*PLEASE READ!!*

**HELENA LABORATORIES**

**PROCEDURE DOWNLOAD END USER AGREEMENT**

In response to customer requests, Helena is pleased to provide the text for procedural package inserts in a digital format editable for your use. The text for the procedure you requested begins on page three of this document. Helena procedures contain the content outlined in the NCCLS (GP2-A#) format, except in the order sequence required by FDA regulations. As the NCCLS format is a guideline, you may retain these procedures as developed by the manufacturer (adding your title/authorization page) or manipulate the text file to produce your own document, matching the NCCLS section order exactly, if preferred.

We also provide the procedure in an Adobe Acrobat PDF format for download at www.helena.com as a “MASTER” file copy. Below are the specifications and requirements for using these digital files. Following the specifications is the procedure major heading sequence as given in the FDA style. Where there is a difference in order, or other notation in the outline, this will be indicated in braces { }.

WHAT YOU NEED TO KNOW:

1) These files represent the most current revision level to date. Your current product inventory could contain a previous revision level of this procedure.

2) The Microsoft Word document provides the text only from the master procedure, in a single-column format.

* It may not contain any illustrations, graphics or captions that may be part of the master procedure included in the kit.
* The master procedure may have contained special formatting characters, such as subscripts, superscripts, degree symbols, mean symbols and Greek characters such as alpha, beta, gamma, etc. These symbols may or may not display properly on your desktop.
* The master procedures may also contain columns of tabbed data. Tab settings may or may not be displayed properly on your desktop.

3) The Adobe Acrobat PDF file provides a snapshot of the master procedure in a printable 8.5 x 11” format. It is provided to serve as a reference for accuracy.

4) By downloading this procedure, your institution is assuming responsibility for modification and usage.

**HELENA LABORATORIES**

**PROCEDURE DOWNLOAD END USER AGREEMENT**

HELENA LABORATORIES LABELING – Style/Format Outline

1. PRODUCT {Test} NAME
2. INTENDED USE and TEST TYPE (qualitative or qualitative)
3. SUMMARY AND EXPLANATION
4. PRINCIPLES OF THE PROCEDURE

 {*NCCLS lists SAMPLE COLLECTION/HANDLING next}*

1. REAGENTS (name/concentration; warnings/precautions; preparation; storage; environment; Purification/treatment; indications of instability)
2. INSTRUMENTS required – Refer to Operator Manual (... for equipment for; use or function; Installation; Principles of operation; performance; Operating Instructions; Calibration\* {\*is next in order for NCCLS – also listed in “PROCEDURE”}’ precautions/limitations/hazards; Service and maintenance information
3. SAMPLE COLLECTION/HANDLING
4. PROCEDURE

 {*NCCLS lists QUALITY CONTROL (QC) next}*

 9) RESULTS (calculations, as applicable; etc.)

10) LIMITATIONS/NOTES/INTERFERENCES

11) EXPECTED VALUES

12) PERFORMANCE CHARACTERISTCS

13) BIBLIOGRAPHY (of pertinent references)

14) NAME AND PLACE OF BUSINESS OF MANUFACTURER

15) DATE OF ISSUANCE OF LABELING (instructions)

For Sales, Technical and Order Information, and Service Assistance,
call Helena Laboratories toll free at 1-800-231-5663.

Form 364

Helena Laboratories

1/2006 (Rev 3)

##

**SPIFE® ImmunoFix-15**

**Cat. No. 3408, 3408T, 3408W**

**INTENDED USE**

SPIFE ImmunoFix-15 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.

**SUMMARY**

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

Alfonso first described immunofixation in the literature in 1964.1 Alper and Johnson published a more practical procedure in 1969 as a result of their work devoted to the detection of genetic polymorphisms of ceruloplasmin and Gc-globulin and the conversion of C3 during activation.2 They later extended their studies to genetic polymorphisms of complement components and the identification of alpha1 antitrypsin.3, 4 Immunofixation has been used as a procedure for the study of immunoglobulins since 1976.5, 6

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

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

**PRINCIPLE**

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

**REAGENTS**

**1. SPIFE IFE-15 Gel
Ingredients:** Each gel contains agarose in tris-barbital/MOPS buffer with a stabilizer and a preservative. **WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. - TOXIC - CAUTION: DO NOT INGEST**. The gel contains barbital which, in sufficient quantity, can be toxic. **Preparation for Use:** The gels are ready for use as packaged. **Storage and Stability:** The gels should be stored horizontally at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. DO NOT REFRIGERATE OR FREEZE. **Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appear­ance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of gel blocks.

