

The SPIFE IgG IEF-20 Kit is intended for the qualitative identification of IgG-specific oligoclonal banding in paired serum and cerebrospinal fluid (CSF) samples by using agarose gel isoelectric focusing and immunoblotting on the SPIFE 3000 system. It is used as an aid in the diagnosis of inflammatory disease of the central nervous system (CNS), such as caused by multiple sclerosis.

## SUMMARY

Intrathecal IgG synthesis within the central nervous system in the proper clinical setting is well established as the diagnostic indicator for multiple sclerosis (MS). In 1994, the Committee of the European Concerted Action for Multiple Sclerosis recommended isoelectric focusing as the most sensitive method for the detection of oligoclonal bands in serum and CSF under certain circumstances.<sup>1</sup>

Isoelectric focusing is considered to be twice as sensitive as the use of the IgG Index.<sup>2</sup> The oligoclonal bands resolved are preferably visualized by IgG specific antibody staining. It should be emphasized that the finding of oligoclonal bands by isoelectric focusing is not specific for MS. It reaches its maximal value in the differential diagnosis only when other known causes of CNS inflammation have been ruled out. The concentration of individual bands may be as low as 2% of the total CSF IgG,<sup>2</sup> but can be seen against the background of the polyclonal IgG present through filtration from the blood. Due to this filtration, it is important to rule out oligoclonal IgG from the blood by running parallel serum samples against CSF samples. The concentration of IgG in the CSF of a normal person is around 25 mg/L,<sup>2</sup> which needs to be doubled for the use of quantitative methods in order to give an abnormal IgG index. With IEF, oligoclonal banding may be detected while the total CSF IgG concentration is within the reference range.<sup>2</sup>

## PRINCIPLE

The SPIFE IgG IEF-20 Kit is based on the method originally described by Keir, G. et al.<sup>3</sup> The Helena method separates serum and CSF proteins according to their isoelectric points in an agarose IEF gel. The proteins are then transferred to nitrocellulose, and the nitrocellulose is then immunofixed to visualize IgG-specific bands. The patterns are interpreted qualitatively by comparing the presence or absence of oligoclonal bands in serum and CSF.

The significance of individual bands in the CSF can only be properly understood in the context of a parallel serum specimen as well as attention to the overall band pattern of all sample tracks on the isoelectric focusing plate. It is important to exclude artifactual bands that are caused by non-linearity of the isoelectric focusing pH gradient. A good practical indicator for these is to compare the serum patterns from several patients.

Bands that are at the same isoelectric point in all specimens of a given run are most likely to be artifacts produced

by the ampholytes used in the separation. The higher the number of these artifactual bands the more difficult it is to recognize not only legitimate abnormal serum bands, but even CSF bands, which can be obscured by interference from the common bands.<sup>1</sup>

## REAGENTS

### WARNING: FOR IN-VITRO DIAGNOSTIC USE

#### 1. SPIFE IgG IEF-20 Gel

**Ingredients:** Each gel contains agarose (1.0%) and separators with a preservative.

**Preparation for Use:** The gel is ready for use as packaged.

**Storage and Stability:** The gels should be stored horizontally at room temperature (15 to 30°C), in the protective packaging, and are stable until the expiration date indicated on the package. **DO NOT REFRIGERATE OR FREEZE THE GELS.**

**Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial or fungal growth indicating contamination. Discard if any of these conditions are present.

#### 2. Anode Solution

**Ingredients:** Contains 0.3 M acetic acid.

**CAUTION: Irritant. Do not ingest.**

**Preparation for Use:** The solution is ready for use as packaged.

**Storage and Stability:** The solution should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.

**Signs of Deterioration:** Discard product if it appears cloudy or contains particulates.

#### 3. Cathode Solution

**Ingredients:** Contains 1 M NaOH.

**WARNING: CORROSIVE** - Causes burns. **DO NOT PIPETTE BY MOUTH.** In case of contact with eyes, rinse immediately with plenty of water and seek medical attention immediately.

**Preparation for Use:** The solution is ready for use as packaged.

**Storage and Stability:** The solution should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.

**Signs of Deterioration:** Discard product if it appears cloudy or contains particulates.

#### 4. Blocking Agent

**Ingredients:** Contains bovine milk protein solids.

**Preparation for Use:** The Blocking Agent is used to prepare two working solutions as follows:

- Blocking Solution A: Dissolve 1 g powder in 50 mL of 0.85% saline.

