more practical procedure published five years later by Alper and Johnson 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 alpha$_1$ antitrypsin.[3, 4] Immunofixation has been used as a procedure for the study of immunoglobulins since 1976.[5,6] The SPIFE Touch Ultra IFE method offers many advantages. These include ease of interpretation, excellent resolution, reagent conservation and rapid turnaround.

In addition, the SPIFE Touch Ultra IFE method offers a larger sample surface area (enabling up to nine 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 6/9 Gel**
   Ingredients: Each gel contains agarose in tris-barbital/MOPS buffer with a stabilizer and a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. 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 L of 10% acetic acid and mix thoroughly. Fill the SPIFE Touch 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. The stain must be replaced after processing ten gels to avoid contamination.
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 Ultra IFE Protein Fixative**
   Ingredients: The fixative contains 10.0% 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 or sheep. 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 Touch analyzer must be used to electrophorese, stain, destain and then dry the gels. The gels may be scanned on a densitometer such as the QuickScan Touch/2000 (Cat. No. 1690/1660). Refer to the appropriate Operator's Manuals 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 as 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, store samples covered at 2 to 8°C for up to 72 hours.

PROCEDURE

Materials Provided: The following materials are provided:

Sample Test Size	**Cat. No.**	**Cat. No.**	
6 Sample	3446	3446T	
9 Sample	3445	3445T	
SPIFE Ultra IFE Gels (10)		Fixative	1 vial
Acid Violet Stain (1 vial)		IgG	1 vial
Tris-Buffered Saline (1 pkg)		IgA	1 vial
Citric Acid Destain (1 pkg)		IgM	1 vial
SPIFE Blotter C (20)		Kappa	1 vial
SPIFE Blotter J (10)		Lambda	1 vial
Blotter Combs (30)			
Applicator Swabs (10)			
Blade Applicator Kit (30)			
or			
SPIFE Urine IFE Templates (30)			
Blotter A-Plus (30)			

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

Item	Cat. No.
SPIFE Touch Analyzer	1068
Quick Scan Touch	1690
Quick Scan 2000	1660
ESH Touch	1380
IFE Controls	9400
REP Prep	
3100	
SPIFE IFE-3/6 Disposable Cups	3368
SPIFE IFE-9/15 Disposable Cups	3363
SPIFE Disposable Cup Tray for IFE-3/6	3377
SPIFE Disposable Cup Tray for IFE-9	3378
Gel Block Remover	1115
SPIFE IFE Multi-Channel Pipettor	1122
Tips for SPIFE IFE Pipettor	3355
Tip Spacers for SPIFE IFE Pipettor	3356
Tip Spacers for SPIFE 3/6 Multi-Channel Pipettor	3349
Tip Spacers for SPIFE 9 Multi-Channel Pipettor	3396
Tips for IFE-3/6 Multi-Channel Pipettor	3402
Tips for IFE-9 Multi-Channel Pipettor	3397
SPIFE IFE-6 Antisera Template	3410
SPIFE IFE-9 Antisera Template	3392
SPIFE IFE-3/6 Antisera Tray	1119
SPIFE IFE- 9 Antisera Tray	3394
SPIFE Urine IFE Alignment Guide	3380
Applicator Blade Weights	3387

Materials and Supplies Needed but not Supplied:

10% Glacial acetic acid
0.85% saline

STEP-BY-STEP METHOD

I. Sample Preparation

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
κ = 1:5 to 1:10
λ = 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 or unconcentrated. However, to achieve higher sensitivity samples may be 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

C. Cerebrospinal Fluid

Concentrate CSF to an IgG level of 100-200 mg/dL for typing oligoclonal bands in CSF. Use concentrated specimen for all patterns. CSF can only be applied to the gel by template method.

II. Sample Application

A. Serum or Urine (Blade Application)

1. Place three Applicator Blades into the vertical slots numbered 4, 10 and 16 in the Applicator Assembly.
   NOTE: 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.
   If testing serum only, follow the instructions marked "**• Serum (Blade Application)**". If testing urine or urine and serum, follow the instructions marked " **• Urine or Urine and Serum (Blade Application)**".
2. Place an Applicator Blade Weight on top of each blade assembly. When placing the weight on the blades, position the weight with the thick side to the right.
3. Slide the Disposable Sample Cups into the appropriate Cup Tray. Pipette 20 µL of urine or diluted serum into the appropriate Sample Cups. Pipette the serum protein dilution into the first well in each row. Use the next five wells for the immunofixation dilutions.
4. Place the Cup Tray into the SPIFE Touch. Align the holes in the tray with the pins on the instrument.
5. Remove the gel from the protective packaging and discard overlay.
6. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
7. 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 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.
8. 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.
9. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
10. Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts. Improper contact between the electrode and the gel block may cause skewed patterns. Close the chamber lid.
11. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP** and proceed to Step III. Once parameters have been verified, proceed to Step IV.A if running serum only or Step IV.B if running urine or urine and serum.

