QuickGel® Immuno-Fix Procedure

Cat. No. 3351, 3551, 3551T

INTENDED USE

QuickGel ImmunoFix 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 or the QuickGel Chamber systems.

Cat. No. 3351 - SPIFE Blade Assembly Application
Cat. No. 3551 - QuickGel Blade Application
Cat. No. 3551T - QuickGel Template Application
For In Vitro Diagnostic use.
Rx Only

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 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 alpha$_1$ antitrypsin.[3,4] Immunofixation has been used as a procedure for the study of immunoglobulins since 1976.[5, 6]

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

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. **QuickGel IFE 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. 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. **QuickGel IFE Protein Fixative**
   Ingredients: The fixative contains 4.0% sulfosalicylic acid, 6.7% trichloroacetic acid, 0.2% glutaraldehyde and 1.7% guanidine HCl.
   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 Analyzer or the QuickGel Chamber must be used to electrophorese, stain, destain and then dry the gels. 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 FOR SPIFE 3000

Materials Provided: The following materials needed for the procedure are contained in the QuickGel IFE Kit (Cat. No. 3351). Individual items are not available.

QuickGel 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
Applicator Blade Assembly (20)	Kappa	1 vial
QuickGel Blotter C (20)	Lambda	1 vial
QuickGel IFE Blotter J (10)		
QuickGel IFE Blotter Combs (10)		

Materials provided but not contained in the kit:

Item	Cat. No.
SPIFE 3000 Analyzer	1088
REP Prep	3100
SPIFE QuickGel IFE Cup Tray	3371
SPIFE IFE 9/15 Disposable Sample Cups	3363
SPIFE Gel Block Remover	1115
SPIFE QuickGel Rigid Antisera Template	3372
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Holder	3358
SPIFE QuickGel IFE Alignment Guide	3373
QuickGel IFE Templates and QuickGel Blotter A	552156

Materials and Supplies Needed but not Supplied:

Pipette and tips
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, 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

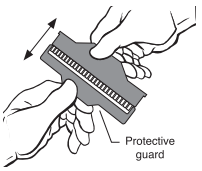
C. Cerebrospinal Fluid

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. Blade Application for Serum and Urine

1. Slide the Disposable Cup Strip into the appropriate Cup Tray (Cat. No. 3371).
2. Pipette 17 μL of diluted serum or 20 μL urine into the shallow wells of the Cup Strip. Samples should be placed in the wells aligned with "SP, G, A, M, K, L".
3. Place the Cup Tray into the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
4. Remove two 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.



5. Place the Applicator Blades into the vertical slots in the Applicator Assembly numbered 6 and 12.

   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.
6. Place the SPIFE QuickGel IFE Alignment Guide on the chamber floor by aligning the round hole over the left pin and the obround hole over the right pin. The two small tabs must be bent upward.
7. 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.
8. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
9. Hold the gel so that the logo is turned to the left side of the chamber. Gently lay the gel down on the REP Prep, starting with the left side, aligning the notch in the gel backing so that it fits around the small tab of the Alignment Guide. Carefully align the right notch in the gel to fit the right upright tab.
10. Place a QuickGel 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.
11. 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.
12. Clean the QuickGel Electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
13. Place a QuickGel Electrode on the outside edge 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. Proceed to Step III.

B. Template Application for CSF

1. Follow Steps II. A. 6-11.
2. Remove one QuickGel IFE template from the package. Hold the template so that the small hole in the corner is toward the front right side of the SPIFE 3000.
3. Carefully place the template on the gel aligning the template slits with the marks on each side of the gel backing. The center hole in the template should align with the indention in the center of the gel. If running two samples, place a second template on the gel.
4. Apply slight fingertip pressure to the 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.**
5. Clean the electrodes with deionized water before and after each use. Wipe with a lint free tissue.
6. 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. Proceed to Step III.