- Blocking Solution B: Dilute 5 mL of Blocking Solution A to 50 mL with 0.85% saline.

**Storage and Stability:** The powder should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.

**Signs of Deterioration:** The Blocking Agent should be free flowing off-white to pale yellow lumpy powder. Any changes to this appearance, or unusual coloration, may indicate deterioration.

## 5. Acetate Buffer Concentrate

**Ingredients:** Contains 0.2 M Acetate Buffer.

**Preparation for Use:** Dilute 2.5 mL of Acetate Buffer Concentrate to 25 mL with deionized water and use immediately.

**Storage and Stability:** The solution should be stored at room temperature (15 to 30°C), and is stable until the expiration date indicated on the label.

**Signs of Deterioration:** Discard product if it becomes cloudy or contains particulates.

## 6. Chromogen

**Ingredients:** Contains 3-amino-9-ethyl-carbazole powder.

**WARNING: TOXIC** - May cause cancer or inheritable genetic damage. Harmful by inhalation and if swallowed. Wear suitable protective clothing, gloves and eye/face protection. In case of exposure, seek medical attention immediately.

**Preparation:** Dissolve contents of the vial in 60 mL of methanol. Use glassware dedicated for this purpose to prevent protein contamination of the chromogen.

**Storage and Stability:** The powder should be stored at 2 to 8°C and is stable until the expiration date indicated on the label. Store 5 mL aliquots of the reconstituted chromogen solution at -20°C in the dark. Frozen aliquots are stable for six (6) months.

**Signs of Deterioration:** The chromogen should be a red-brown color. Changes from this appearance or failure of the chromogen to perform in the test may indicate product deterioration. Discard if either of these conditions are present.

## 7. Single Antibody

**Ingredients:** Contains sheep anti-human IgG peroxidase conjugate with a preservative.

**WARNING:** Harmful if swallowed.

**Preparation for Use:** The antiserum is ready for use as packaged.

**Storage and Stability:** The antiserum should be stored refrigerated at 2 to 8°C and is stable until expiration date indicated on the label.

**Signs of Deterioration:** Discard the vial if it becomes cloudy or contains particulates.

## INSTRUMENT

A SPIFE 3000 Analyzer must be used to electrophorese the gel. Refer to the Operator's Manual for detailed instructions.

## SPECIMEN COLLECTION AND HANDLING

**Specimen:** Freshly collected serum and CSF are the specimens of choice. Both serum and CSF should be collected at the same time using conventional collection methods for clinical laboratory testing.

**Handling:** CSF samples should be applied neat. Serum samples should be diluted 1:300 in 0.85% saline.

**Storage and Stability:** Samples can be stored refrigerated at 2 to 8°C for up to 3 days or 2 weeks at -20°C.

## PROCEDURE

**Materials Provided:** The following materials needed for the procedure are contained in the SPIFE IgG IEF-20 Kit (Cat. No. 3385).

- SPIFE IgG IEF Gel (10)
- SPIFE IgG IEF Anode Solution (1 vial)
- SPIFE IgG IEF Cathode Solution (1 vial)
- SPIFE IgG IEF Blocking Agent (1 vial)
- SPIFE IgG IEF Acetate Buffer Concentrate (1 vial)
- SPIFE IgG IEF Chromogen (1 vial)
- SPIFE IgG IEF Antibody (1 vial)
- Transfer Membranes (20)
- Blotter C (20)
- Blotter D (40)
- Electrode Wicks (20)
- Wick Blotters (40)
- Soak-away Blotters (20)
- Center Electrode Wicks (5)
- Center Wick Blotters (10)

**Materials Provided but not contained in the kit:**

| Item                                      | Cat. No. |
|---|----------|
| SPIFE 3000                                | 1088     |
| REP Prep                                  | 3100     |
| SPIFE IgG IEF Electrodes (1 gel)          | 3383     |
| SPIFE IgG IEF Square Electrodes           | 3703     |
| SPIFE IEF Adapter and Electrodes (2 gels) | 3704     |
| TITAN GEL High Resolution Protein Marker  | 5141     |

**Materials required but not provided:**

- 30% Hydrogen Peroxide
- Methanol
- 0.85% Saline
- Pipette to deliver 5 µL

## STEP-BY-STEP PROCEDURE

Two (2) gels may be run in the SPIFE simultaneously, however the test parameters, technique, electrodes and some paper components are different from those used when electrophoresing one gel.