**2. Acid Violet Stain**

**Ingredients:** The stain is comprised of Acid Violet stain. **WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
Preparation for Use:** Dissolve the dry stain in 1 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. 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. Clear Wash
Ingredients:** The powder contains anionic and nonionic surfactants, sodium carbonate, enzymes 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.

**6. 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.

**7. Antisera to Human IgG, IgA, IgM, Kappa Light Chain and Lambda Light Chain
Ingredients:** Antisera vials in the kit contain monospecific antisera to human immunoglobulin heavy chains, IgG, IgM, IgA and to human light chains, Kappa and Lambda. The antisera have been prepared in goat. Each vial of antiserum contains a stabilizer and sodium azide as a preservative. **WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY**. To prevent the formation of toxic vapors, do not mix with acidic solutions. When discarding, always flush sink with copious amounts of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH. **Preparation for Use:** The antisera are ready for use as packaged. **Storage and Stability:** The antisera should be stored at 2 to 8°C and are stable until the expiration date indicated on the vial. **Signs of Deterioration:** Extremely cloudy antisera may be indicative of bacterial contamination.

**INSTRUMENT**

A SPIFE 3000 instrument must be used to electrophorese, stain, destain, and then dry the gels. The gels may be scanned on a densitometer such as the QuickScan 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:** If storage is necessary, samples may be stored covered at 2 to 8°C for up to 72 hours.

**PROCEDURE**

**Materials Provided:** The following materials needed for the procedure are contained in the SPIFE IFE-15 Kit (Cat. No. 3408, 3408T, 3408W). Individual items are not available.

**Cat. No. 3408 - Blade Application**

SPIFE IFE Gels (10) Fixative

 Acid Violet Stain (1 vial) IgG

 Tris-Buffered Saline (1 pkg) IgA

 Citric Acid Destain (1 pkg) IgM

 SPIFE Blotter C (20) Kappa

 SPIFE Blotter D (20) Lambda

 IFE-15 Blotter Combs (10)

 Applicator Blade Assembly (50)

 or

**Cat. No. 3408T - Template Application**

 SPIFE Urine IFE Templates (50)

 Blotter A-Plus (50)

 or

**Cat. No. 3408W – Optional Clear Wash**

SPIFE IFE Gels (10) Fixative

 Acid Violet Stain (1 vial) IgG

 Clear Wash (1 pkg) IgA

 Citric Acid Destain (1 pkg) IgM

 SPIFE Blotter C (20) Kappa

 SPIFE Blotter D (20) Lambda

 IFE-15 Blotter Combs (10)

 Applicator Blade Assembly (50)

**Materials provided by Helena Laboratories but not contained in the kits above:**  **Item Cat. No.**

 SPIFE 3000 Analyzer 1088

 QuickScan Touch 1690

 QuickScan 2000 1660

 REP Prep 3100

 Gel Block Remover 1115

 SPIFE IFE Multi-Channel Pipettor 1122

 Pipette Tips for SPIFE IFE Pipettor 3355

 Tip Spacers 3356

 SPIFE IFE-15 Antisera Template 3352

 SPIFE IFE Antisera Tray 3394

 SPIFE IFE-9/15 Disposable Cups 3363

 SPIFE 3000 IFE-15 Disposable Cup Tray 3362

 SPIFE Urine IFE-15 Alignment Guide 3407

 SPIFE IFE Weight 3470

**Materials and Supplies Needed but not Supplied**

 10% 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. **NOTE:** 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 = undiluted to 1:10

 L = undiluted to 1:5
The more concentrated samples are more likely to prozone while the more diluted samples may not exhibit desired sensitivity.

 **B. Urine**

 Urine samples may be run diluted, neat or concentrated.

 Shake samples to homogenize. Centrifuge desired volume at 2000 x g for 5 minutes. Remove supernatant and concentrate as follows:

 Total Protein (mg/dL) Conc. Factor

 < 50 100x

 50-100 50x

 100-300 25x

 300-600 10x

 > 600 5x

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

**II. Sample Application**

 **A. Serum or Urine Blade Application**

 1. Slide the Disposable Cup strips into the IFE-15 Cup Tray.

 2. Pipette 17 µL of diluted serum or 20 µL of urine into the Disposable Cups.

 3. Place the Cup Tray into the SPIFE 3000. Align the holes in the tray with the pins on the instrument.

 4. Remove five disposable Applicator Blade Assemblies from the packaging. Remove the protective guard from the blades by gently bending the protective piece back and forth until it breaks free.

