SPIFE® IFE Pentavalent Kit Procedure

Cat. No. 3457

INTENDED USE

SPIFE IFE Pentavalent Kits are intended for the qualitative in-vitro diagnostic separation of abnormal immunoglobulins in serum using protein electrophoresis and immunofixation on the SPIFE 3000 system. The test is used as a screening aid for abnormal proteins, but must be used in conjunction with other clinical findings.

All specimens exhibiting a band in the immunofix lane with the Pentavalent Antisera must be retested with corresponding monospecific SPIFE IFE Antisera (G,A,M,K,L) to identify the abnormal protein.

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 SPIFE 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. **SPIFE IFE Pentavalent Protein Fixative**
   Ingredients: The fixative contains 4.0% sulfosalicylic acid, 6.7% trichloroacetic acid, 0.002% 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.
2. **SPIFE IFE Pentavalent Antisera**
   Ingredients: The Pentavalent Antisera vial contains pooled antisera to human immunoglobulin heavy chains, IgG, IgM, IgA and to human light chains, Kappa and Lambda, both free and bound. The antisera have been prepared in goat and contain a stabilizer and sodium azide as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. Refer to the Sodium Azide Warning.
   Preparation for Use: The antisera is ready for use as packaged.
   Storage and Stability: The antisera should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.
   Signs of Deterioration: Extremely cloudy antisera may be indicative of bacterial contamination.
3. **SPIFE 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. Refer to the Sodium Azide Warning.
   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.
4. **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.
5. **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.
6. **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.

Sodium Azide Warning

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.

INSTRUMENT

A SPIFE 3000 instrument 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 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 is the specimen of choice. 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 Pentavalent Kit. Individual items are not available.

SPIFE IFE Gels (10)	SPIFE IFE Pentavalent Fixative
Acid Violet Stain (1 vial)	SPIFE IFE Pentavalant Antisera
Tris-Buffered Saline (1 pkg)	SPIFE Blotter C
Citric Acid Destain (1 pkg)	SPIFE Blotter J
Applicator Blade Assembly	
SPIFE Blotter C	
SPIFE Blotter D	
IFE Blotter Combs	

Number of Samples Tested	Cat. No.	Name of Kit
27 Samples	3457	SPIFE IFE-9 Pentavalent Kit

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

Item	Cat. No.
SPIFE 3000 Analyzer	1088
SPIFE IFE-9 Kit	3409
REP Prep	3100
SPIFE Gel Block Remover	1115
SPIFE IFE Pipettor	1122
Pipette Tips for SPIFE IFE Pipettor	3355
Tip Spacers for IFE 9/15	3356
SPIFE IFE Antisera Tray	3394
SPIFE IFE-9/15 Disposable Cups	3363
SPIFE 2000/3000 Disposable Cup Tray for IFE 9	3378
SPIFE IFE-9 Antisera Template	3392

Materials Needed but not supplied:

10% Glacial Acetic Acid
0.85% saline

STEP-BY-STEP METHOD

NOTE: Any specimen exhibiting a band in the immunofix lane must be retested to identify the abnormal protein using one of the following SPIFE IFE Kits.

Name	Cat. No.
SPIFE IFE-3	3406
SPIFE IFE-6	3401
SPIFE IFE-9	3409
SPIFE IFE-15	3408

I. Sample Preparation

The patient serum samples are diluted 1:3 (1 part serum with 2 parts 0.85% saline) for serum protein lanes and diluted 1:10 (1 part serum with 9 parts 0.85% saline) for pentavalent lanes.

1. Remove the appropriate number of 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.
2. Place the Applicator Blades into the vertical slots in the Applicator Assembly numbered 4, 10 and 16.

   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.

II. Sample Application

1. Slide the Disposable Cup strips into the appropriate Cup Tray. Two (2) cups are required for each sample tested.
2. Pipette 17 µL of each diluted serum into the two Disposable Cups.

1st Cup	2nd Cup
1:3(SP)	1:10 (Pent.)

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

III. Gel Preparation

1. Remove the gel from the protective packaging and discard overlay.
2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
3. 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.
4. Using a SPIFE Blotter C, gently blot the entire surface of the gel using slight fingertip pressure on the blotter. Remove the blotter.
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 electrodes and the gel blocks may cause skewed patterns. Close the chamber lid.
7. Press the **TEST SELECT/CONTINUE** buttons located on the Electrophoresis and Stainer sides of the instrument until the **SERUM IFE** option appears on the displays.

IV. Electrophoresis

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

***A Blot 1 time of 3 minutes is recommended. Due to variations in environmental conditions, a Blot time range of 2-5 minutes is acceptable.**

****A Fixative/Antisera absorption time of 1-3 minutes is acceptable.**

*****An Electrophoresis time of 7:00 minutes is recommended, but a range of 6:30 - 7:30 minutes is acceptable.**

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

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			

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.

V. Immunofixation

1. When electrophoresis is complete, open the chamber lid. Remove the carbon electrodes.
2. Using the SPIFE Gel Block Remover, remove and discard both gel blocks.
3. Pour the contents of the Fixative vial and the Pentavalent vial into alternating wells of the Antisera Tray. Cover the tray when not in use. Store tray and antisera at 2 to 8°C.
4. Place 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.
5. 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 50 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 incubation. After the **2 minute incubation time, the SPIFE will beep.
8. When antisera incubation is complete, open the chamber lid. Place the Blotter Comb(s) 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 *3 minutes.
9. After *3 minutes, the instrument will beep. Remove the Blotter Comb(s) and the Antisera Template. Gently blot the gel surface with a Blotter C, then remove the blotter. Place a Blotter J on the surface of the gel. Place the Antisera Template on top of the Blotter J. Close the chamber lid and press the **TEST SELECT/CONTINUE** button. The final blot will be timed for 5 minutes.
10. When the beeper sounds, open the chamber lid and remove the Antisera Template and the blotter. 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.

VI. Washing, Staining, and Destaining

1. With **SERUM 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.
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.

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. 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. Occasionally, an application phenomenon occurs at the application site, but does not interfere with pattern interpretation.

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. This method has been optimally developed to minimize the antigen excess phenomenon.
2. Testing has not been conducted for cross-reacting or interfering substances.

Further Testing Required:

1. If a band is seen in the Pentavalent lane, IFE testing must be run for specific identification.
2. A CRP band may be detected in patients with acute inflammatory response.[1,2] 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.

PERFORMANCE CHARACTERISTICS

Sixty serum specimens containing monoclonal and polyclonal proteins were tested using the SPIFE IFE Pentavalent Antisera and the SPIFE IFE system. Forty-five specimens demonstrated monoclonal and polyclonal patterns with both methods. The remaining fifteen specimens were exclusively polyclonal. Subtle differences in pattern presentation are within acceptable procedural variation and create no conflict regarding monoclonal or polyclonal interpretation.

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