### I. Procedure for One Gel

#### A. Gel Preparation

1. Remove two Electrode Wicks from the packaging. Soak one Electrode Wick in the Anode Solution and one wick in the Cathode Solution. Take care not to mix the anode and cathode solutions.
2. Remove the gel from the packaging and place on a paper towel. Carefully remove the transparent protective overlay. Using slight fingertip pressure, gently blot the gel surface with a Blotter C and discard blotter.

#### B. Electrophoresis

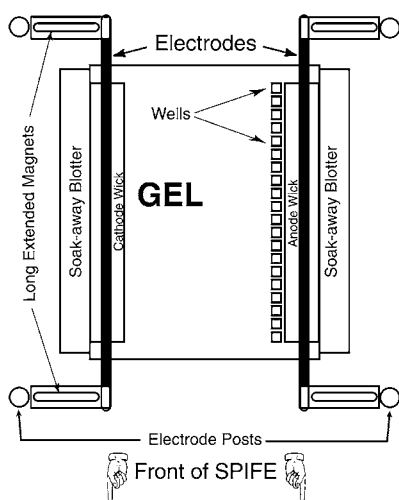
1. Using the instructions provided in the SPIFE 3000 Operators Manual, set up the parameters as follows:

## Electrophoresis Unit

- 1) No Prompt  
Electrophoresis 1 45:00 15°C 700V 80mA
- 2) No Prompt  
END OF TEST

2. Dispense 0.5 mL of REP Prep onto the chamber floor.
3. Position the gel in the center of the chamber floor with the sample wells toward the anode (right) electrode. Wipe excess REP Prep from around the edges of the gel.
4. Place the wick in the Anode Solution between two Wick Blotters and blot almost dry.
5. Position the anodal wick on the gel surface under the anode (right) electrode.

6. Repeat Step 4 for the wick in the Cathode Solution, and place the cathodal wick on the gel under the cathode (left) electrode.
7. Place an electrode (Cat. No. 3383) over each wick ensuring that the magnetic extenders contact the inside of the magnetic posts. The gel may need to be repositioned slightly so that the electrodes are directly over the wicks.



8. Position one Soak-away Blotter against the edge of the gel on the cathode end and one Soak-away Blotter against the gel on the anode end to act as excess fluid blotters. Ensure these Soak-aways are in contact with the gel.
9. Apply 5  $\mu$ L of sample to each sample well. Close the chamber lid.
10. With **CSF-IEF** on the display, press **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin electrophoresis.
11. Continue to Step III.

## II. Procedure for Two Gels

### A. Gel Preparation

1. Remove two Electrode Wicks and one Center Electrode Wick from the packaging. Soak the Center Electrode Wick in the Anode Solution and two Electrode Wicks in the Cathode Solution. Take care not to mix the anode and cathode solutions.
2. Remove two gels from the packaging and place on a paper towel. Carefully remove the transparent protective overlay from each gel. Using slight fingertip pressure, gently blot the gel surface of each gel with a Blotter C and discard blotters.

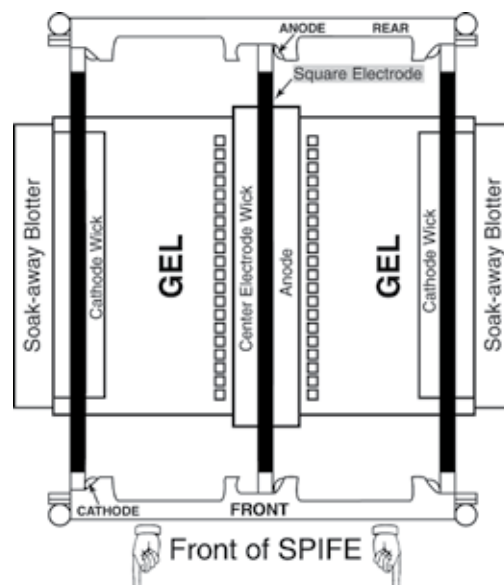
## B. Electrophoresis

1. Using the instructions provided in the SPIFE 3000 Operators Manual, set up the parameters as follows:

### Electrophoresis Unit

- 1) No Prompt  
Electrophoresis 1 45:00 15°C 700V 160mA
- 2) No Prompt  
END OF TEST