 5. Place the five Applicator Blades into the vertical slots numbered 1, 5, 8, 12, and 15 in the Applicator Assembly.  **Please note that the blade assembly will only fit into the slots in the Applicator Assembly one way; do not try to force the Applicator Blade into the slots.**

 6. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.

 7. Remove the gel from the protective packaging and discard overlay.

 8. Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.

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

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

 11. Place a carbon electrode on the outside edge of each gel block outside the magnetic posts. Improper contact between the electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.

 12. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **SERUM/URINE IFE** option appears on the displays. Proceed to Step III.

 **B. Urine or CSF Template Application**

 1. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.

 2. 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.

 3. Place the Urine IFE Templates on the gel aligning the application slits with the appropriate set of pins on the sides of the Alignment Guide. The templates have been marked with a hole in one cor-ner. Place the marked corner in the lower left position. Apply slight fingertip pressure to the template, making sure there are no air bubbles under it. Up to five templates can be placed on a gel at one time. **NOTE:** If wearing rubber gloves to perform this step, place a Blotter A-Plus over the template and then apply fingertip pressure to the template. Powder from the gloves can produce gel artifacts. Remove the blotter.

 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 edge of each gel block outside the magnetic posts. 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 displays.

**III. Electrophoresis**
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.

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

 **• Serum (Blade Application)**

**Electrophoresis Unit**

 1) No prompt

 Load Sample 1 00:30 21°C SPD6

 2) No prompt

 Apply sample 1 00:30 21°C SPD1 LOC 2

 3) No prompt

 Electrophoresis 1 5:30 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 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.

**Electrophoresis Unit**

 1) No prompt

 Load sample 1 00:25 21°C SPD6

 2) No prompt

 Apply sample 1 00:25 21°C SPD6 LOC2

 3) No prompt

 Load sample 2 00:25 21°C SPD6

 4) No promp

 Apply sample 2 00:25 21°C SPD6 LOC2

 5) No prompt

 Load sample 3 00:25 21°C SPD6

 6) No prompt

 Apply sample 3 00:25 21°C SPD6 LOC2

 7) No prompt

 Absorb 1 2:00 21°C

 8) No prompt

 Electrophoresis 1 5:30 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 or 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 5:30 21°C 650V 160 mA

 3) Remove gel blocks, 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 or 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

 **A. Serum or Urine Blade Application**

 1. 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.

 2. The SPIFE 3000 will apply samples onto the gel and electrophorese, then beep when completed. Dispose of blades and cups as biohazardous waste. Proceed to Step IV.

 **B. Urine or CSF Template Application**

 1. With **URINE IFE** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press START/STOP to begin. Open the chamber lid.

 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 5 minutes.

 4. After sample application is complete, open the chamber lid and gently blot the 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. SPIFE will beep when electrophoresis is complete.

**IV. Immunofixation**

 1. When electrophoresis is complete, open the chamber lid. Remove the carbon electrodes.

 2. Using the Gel Block Remover, remove and discard both gel blocks.

 3. 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.

 4. Place six (6) tips onto the SPIFE IFE Pipettor. Note that one side of the Tip Spacer is concave around the holes. Holding the pipettor with the tips up, slide the concave side of the spacer down over the tips so that the tips squeeze together.

 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. Pipette 250 µL of Fixative and Antisera from the Antisera Tray and quickly dispense them into the oval slots at the right end of each antisera channel in the template.

 7. Close the chamber lid and press the **TEST SELECT/CONTINUE** button to continue with antisera absorption. After the 10 minute absorption time, the SPIFE 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 **TEST SELECT/CONTINUE** button. After the preliminary blot, the instrument will beep.

 9. Remove the Blotter Comb and the Antisera Template. Gently blot the gel surface with a Blotter C, then remove the blotter. Place a Blotter D on the surface of the gel. Place the Antisera Template on top of the Blotter D.  **NOTE:** If a clearer background is desired, place 2 Blotter Ds on the surface of the gel. Place the Antisera Template on top of the Blotter Ds and place the SPIFE IFE Weight on top of the Antisera Template.

