QuickGel® Touch ImmunoFix Procedure

Cat. No. 3351

INTENDED USE

QuickGel Touch ImmunoFix Procedure is intended for the qualitative identification of monoclonal gammopathies in serum, cerebrospinal fluid (CSF) or urine using protein electrophoresis and immunofixation on the SPIFE Touch system.

SUMMARY

Immunofixation electrophoresis (IFE) is a two stage procedure using agarose gel high resolution electrophoresis in the first stage and immunoprecipitation in the second. There are numerous applications for IFE in research, forensic medicine, genetic studies and clinical laboratory procedures. The greatest demand for IFE is in the clinical laboratory where it is primarily used for the detection of monoclonal gammopathies. A monoclonal gammopathy is a primary disease state in which a single clone of plasma cells produces elevated levels of an immunoglobulin of a single class and type. Such immunoglobulins are referred to as monoclonal proteins, M-proteins or paraproteins. Their presence may be of a benign nature or of uncertain significance. In some cases they are indicative of a malignancy such as multiple myeloma or Waldenstrom's macroglobulinemia. Differentiation must be made between polyclonal and monoclonal gammopathies 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 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.
   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. **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 Touch must be used to electrophorese, stain, destain and then dry the gels. Refer to the appropriate 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 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
Blade Applicator Kit (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 Touch	1068
REP Prep	3100
SPIFE QuickGel IFE Cup Tray	3371
SPIFE IFE 9/15 Dispo Cups	3363
Gel Block Remover	1115
SPIFE QuickGel IFE Antisera Template	3372
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
SPIFE QuickGel IFE Alignment Guide	3373
QuickGel IFE Templates and Blotter A (for CSF)	552156
Applicator Blade Weights	3387

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. Serum or Urine (Blade Application)

1. Slide the Disposable Cup Strip into the appropriate Cup Tray (Cat. No. 3371).
2. Pipette 20 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 Touch. Align the holes in the tray with the pins on the instrument.
4. Remove two disposable Applicator Blades from the packaging.
5. Place the Applicator Blades into the vertical slots in the Applicator Assembly numbered 6 and 12.
   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.
   If testing serum only, follow the instructions marked "**• Serum (Blade Application)**". If testing urine or urine and serum, follow the instructions marked "**• Urine or Urine and Serum (Blade Application)**".
6. Place an Applicator Blade Weight on top of the blade assembly. When placing the weight on the blade, position the weight with the thick side to the right.
7. 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.
8. 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.
9. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
10. 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.
11. 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.
12. 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.
13. Clean the QuickGel Electrodes with deionized water before and after each use. Wipe with a lint-free tissue.
14. 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.
15. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP** then proceed to Step III. Once parameters have been verified, proceed to Step IV.A if running serum only or IV.B if running urine or urine and serum.

B. CSF (Template Application)

1. Follow Steps II. A. 7-12
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 Touch.
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.
7. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP** then proceed to Step III. Once parameters have been verified, proceed to Step IV.C.

III. Electrophoresis Parameters

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

- **Serum (Blade Application)**

	Separator Unit
Load Sample	Prompt: None Time: 0:30 Temperature: 21°C Speed: 6
Apply Sample	Prompt: None Time: 0:30 Temperature: 21°C Speed: 1 Location: 1
Electrophoresis	Prompt: None Time: 8:00 Temperature: 21°C Voltage: 350 V mA: 60 mA
Absorb	Prompt: Remove Gel Blocks, Apply Antisera Time: 2:00 Temperature: 21°C
Blot 1	Prompt: Remove Excess Antisera Time: 2:00 Temperature: 21°C
Blot 2	Prompt: Remove Template, Install Blotter Time: 5:00 Temperature: 40°C
Dry	Prompt: Remove Blotter Time: 8:00 Temperature: 50°C
End	

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

	Separator Unit
Load Sample 1	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6
Apply Sample 1	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6 Location: 1
Load Sample 2	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6
Apply Sample 2	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6 Location: 1
Load Sample 3	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6
Apply Sample 3	Prompt: None Time: 0:25 Temperature: 21°C Speed: 6 Location: 1

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

Electrophoresis
Prompt: None
Time: 8:00
Temperature: 21°C
Voltage: 350 V
mA: 60 mA

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

Blot 1
Prompt: Remove Excess Antisera
Time: 2:00
Temperature: 21°C

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

Dry
Prompt: Remove Blotter
Time: 8:00
Temperature: 50°C

End

- **CSF (Template Application)**

Separator Unit

Pause
Prompt: None
Time: 2:00
Temperature: 21°C

Electrophoresis
Prompt: None
Time: 8:00
Temperature: 21°C
Voltage: 350 V
mA: 60 mA

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

Blot 1
Prompt: Remove Excess Antisera
Time: 2:00
Temperature: 21°C

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

Dry
Prompt: Remove Blotter
Time: 8:00
Temperature: 50°C

End

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

Stainer Unit

Wash 1
Prompt: Plate Out, Gel Holder In
Time: 0:30
Recirculation: On
Valve: 1
Fill, Drain

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

Stain
Prompt: None
Time: 4:00
Recirculation: Off
Valve: 5
Fill, Drain

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

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

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

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

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

End

IV. Electrophoresis

A. Serum (Blade Application)

1. Open the chamber lid and place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples, electrophorese and beep when completed. Proceed to Step V.

B. Urine or Urine and Serum (Blade Application)

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

C. CSF (Template Application)

1. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. Open the chamber lid and apply CSF by placing 3 µL of each sample onto slits in the template (one protein and five immunofixation) for each patient.
3. Close the chamber lid and press the **CONTINUE** button for the electrophoresis chamber. Sample application will be timed for 2 minutes.
4. Open the chamber lid and gently blot the template with a QuickGel Blotter A and carefully remove the blotter and template. Dispose of templates as biohazardous waste.
5. Close the chamber lid and press the **CONTINUE** button to start electrophoresis. The SPIFE Touch will beep when electrophoresis is complete.

V. Immunofixation

1. When electrophoresis is complete, open the chamber lid. Remove the QuickGel electrodes.
2. Using the 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 **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 **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 **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 **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.

VI. Washing, Staining, and Destaining

1. Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed.
2. After the chamber has been rinsed, remove the Gel Holder from the stainer chamber. 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.

3. Place the Gel Holder with the attached gel facing backwards into the stainer chamber, with the front of the Gel Holder facing the operator. The gel should face away from the operator.
4. Press the **CONTINUE** button to begin the staining process. The instrument will wash, stain, destain and dry the gel.
5. When the process is completed, the instrument will beep. Carefully remove the Gel Holder from the stainer because the metal piece on the holder will be hot.

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

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

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