2. Dispense 0.25 to 0.5 mL of REP Prep at each end of the chamber floor.
3. Place a gel on the left side and right side of the chamber floor with the sample wells toward the center of the chamber. The edges of the gel backing should touch, but not overlap, at the center of the chamber floor. Wipe the excess REP Prep from around the edges of the gel backing.
4. Place the Center Electrode Wick, in the Anode Solution, between two Center Wick Blotters and blot almost dry.
5. Position the Center Electrode Wick on the gel surface so that it overlaps both gels at the center of the chamber.
6. Place the wicks, in the Cathode Solution, between two Wick Blotters and blot almost dry.
7. Place one Electrode Wick on the gel at the outer edge of each gel.
8. Insert the SPIFE IEF Electrode Adapter marked Front between the two magnetic posts (Cat. No. 3704) located at the front of the chamber floor. Insert the IEF Electrode Adapter marked Rear between the two magnetic posts located at the back of the chamber floor.



9. Clean and wipe the three (3) IEF electrodes with a lint-free tissue.
10. Place a round electrode into the slots created by the adapter – one on each outside edge. Place the square electrode in the middle. Be sure all 3 electrodes are seated firmly against the electrode wicks.

11. Position one Soak-away Blotter against the edge of the gel on the left end of the chamber and one Soak-away Blotter against the gel on the right end of the chamber to act as excess fluid blotters. Ensure these Soak-aways are in contact with the gel.
12. Apply 5  $\mu\text{L}$  of sample to each sample well. Close the chamber lid.
13. With **CSF-IEF** on the display, press **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin electrophoresis.

### III. Immunoblotting

Following the focusing step, each gel should be processed according to the following procedures.

1. Remove the gel from the chamber floor, and place it on a Blotter D with sample number one on the left. Remove and discard the electrode wicks and blotters.
2. Pre-wet a sheet of Transfer Membrane in deionized water. Remove excess water by gently blotting between two paper towels or tissues. Gently place the Transfer Membrane onto the gel surface, red dot down. Allow the membrane to take up moisture as it is placed on the gel surface to prevent the production of air pockets. Leave for 10 seconds and discard.
3. Pre-wet a second sheet of Transfer Membrane in 10% methanol in water. Allow excess fluid to drain from the membrane by holding the membrane vertical for 10-20 seconds. Carefully lay this Transfer Membrane onto the gel surface, red dot down in the lower left corner. The dot will be by sample number one. Gently move your finger across the surface of the membrane to ensure good gel contact and to remove air pockets between the gel and the membrane (avoid excessive pressure which could damage the gel). Place one Blotter C, three Blotter D's and a weight on top of the gel. Press for 30 minutes. (The weight should weigh between 2 and 2.5 kg.)
4. Remove the blotters and weight and discard the blotters. Place the Transfer Membrane, red dot up, into a staining dish containing 20 mL of Blocking Solution A. Ensure that the entire surface of the membrane is covered. Gently agitate for 30 minutes.
5. Wash the membrane in water three times.
6. Pour 15 mL of Blocking Solution B into a staining dish. Add 15  $\mu\text{L}$  of Single Antibody and mix.
7. Place the membrane in this solution for 30 minutes and gently agitate.
8. Wash the membrane in water three times and, finally, in saline for 5 minutes. Rinse in deionized water.

### IV. Visualization

1. Pour 25 mL of diluted Acetate Buffer into a dish. Add 5 mL Chromogen Solution and 25  $\mu\text{L}$  of 30% hydrogen peroxide and mix quickly.
2. Place the membrane in this solution and gently agitate. Allow color to develop for 20 minutes. It is normal for precipitation of chromogen to occur during this step.
3. Rinse in water three times and allow the membrane to air dry.

4. Transfer membranes may be visually inspected for qualitative evaluation even after being kept an indefinite period of time after being processed. Transfer membranes that have darkened after long-term storage should not be used for evaluation.