 10. When the beeper sounds, open the chamber lid and remove the Antisera Template and the blotter. Lay one electrode across each end of the gel to prevent curling during the drying step. Close the chamber lid and press the **TEST SELECT/CONTINUE** button. The gel will be predried in the electrophoresis chamber.
**NOTE:** Do not allow antisera to dry in the template. The Antisera Template should be cleaned with a mild biocidal detergent. The template may also be scrubbed with a soft brush to remove any antisera residue. Rinse with deionized water.

 11. After the gel has been predried, carefully remove the gel from the electrophoresis chamber.

**V. Washing, Staining, and Destaining
NOTE:** If a clearer background is desired, an optional Clear Wash (3408W) is available to be used in the place of the Tris-Buffered Saline.

 1. With **SERUM/URINE IFE** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin.

 2. Press the **TEST SELECT/CONTINUE** button. This will initiate the chamber prerinse 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 on the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.

 4. Place the gel holder with attached gel into the stainer chamber. The gel should face backwards in the stainer.

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

**INTERPRETATION OF RESULTS**

The majority of monoclonal proteins migrate in the cathodic (gamma) region of the protein pattern. But, due to their abnormality, they may migrate anywhere within the globulin region on protein electrophoresis. The monoclonal protein band on the immunofixation pattern will occupy the same migration position and shape as the monoclonal band on the reference protein electrophoresis pattern. The abnormal protein is identified by the corresponding antiserum used. Because of the increased sensitivity of the procedure, it is not uncommon to see a fixed band that is not visible in the serum protein procedure.

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

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

**LIMITATIONS**

1. Antigen excess will occur if there is not a slight antibody excess or antigen/antibody equivalency at the site of precipitation. Antigen excess in IFE is usually due to a very high level of immunoglobulin in the patient sample. The dissolution of immunoprecipitation is manifested by a loss of protein at the point of highest antigen concentration, resulting in staining in the margins and leaving the central area with little demonstrable protein stain. In this case it may be necessary to adjust the protein content of the sample by dilution. Electrophoresing excessive amounts of antigen decreases resolution and requires higher concentrations of antibody. For optimum separation and sufficient intensity for visual detection, care must be taken in adjusting antibody content, sample concentration, time and voltage. The SPIFE ImmunoFix method has been optimally developed to minimize the antigen excess phenomenon.

2. Monoclonal proteins may occasionally adhere to the gel matrix, especially IgM. These bands will appear in all five antisera reaction areas of the gel. However, where the band reacts with the specific antisera for its heavy chain and light chain, there will be a marked increase in size and staining activity, allowing the band to be identified.

**Further Testing Required:**

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

1. Serum samples which have a precipitin band with Kappa or Lambda Light Chain Antisera but none corresponding with IgG, IgA or IgM antisera may have a free light chain or they may have an IgD or IgE monoclonal protein. Such sera should be tested with ImmunoFix IgD and IgE antisera.

2. A CRP band may be detected in patients with acute inflammatory response.7, 8 CRP appears as a narrow band on the most cathodic end of the high resolution agarose protein electrophoresis pattern. Elevated alpha1 antitrypsin and haptoglobin (acute phase proteins) are supportive evidence for the presence of a CRP band. Patients with a CRP band will have a positive CRP by latex agglutination or an elevated quantitative CRP.

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

**PERFORMANCE CHARACTERISTICS**

Twenty-one abnormal and nine normal serum specimens were tested using the SPIFE ImmunoFix-15 Procedure and the SPIFE ImmunoFix-9 Procedure. Of the test results, 97% showed good agreement between methods. The remaining samples differed slightly with the presence of multiclonal specimens. Ten abnormal and five normal urine specimens were also tested using both procedures. Of the test results, 87% showed good agreement between methods. The remaining samples differed slightly with the presence of multiclonal specimens with the IFE-15 procedure being slightly more sensitive.

**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.

For Sales, Technical and Order Information and Service Assistance, call 800-231-5663 toll free.

Helena Laboratories warrants its products to meet our published specifications and to be free from defects in materials and workmanship. Helena’s liability under this contract or otherwise shall be limited to replacement or refund of any amount not to exceed the purchase price attributable to the goods as to which such claim is made. These alternatives shall be buyer’s exclusive remedies.

In no case will Helena Laboratories be liable for consequential damages even if Helena has been advised as to the possibility of such damages.

The foregoing warranties are in lieu of all warranties expressed or implied including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

Shading indicates that text has been modified, added or deleted.

Pro 200

3/20(7)