III. Electrophoresis

If testing only serum samples, follow the instructions marked "Serum". If testing only urine or serum with urine, follow instructions marked "Urine or Urine and Serum". The blade used for serum application will be added after the second application of urine.

Due to variation in environmental conditions,
***a Blot 1 time range of 2 to 5 minutes is acceptable.**
****an antisera absorption time of 1 to 3 minutes is acceptable.**

Using the instructions provided in the Operator's Manual, set up parameters as follows.

- **Serum**

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 1
3) No prompt
   Electrophoresis 1 8:00 21°C 350V 60 mA
4) Remove gel blks, apply antisera (continue)
   Absorb 1 **2:00 21°C
5) Remove excess antisera (continue)
   Blot 1 *2:00 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**

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 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 8:00 21°C 350V 60 mA
9) Remove gel blks, apply Antisera (continue)
   Absorb 2 **2:00 21°C
10) Remove excess Antisera (continue)
    Blot 1 *2:00 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

1. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **QG SERUM/URINE IFE** option appears on the displays.
2. 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.
3. 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.

- **CSF (Template Application)**

Electrophoresis Unit

1) Apply sample to template, (continue)
   Absorb 1 2:00 21°C
2) Blot and remove template, (continue)
   Electrophoresis 1 8:00 21°C 350V 60 mA
3) Remove gel blocks, apply antisera, (continue)
   Absorb 2 **2:00 21°C
4) Remove excess antisera, (continue)
   Blot 1 *2:00 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

1. With **QG 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** 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.
3. Close the chamber lid, and press the **TEST SELECT/CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 2 minutes.
4. After sample application is complete, open the chamber lid and gently blot the template with a QuickGel Blotter A.
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. SPIFE will beep when electrophoresis is complete.

- **Serum, CSF and Urine**

Stainer Unit

1) Plate Out, Holder In, Press (Continue)
   Wash 1 00:30 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. Immunofixation

1. When electrophoresis is complete, open the chamber lid. Remove the QuickGel electrodes.
2. Using the SPIFE Gel Block Remover, remove and discard both gel blocks. Wipe around the edges of the gel to remove excess moisture.
3. Holding the Antisera Template by the handles, gently place it 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.
4. Quickly pipette 140 µL of Fixative and Antisera into the oval slots at the right end (anode) of each antisera channel in the template.
5. Close the chamber lid and press the **TEST SELECT/CONTINUE** button to continue with antisera absorption. After the **2 minute absorption time, the SPIFE will beep.
6. When antisera absorption is complete, open the chamber lid. Place one QuickGel 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. The preliminary blot will be timed for *2 minutes and the instrument will beep.
7. Remove the Blotter Combs and the Antisera Template. Gently blot the gel with a QuickGel Blotter C and 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.

8. When the beep sounds, open the chamber lid and remove the Antisera Template and discard the blotter. Replace the QuickGel Electrodes on the ends of the gel to prevent curling. 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.
9. After the gel has been predried, carefully remove the gel from the electrophoresis chamber.

V. Washing, Staining, and Destaining

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 prerinse cycle.
3. After the chamber has been rinsed, remove the QuickGel Holder from the Stainer Unit. 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.
4. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
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 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.

PROCEDURE FOR QuickGel® CHAMBER

The following instructions are for using the QuickGel Chamber (Cat. No. 1284) for electrophoresis.

Materials Provided: The following materials needed for the procedure are contained in the QuickGel IFE Kits (Cat. No. 3551 or 3551T). Individual items are not available.