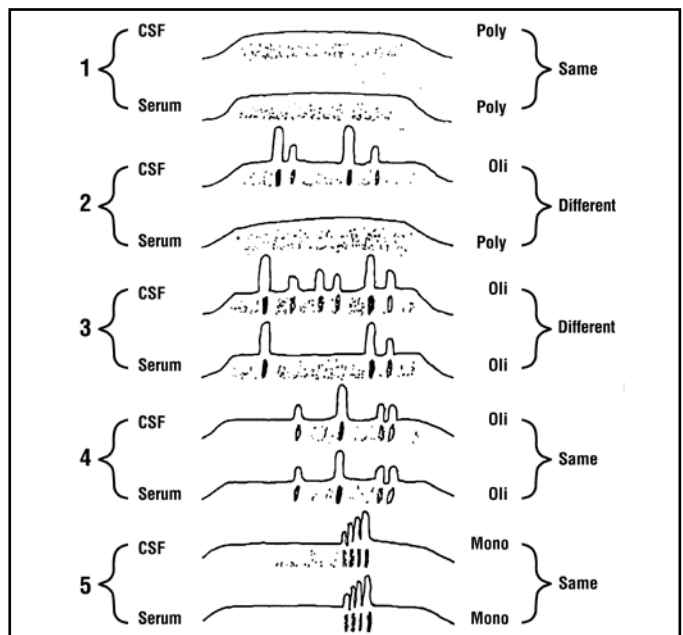
### QUALITY CONTROL

It is recommended that the TITAN GEL High Resolution Protein Marker (Cat. No. 5141) be used as a positive control for oligoclonal bands. It should be run on each gel to ensure that all reagents and procedures perform properly. Dilute the marker 1:30 with saline before use. If no oligoclonal bands are detected, the test is considered invalid and should be repeated.

Additional controls may be included according to guidelines and requirements of local, state and/or federal regulations or accrediting organizations.

### INTERPRETATION OF RESULTS<sup>2,3</sup>

For a full review of the interpretation of IgG IEF patterns, see Andersson, M. et al.<sup>1</sup> The IgG bands become visible as a red/red brown color on the transfer membrane. Comparison of paired serum/CSF samples should always be the standard procedure. The interpretation of the patterns obtained should be performed in the context of the clinical history of the patient and appropriate biochemical and/or microbiological testing. Most of the clinical interest in CSF electrophoresis is focused on the gamma region. Oligoclonal bands are multiple distinct bands in the gamma zone of the CSF pattern, which are not present in serum.



**Figure 1:** Diagram showing idealized CSF and serum isoelectric focusing patterns. Different CSF and serum patterns denote local IgG synthesis. Densitometric scans of the patterns show the differences in optical density (relative amount of protein per band). Type 1 is normal. Type 2 is found in multiple sclerosis. Type 3 is found in multiple sclerosis and brain inflammation in systemic disease-for example sarcoidosis. Type 4 is found in systemic inflammation-for example Guillain-Barré. Type 5 is found in myeloma or monoclonal gammopathy of uncertain significance. The pH gradient is from 6-9, and the cathode is on the right. Poly = Polyclonal; Oli = Oligoclonal; Mono = Monoclonal.

There are five different IEF patterns that may be seen.

1. Normal CSF - no bands present in the CSF (see picture - Type 1).
2. CSF with restricted oligoclonal bands not seen in the serum (see picture - Type 2).
3. CSF with restricted oligoclonal bands with additional bands seen in both the CSF and serum (see picture - Type 3).
4. Identical oligoclonal bands in the CSF and serum (see picture - Type 4).
5. Monoclonal bands in both the CSF and serum (see picture - Type 5).

Of these five different patterns only pattern numbers 2 and 3 represent local synthesis of IgG within the central nervous system. If no oligoclonal bands are found in either CSF or serum, then no pathological process has been detected.

Oligoclonal bands that are present in the CSF only are associated with a variety of inflammatory brain diseases, the most common example of which is MS. Oligoclonal bands appear in 90% of MS patients sometime during the course of their disease.

Increased IgG in the CSF is not specific for MS, but is an indication of chronic neural inflammation. Oligoclonal bands have been reported in cases of non-treated neurosyphilis, progressive rubella panencephalitis, trypanosomiasis, acute bacterial or viral meningitis, optic neuritis, subacute sclerosing panencephalitis, polyneuritis, Guillain-Barré Syndrome, meningeal carcinomatosis, headaches, systemic lupus erythematosus, and other infectious autoimmune diseases.