B. Urine or CSF (Template Application)

1. Remove the gel from the protective packaging and discard overlay. Carefully place the gel on the SPIFE Urine IFE Alignment Guide. 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.
2. Place the Urine IFE Template(s) 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 three 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.
3. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
4. 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 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.
5. Clean the electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
6. Place a carbon electrode on the outside ledge of each gel block outside the magnetic posts. Improper contact between the electrode and the gel block may cause skewed patterns. Close the chamber lid.
7. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP** and proceed to Step III. Once parameters have been verified, proceed to Step IV.C.

III. Electrophoresis Parameters

Using the instructions provided in the 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 range of 2 to 5 minutes is acceptable.**

Separator Unit

• Serum (Blade Application)

Load Sample	Prompt: None Time: 0:30 Temperature: 21°C Speed: 6
Apply Sample	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 1
Electrophoresis	Prompt: None Time: 7:00 Temperature: 21°C Voltage: 650 V mA: 160 mA
Absorb	Prompt: Remove Gel Blocks, Apply Antisera Time: 2:00 Temperature: 21°C
Blot 1	Prompt: Remove Excess Antisera Time: 3:00* Temperature: 21°C
Blot 2	Prompt: Remove Template, Install Blotter Time: 5:00 Temperature: 40°C
Dry	Prompt: Remove Blotter Time: 15:00 Temperature: 50°C
End	

- **Urine or Urine and Serum (Blade Application)**

Load Sample 1
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6

Apply Sample 1
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6
Location: 1

Load Sample 2
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6

Apply Sample 2
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6
Location: 1

Load Sample 3
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6

Apply Sample 3
Prompt: None
Time: 0:25
Temperature: 21°C
Speed: 6
Location: 1

Absorb 1
Prompt: None
Time: 2:00
Temperature: 21°C

Electrophoresis
Prompt: None
Time: 7:00
Temperature: 21°C
Voltage: 650 V
mA: 160 mA

Absorb
Prompt: Remove Gel Blocks, Apply Antisera
Time: 2:00
Temperature: 21°C

Blot 1
Prompt: Remove Excess Antisera
Time: 3:00*
Temperature: 21°C

Blot 2
Prompt: Remove Template, Install Blotter
Time: 5:00
Temperature: 40°C

Dry
Prompt: Remove Blotter
Time: 15:00
Temperature: 50°C

End

- **Urine or CSF (Template Application)**

Pause
Prompt: None
Time: 5:00
Temperature: 21°C

Electrophoresis
Prompt: To Continue
Time: 7:00
Temperature: 21°C
Voltage: 650 V
mA: 160 mA

Absorb
Prompt: Remove Gel Blocks, Apply Antisera
Time: 2:00
Temperature: 21°C

Blot 1
Prompt: Remove Excess Antisera
Time: 3:00*
Temperature: 21°C

Blot 2
Prompt: Remove Template, Install Blotter
Time: 5:00
Temperature: 40°C

Dry
Prompt: Remove Blotter
Time: 15:00
Temperature: 50°C

End

Stainer Unit

- **Serum, CSF or Urine (Both Application Methods)**

Wash 1
Prompt: Plate Out, Gel Holder In
Time: 0:03
Recirculation: On
Valve: 1
Fill, Drain

Wash 2
Prompt: Plate In, Gel Holder In
Time: 10:00
Recirculation: On
Valve: 1
Fill, Drain

Stain
Prompt: None
Time: 4:00
Recirculation: Off
Valve: 5
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

Dry 1
Prompt: None
Time: 8:00
Temperature: 63°C

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

Dry 2
Prompt: None
Time: 5:00
Temperature: 63°C

End

IV. Electrophoresis

A. Serum (Blade Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. The SPIFE Touch will apply samples onto the gel and start electrophoresis, then beep when electrophoresis is complete. Proceed to Step V.

B. Urine or Urine and Serum (Blade Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an option to proceed. **NOTE:** Serum and urine samples may be run on the same gel on different rows by pipetting 20 µL of urine or diluted serum into the cups. Change Load Sample 3 “Prompt: None” to “Prompt: To Continue”. Place Applicator Blades into the slots that correspond to the urine samples. After the second urine application, the instrument will beep and stop. Open the chamber lid and add an Applicator Blade into the remaining slot for the serum samples. Close the chamber lid and press **CONTINUE** to proceed.
2. When electrophoresis is complete, the instrument will beep. Proceed to Step V.

C. Urine or CSF (Template Application)

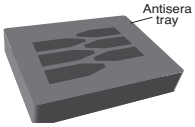
1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. Place 3 µL of each sample onto the slits in the template (one protein and five immunofixation) for each patient. Apply the samples as quickly as possible.
3. Close the chamber lid and press the **CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 5 minutes.
4. After sample application is complete, open the chamber lid and gently blot each template with a Blotter A-Plus.
5. Carefully remove the blotter(s) and template(s) and discard as biohazardous waste.
6. Close the chamber lid and press the **CONTINUE** button to start electrophoresis. The SPIFE Touch will beep when electrophoresis is complete.

