SPIFE® 3000 ImmunoFix Procedure for Plastic Applicators

Cat. No. 3401, 3401T, 3406, 3406T, 3409, 3409T

INTENDED USE

The SPIFE 3000 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 3000 system.

Cat. No. 3401, 3406, 3409 - Blade Assembly Application

Cat. No. 3401T, 3406T, 3409T - Template Application

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

In addition, the SPIFE 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.

COMPONENTS

1. **SPIFE IFE 3/6/9 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 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 2.5% sulfosalicylic acid, 1.0% trichloroacetic acid and 0.25% 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.

INSTRUMENTS

A SPIFE 3000 analyzer must be used to electrophorese, stain, wash, 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 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: Fresh serum, CSF or urine is the specimen of choice. 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.
3 Sample	3406	3406T
6 Sample	3401	3401T
9 Sample	3409	3409T

SPIFE 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 D (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 above:

Item	Cat. No.
SPIFE 3000 Analyzer	1088
QuickScan 2000	1660
QuickScan Touch	1690
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
Pipette 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-3 Antisera Template	3395
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 Applicator Blade will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.
2. Place an Applicator Blade Weight on top of each blade assembly. When placing the weight on the blade, 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 patient serum into the appropriate Sample Cups. For IFE-3 gels, use the first set of wells on each row (PT1, PT3, PT5). 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 3000. 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 side and ending on the right, 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. 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. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **SERUM IFE** or **URINE IFE** option appears on the displays. Proceed to Step III.

B. Urine/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. Remove the blotter.
2. Place one Urine IFE Template on the gel aligning the application slits with the upper 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 then 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 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.
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 results. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **URINE IFE** option appears on the display.

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.**
- **** an Electrophoresis time of 6:30 minutes is recommended, but a range of 6:00 to 7:00 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	LOC1

3) No prompt
Electrophoresis 1 **6:30 21°C 650V 160 mA
4) Remove gel blks, apply antisera (continue)
Absorb 1 10:00 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 8:00 50°C
8) No prompt
END OF TEST

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

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

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

Electrophoresis Unit

1) No prompt
Load Sample 1 00:25 21°C SPD6
2) No prompt
Apply Sample 1 00:25 21°C SPD6 LOC1
3) No prompt
Load Sample 2 00:25 21°C SPD6
4) No prompt
Apply Sample 2 00:25 21°C SPD6 LOC1
5) No prompt
Load Sample 3 00:25 21°C SPD6
6) No prompt
Apply Sample 3 00:25 21°C SPD6 LOC1
7) No prompt
Absorb 1 2:00 21°C
8) No prompt
Electrophoresis 1 **6:30 21°C 650V 160 mA
9) Remove gel blks, apply antisera (continue)
Absorb 2 10:00 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 8:00 50°C
13) No prompt
END OF TEST

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

Electrophoresis Unit

1) Apply sample to template (continue)
Absorb 1 5:00 21°C
2) Blot and remove template (continue)
Electrophoresis 1 **6:30 21°C 650V 160 mA
3) Remove gel blks, apply antisera (continue)
Absorb 2 10:00 21°C
4) Remove excess antisera (continue)
Blot 1 *3:00 21°C
5) Remove template, install blot (continue)
Blot 2 5:00 40°C
6) Remove blotter (continue)
Dry 1 8:00 50°C
7) No prompt
END OF TEST

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

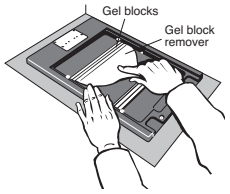
Stainer Unit

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

IV. Electrophoresis

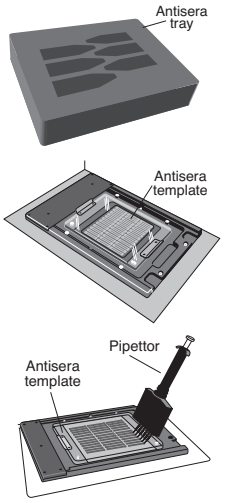
A. Serum or Urine (Blade Application)

1. With the appropriate test name 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.
2. The SPIFE 3000 will apply samples onto the gel and start electrophoresis, then beep when electrophoresis is complete. Proceed to Step V.



B. Urine/CSF (Template Application)

1. With the appropriate test name 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.
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. For IFE-3 gels, pipette the urine protein sample into the first slit in each template. Use the next five slits for the immunofixation samples.
3. Close the chamber lid and press the **TEST SELECT/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 **TEST SELECT/CONTINUE** button to start electrophoresis. The instrument will beep when electrophoresis is complete.



V. Immunofixation

Serum, CSF and 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. Place six (6) tips onto the appropriate 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 are aligned properly.
6. 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.
7. 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.

8. Close the chamber lid and press the **TEST SELECT/CONTINUE** button to continue with antisera absorption. After the 10 minute absorption time, the instrument will beep.
9. 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, the instrument will beep.
10. 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 D on the surface of the gel. Place the Antisera Template on top of the Blotter D. Close the chamber lid and press the **TEST SELECT/CONTINUE** button. The final blot will be timed for 5 minutes.
11. When the beeper sounds, open the chamber lid and remove the Antisera Template and the blotters. Lay one electrode across each end of the gel to prevent curling during the drying step. Close the chamber and press the **TEST SELECT/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.
12. After the gel has been predried, carefully remove the gel from the electrophoresis chamber.

VI. Washing, Staining and Destaining

Serum, CSF or Urine

1. With the appropriate test name 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.
2. Press the **TEST SELECT/CONTINUE** button. This will initiate the chamber pre-rinse cycle.
3. 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.
4. 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.
5. Press the **TEST SELECT/CONTINUE** button to begin the staining process. The instrument will wash, stain, destain and dry the gel.
6. 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. But, 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.

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 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 immunoglobulins. Gamma-trace is often detected in normal cerebrospinal fluid.[9, 10]

PERFORMANCE CHARACTERISTICS

Nine abnormal and three normal serum specimens and four urine specimens were tested using the SPIFE Ultra ImmunoFix with plastic blades procedure and SPIFE Ultra ImmunoFix with metalized blades procedures. The test results showed good agreement between methods.

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 Fluid, Clin Chem, 29(10):1842-1844, 1983.

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Test System Code: 25288
Analyte Code: 2802
Complexity: High

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