If oligoclonal bands are found in both the CSF and serum, and the patterns are the same in both fluids, then the primary pathology is outside the central nervous system. The CSF is simply reflecting the abnormalities in the serum gammaglobulins. Examples of this include systemic infection by viruses such as HIV or measles, and post-infection polyneuropathy. Autoimmune diseases, such as Behcet's, SLE and sarcoidosis can also give this pattern, as can paraneoplastic syndromes and paraproteinemias.

Since 80% of CSF is derived from serum, patients with monoclonal proteins may have a corresponding band in their CSF. If the pattern of the bands in the CSF and serum is monoclonal, rather than oligoclonal, then the possibility of a B-cell tumor should be considered.

When the band patterns of the CSF and serum differ and there are more bands in the CSF than the serum, this suggests a specific involvement of the CNS in the pathology. The causes are the same as those listed above but the evidence of CNS involvement is superimposed upon the systemic response.

Oligoclonal bands may be present in the serum of patients with Hodgkin's disease and approximately 5% of MS patients will show faint bands in the serum. The reason for this is unclear but may be associated with underlying infection or release of CNS antigens into the peripheral blood system, where they induce antibody production. Approximately 2-3% of clinically confirmed MS patients show no evidence of oligoclonal bands in the CSF. For these patients the oligoclonal pattern is still evolving and a second test run at a later date may show they have developed oligoclonal bands.<sup>2</sup>

## LIMITATIONS

1. It is imperative that serum and CSF samples be collected from the patient at the same time. Any treatment of the samples that might alter the concentration of the immunoglobulins (other than those required under the Sample Storage and Handling section of this procedure) must be avoided.
2. The use of antisera other than the one provided in the kit may affect the results.
3. It is important to exclude artifactual bands that are caused by non-linearity of the isoelectric focusing pH gradient. A good practical indicator for these is to compare the serum protein patterns from several patients. Bands that are at the same isoelectric point in all specimens of a given run are most likely to be artifacts produced by the ampholytes used in the separation.<sup>1</sup>

## TROUBLESHOOTING

The following table details possible reasons and solutions to some of the most commonly encountered problems associated with isoelectric focusing followed by immunoblotting.

| <b>Problem Observed</b>                             | <b>Possible Reasons and Solutions</b>   |
|---|---|
| 1. No staining of sample lanes                      | a) No samples applied.  |
| a) Background unstained                             | b) Antibody omitted or degraded.<br>c) No hydrogen peroxide added.  |
| b) Background heavily and uniformly stained.        | a) Blocking step omitted.<br>b) Inadequate washing of the transfer membrane during the immunofixation and coloration steps.   |
| c) Background heavily but unevenly stained          | a) Omitted or inadequate pre-blot step.   |
| 2. Sample lanes stained but faint                   | a) Insufficient sample applied.<br>b) Insufficient staining time.<br>c) Old or contaminated hydrogen peroxide.<br>d) Decay of antibody due to incorrect storage.<br>e) Oxidized chromogen (will appear black).<br>f) pH of acetate buffer incorrect (should be 5.0 to 5.2). |
| 3. Sample lanes intensely stained losing resolution | a) Excessive sample applied.<br>b) Overdeveloped (rare).  |
| 4. Sample lanes distorted/smeared                   | a) No soak-always used.<br>b) Condensation on gel surface due to excessive cooling of the gel.<br>c) Excessive sample volume.<br>d) Inadequate cooling during IEF.  |
| 5. All staining in the sample well                  | a) No power applied during electrophoresis.   |
| 6. White spots in the stained sample                | a) Air bubbles between gel and membrane at press stage. Ensure a pre-wet membrane is used and that bubbles are rubbed out from between the membrane and gel.  |

## PERFORMANCE CHARACTERISTICS

### Accuracy

Sixty paired CSF and serum samples with known clinical diagnoses were tested using the SPIFE 3000 and the SPIFE IgG IEF procedure. The samples could be subdi-

vided into 34 MS, 12 possible MS and 14 other inflammatory brain conditions. Results were considered positive if oligoclonal bands were observed in the CSF and not in the serum. Of the 34 patients diagnosed with MS, 29 were positive as compared to 7 in the possible MS patients. For patients with other inflammatory brain conditions, 2 of 14 were positive. Excluding the possible MS patients, the assay sensitivity and specificity are 85% and 80% respectively.