Cat. No. 3551T

QuickGel 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
QuickGel IFE Templates (20)	Kappa	1 vial
QuickGel Blotter A (20)	Lambda	1 vial
QuickGel Blotter C (20)		
QuickGel IFE Blotter J (10)		
QuickGel IFE Blotter Combs (10)		

Cat. No. 3551

QuickGel 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
QuickGel IFE Applicator Blades (20)	Kappa	1 vial
QuickGel Blotter C (20)	Lambda	1 vial
QuickGel IFE Blotter J (10)		
QuickGel IFE Blotter Combs (10)		
QuickGel Dispo Sample Cups (10)		

Materials provided but not contained in the kit:

Item	Cat. No.
QuickGel Chamber	1284
QuickGel IFE Rigid Antisera Template	3552
REP Prep	3100
QuickGel Gel Block Remover	1262
Staining Dish	4061
QuickGel Applicator	1265
QuickGel Applicator Base	1266
QuickGel Applicator Weights	1267
QuickGel Dispo Cup Tray	1268
QuickGel IFE Dispo Sample Cups (50/pkg)	1258
QuickGel Applicator Kit (includes Applicator, Applicator Base, Weights and Cup Tray)	1274
QuickGel IFE Applicator Blades (10/pkg)	1272

Materials needed but not provided:

Pipette and tips
10% acetic acid
0.85% saline
Power Supply capable of providing at least 350 Volts

STEP BY STEP METHOD

I. Sample Preparation

Sample application for serum and urine may be done with applicator blades or templates. CSF must be done with templates only

NOTE: Helena's recommendation for sample dilutions are given below. However, due to desired sensitivity variations or sample volumes, serum samples may also be diluted more or less as needed. The more concentrated samples are more likely to prozone while the more diluted samples may not exhibit desired sensitivity.

A. Serum

1. Template Application

The patient serum samples are diluted 1:5 (1 part serum with 4 parts 0.85% saline) for serum protein lanes and diluted 1:10 (1 part serum with 9 parts 0.85% saline) for immunofix lanes.

2. Blade Application

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

B. Urine (Template or Blade Application)

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

C. Cerebrospinal Fluid (Template Method)

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

II. Chamber Preparation

1. The QuickGel Chamber must be plugged into a power supply. Set a timer for *7:30 minutes and the power at 350 Volts.
   *An electrophoresis time of 7:00 to 8:00 minutes is acceptable.
2. Snap the Electrophoresis Lid into place on the chamber.
3. Ensure that the chamber floor is cool (room temperature) before starting the test.

III. Gel Preparation

1. Carefully cut open one 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 remaining gel. Remove the gel to be used from the plastic mold and discard the mold.
2. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Hold the gel so that the logo is turned to the left side of the chamber. Place the left notch of the gel so that it fits the left pin of the chamber floor and gently roll the gel to the right side fitting the right notch to the right pin of the chamber floor. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess REP Prep. Make sure that no bubbles remain under the gel.
4. Place a QuickGel Blotter C on the gel with the longer sides parallel with the gel blocks. Gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.

IV. Sample Application

A. Template Application

1. Remove two QuickGel IFE Templates from the package. Hold the template so that the small hole in the corner is toward the front right side of the chamber.

2. Carefully place the template on the gel aligning the template slits with the marks on each side of the gel backing. The center hole in the template should align with the indention in the center of the gel. Two samples can be run on one gel.
3. Apply slight fingertip pressure to the 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.**
4. Apply 3 μL of the appropriate serum dilution, CSF or urine to the template slits. Wait 2 minutes after the last sample application to allow proper absorption.
5. Use the QuickGel Blotter A to gently blot the excess sample from the template. Then carefully remove the template.
6. Close the lid, press the power switch to turn on the chamber and start the power supply.