V. Immunofixation

Serum, CSF or Urine

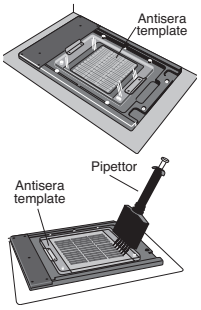
1. When electrophoresis is complete, open the chamber lid and remove the carbon electrodes.
2. Using the Gel Block Remover, remove and discard both gel blocks. Use a lint-free tissue to wipe around the edges of the gel backing to remove any excess moisture.

3. Apply IFE Controls
   a. Carefully blot the control wells with an Applicator Swab to remove excess buffer.
   b. Apply 1 µL of the control to the appropriate wells. The IgG Kappa control is applied to the “G” and “Kappa” wells. The IgA Lambda control is applied to the “A” and “Lambda” wells and the IgM control is applied to the “M” well only.



   c. Close the chamber lid and allow the controls to absorb into the agarose for three minutes.
4. 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.

5. 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.
6. Using a pipettor, aspirate 50 µL of Fixative and Antisera from the Antisera Tray. Dispense the fixative and antisera quickly into the oval slots at the right end of each antisera channel in the template.
7. Close the chamber lid and press the **CONTINUE** button to continue with antisera absorption. After the absorption time, the instrument will beep.



8. 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 **CONTINUE** button. After the preliminary blot, the instrument will beep. Open the chamber lid.
9. Remove the Blotter Combs and the Antisera Template. Gently blot the gel surface with a Blotter C, then remove the blotter. Place a SPIFE 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 **CONTINUE** button. The final blot will be timed for 5 minutes.
10. When the beeper sounds, open the chamber lid and remove the Antisera Template and blotter. Lay one electrode across each end of the gel to prevent curling during the drying step. Close the chamber lid and press the **CONTINUE** button. The gel will be predried in the electrophoresis chamber.
    NOTE: Do not allow antisera to dry on 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 and wipe completely dry.
11. After the gel has been predried, carefully remove the gel from the electrophoresis chamber.

VI. Washing, Staining and Destaining

Serum, CSF or Urine

1. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. 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 over the left pin and the obround hole over the right pin.
3. Place the Gel Holder with attached gel into the stainer chamber, with the front of the Gel Holder facing the operator. The gel should face away from the operator.
4. Press the **CONTINUE** button to begin the staining process. The instrument will wash, stain, destain and dry the gel.
5. 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: The ImmunoFix Controls (Cat. No. 9400) are recommended for use as qualitative controls for verification of the appropriate reactivity of the antisera. The set contains three monoclonal proteins; IgG Kappa, IgA Lambda and IgM.

INTERPRETATION OF RESULTS

The majority of monoclonal proteins migrate in the cathodic (gamma) region of the protein pattern. However, due to their abnormality, they may migrate anywhere within the globulin region during 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 Touch Ultra 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 alpha$_1$ 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 immunoglbulins. Gamma-trace is often detected in normal cerebrospinal fluid. [9,10]

PERFORMANCE CHARACTERISTICS

Fourteen serum samples containing monoclonal and polyclonal proteins were tested using the SPIFE 3000 and SPIFE Touch instruments. The test results showed complete agreement between instruments. Additionally, six urine and three serum samples were tested on the SPIFE 3000 and SPIFE Touch instruments using the blade application method with results showing complete agreement between instruments. Five urine and four CSF samples were tested on SPIFE 3000 and SPIFE Touch instruments using the template application method with results showing complete agreement between instruments.

BIBLIOGRAPHY

1. Alfonso, E., Quantitation Immunoelectrophoresis of Serum Proteins, Clin Chem Acta, 10:114-122, 1964.
2. Alper, C.A. and Johnson, A.M., Immunofixation Electrophoresis: A Technique for the Study of Protein Polymorphism. Vo Sang 17:445-452, 1969.
3. Alper, C.A., Genetic Polymorphism of Complement Components as a Probe of Structure and Function. Progress in Immunology, First International Congress of Immunology. Edited by New York, Academic Press, 609-624, 1971.
4. Johnson, A.M. Genetic Typing of Alpha(1)-Antitrypsin in Immunofixation Electrophoresis. Identification of Subtypes of P.M., J Lab Clin Med, 87:152-163, 1976.
5. Cawley, L.P. et al., Immunofixation Electrophoretic Technique Applied to Identification of Proteins in Serum and Cerebrospinal Fluid, Clin Chem, 22:1262-1268, 1976.
6. Ritchie, R.F. and Smith, R., Immunofixation III, Application to the Study of Monoclonal Proteins, Clin Chem, 22:1982-1985, 1976.
7. Jeppsson, J.E., et al., Agarose Gel Electrophoresis, Clin Chem, 25(4):629-638, 1979.
8. Killingsworth, L.M., et al., Protein Analysis, Diagnostic Medicine, 3-15, Jan/Feb., 1980.
9. Keshgegian, A.A., et al., Oligoclonal Immunoglobulins in Cerebrospinal Fluid Multiple Sclerosis, Clin Chem, 26(9):1340-1345, 1980.
10. Papadopoulos, N.M., et al., A Unique Protein in Normal Human Cerebrospinal

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