**Method Comparison**

Sixty paired CSF and serum patient samples were tested using the SPIFE IgG IEF kit and a commercially available electrophoresis and immunofixation kit. Both the serum and CSF samples were pretreated according to the manufacturer's specifications.

These samples included 34 MS, 12 possible MS and 14 other inflammatory brain conditions (headache, fibromyalgia, optic neuropathy, visual blurring, trigeminal neuralgia, dementia and vertigo). Eighteen paired samples (17 MS and 1 possible MS) and 3 serum samples were excluded due to prozone effect when analyzed with the commercially available kit. Results were tabulated below.

|               | Electrophoresis/Immunofixation |                |
|---------------|--------------------------------|----------------|
|               | Positive                       | Negative       |
| SPIFE IgG Kit | Positive 18                    | 4 <sup>a</sup> |
|               | Negative 2 <sup>b</sup>        | 18             |
|               | Total 20                       | 22             |

<sup>a</sup> two MS and 2 possible MS

<sup>b</sup> 1 MS and 1 possible MS

|                    |       |         |
|--------------------|-------|---------|
| Overall agreement  | 85.7% | (36/42) |
| Positive agreement | 90.0% | (18/20) |
| Negative agreement | 81.8% | (18/22) |

**Analytical Sensitivity**

The SPIFE IgG IEF method will detect IgG type monoclonal bands at concentrations greater than 0.28 mg/L. An IgG lambda serum sample was assayed using Radial ImmunoDiffusion (RID) to determine its IgG concentration. The sample was then serially diluted and run on the IEF method. The patterns were visually inspected to see when the patterns were no longer visible. That sample's dilution was back calculated to determine its concentration.

**Reproducibility**

**Within Gel Reproducibility**

A CSF and serum sample from two different patients (one with oligoclonal bands, one without) were run in replicate on a single gel. The patterns were visually inspected and found to be qualitatively identical. In each lane the oligoclonal bands were correctly identified. No false negatives or positives were observed.

**Within Lot (Gel to Gel) Reproducibility**

A CSF and serum sample from two different patients (one with oligoclonal bands, one without) were run in replicate on five separate gels. The patterns were visually inspected and found to be qualitatively identical. In each lane the oligoclonal bands were correctly identified. No false negatives or positives were observed.

**BIBLIOGRAPHY**

1. Andersson, M. et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol, Neurosurg. Psychiatry, 1994; 57:897-902.
2. Keir, G. Report on the Evaluation of Helena Laboratories' Method for Agarose Isoelectric Focusing of Immunoglobulin G. Private communication.
3. Keir, G. et al. Isoelectric focusing of cerebrospinal fluid immunoglobulin G: An annotated update. Ann. Clin. Biochem., 1990; 27:436-443.

| SPIFE IgG IEF-20 KIT   |                 |
|--|-----------------|
| <b>Cat. No. 3385</b>   |                 |
| SPIFE IgG IEF Gel (10)   |                 |
| SPIFE IgG IEF Anode Solution (1 vial)  |                 |
| SPIFE IgG IEF Cathode Solution (1 vial)  |                 |
| SPIFE IgG IEF Blocking Agent (1 vial)  |                 |
| SPIFE IgG IEF Acetate Buffer Concentrate (1 vial)  |                 |
| SPIFE IgG IEF Chromogen (1 vial)   |                 |
| SPIFE IgG IEF Antibody (1 vial)  |                 |
| Transfer Membranes (20)  |                 |
| Blotter C (20)   |                 |
| Blotter D (40)   |                 |
| Electrode Wicks (20)   |                 |
| Wick Blotters (40)   |                 |
| Soak-away Blotters (20)  |                 |
| Center Electrode Wicks (5)   |                 |
| Center Wick Blotters(10)   |                 |
| <b>Other Supplies and Equipment</b>  |                 |
| The following items, needed for the performance of the SPIFE IgG IEF-20 Kit, must be ordered individually. |                 |
| <b>Item</b>  | <b>Cat. No.</b> |
| SPIFE 3000   | 1088            |
| REP Prep   | 3100            |
| SPIFE IgG IEF Electrodes (1 gel)   | 3383            |
| SPIFE IgG IEF Square Electrodes  | 3703            |
| SPIFE IEF Adapter and Electrodes (2 gels)  | 3704            |
| TITAN GEL High Resolution Protein Marker   | 5141            |

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