B. Blade Application

1. Remove one QuickGel Applicator Blade from the packaging. If testing two samples, remove two Applicator Blades from the packaging.
2. Urine samples must be applied on the gel three times. When testing serum with urine samples, serum application is made after the second urine application. Therefore the blade for serum application is not added until after the second urine application.
3. Place an Applicator Weight on top of each Applicator Blade. If using two Applicator Blades, place them into the vertical slots A and C of the Applicator. One blade should be placed into the slot corresponding to cup placement.
4. Place the appropriate number of QuickGel IFE Dispo Sample Cups into either or both Row A and Row C of the Dispo Cup Tray. Pipette 17 μL of dilutent serum or 20 μL urine into the Sample Cups and cover tray until ready to use. When ready, place the Dispo Cup Tray into the Applicator Base.
5. If testing only serum samples, follow steps 6 through 11. If testing serum and urine on the same gel, perform steps 6 through 10 twice for the urine samples. Place the Modified Blade for serum samples into the applicator, and repeat steps 6 through 10 for a total of 3 applications for urine samples and 1 application for serum samples.
6. While holding the white Applicator knob up, place the Applicator into the designated slot on the Applicator Base aligning the small red dots on the Applicator with those on the Base.
7. Slowly lower the Applicator Knob allowing the blades to enter the sample cups, and immediately start a timer for 30 seconds.
8. After 30 seconds, lift the Applicator Knob. Immediately place the Applicator into the slot on the chamber floor, aligning the red dots.
9. Slowly lower the Applicator Knob to apply sample to the gel. Set a timer for 30 seconds.
10. After the 30 seconds application, raise the Applicator Knob and remove the Applicator from the chamber.
11. Close the lid, press the power switch to turn on the chamber and start the power supply.

V. Electrophoresis and Staining

1. Electrophorese the gel for *7:30 minutes at 350 Volts.
2. Turn off the Power Supply and the QuickGel Chamber. Remove the lid.
3. Using the QuickGel Gel Block Remover, carefully remove just the two gel blocks. Antisera leakage can occur if more than just the gel blocks are removed. Again, use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture.
4. Replace the Electrophoresis Lid with the Drying Lid. Clean the two electrodes on the Electrophoresis Lid with deionized water after each use. Wipe with a lint free tissue.
5. Holding the Antisera Template in the up position, place the template into the appropriate slots in the chamber.
6. Gently lower the Antisera Template onto the surface of the gel. No further pressure is needed.
7. Quickly pipette 140 μL of Fixative and Antisera into the slots at the right end (anode) of each Antisera channel in the template.
8. After 2 minutes (1 to 3 minutes is acceptable) absorption time, place one Blotter Comb into the same slots as the Antisera application. After 2 minutes, remove the blotter comb and the Antisera Template.
9. Gently blot the gel with a QuickGel Blotter C and remove the blotter. Place a QuickGel Blotter J on the surface of the gel. Place the Antisera template on top of the Blotter J. After 5 minutes, remove the Antisera Template and Blotter J. Close the lid.
10. Dry the gel for 8 minutes or until dry by turning on only the QuickGel Chamber. After drying is complete, turn the chamber off and remove the gel.
11. Fill a Staining Dish with prepared stain. Fill another one with Destain solution. Fill a third dish with Tris-Buffered Saline (TBS).
12. Place the gel in TBS and shake gently on a rotator for 10 minutes. Remove the gel from the TBS and allow it to drain on a blotter.
13. Place the gel into the Staining Dish containing the prepared stain for 4 minutes. Remove the gel from the stain and allow it to drain on a blotter.
14. Destain the gel in two consecutive washes of Destain solution. Use a gentle alternately rocking and swirling technique. Allow the gel to remain in each wash for 1 minute. The gel background should be completely clear. Tap the gel to remove the excess destain solution.
15. Ensure that the chamber floor is clean. Replace the gel onto the QuickGel Chamber floor. Close the Drying Lid, turn on the QuickGel Chamber and dry for 10 minutes or until dry.
16. Remove the gel from the chamber, and place it into the Destain again for 1 minute. Tap the gel to remove excess destain solution.
17. Place the gel back into the chamber and dry for 5 minutes.
18. Turn off the chamber and remove the gel.

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. 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 “Immuno- Fixation 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 QuickGel 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. Evaluated $alpha_1$ 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

Serum, urine and CSF specimens containing monoclonal and polyclonal proteins were tested using the SPIFE ImmunoFix and the QuickGel ImmunoFix procedures. The test results showed good agreement between the methods.

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