HYDRAGEL 3 CSF ISOFOCUSING
Ref. 4353

HYDRAGEL 9 CSF ISOFOCUSING
Ref. 4355
INTENDED USE

The HYDRAGEL 3 CSF ISOFOCUSING and HYDRAGEL 9 CSF ISOFOCUSING kits are designed for the qualitative detection and identification of "oligoclonal" bands in the electrophoretic patterns of cerebrospinal fluid (CSF) and confirmation of their immunoglobulin character. The procedure includes isoelectrofocusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with anti-Ig G antiserum. The use of enzyme labeled anti-Ig G antiserum permits to detect only the "true" Ig G oligoclonal banding at increased sensitivity of detection so that the analysis can be generally performed on unconcentrated CSF. The Ig G immunofixation patterns of CSF and serum from the same patient are then visually compared. This allows detection of oligoclonal banding that represents intrathecal synthesis of immunoglobulins.

The HYDRAGEL 3 CSF ISOFOCUSING and HYDRAGEL 9 CSF ISOFOCUSING kits are indicated when certain diseases of the central nervous system (CNS), such as multiple sclerosis, are suspected and the detection of oligoclonal banding and inflammatory processes (intrathecal synthesis of immunoglobulins) can aid to the diagnosis.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

Many disorders of the central nervous system are associated with increased concentration of CSF proteins either due to increased permeability of blood-CSF barrier or to synthesis of immunoglobulins, primarily Ig G, within the central nervous system (CNS). In the latter case, such intrathecal synthesis of immunoglobulins (Ig's) is often associated with restricted heterogeneity which manifests itself as "oligoclonal banding" seen in the gamma zone of high resolution electrophoretic migration patterns. The bands that are not Ig's can also be present in the gamma globulin zone but do not have the same diagnostic significance. Immunofixation is a choice detection technique since it can prove the Ig character of the oligoclonal bands, identify the Ig involved and provide the necessary test specificity. Since only Ig G oligoclonal banding has a routine diagnostic significance the SEBIA test is primarily concerned with the detection of Ig G oligoclonal bands using specific anti-Ig G antiserum.

Presence of intrathecal Ig G suggests inflammatory disease of the CNS, such as caused by multiple sclerosis (MS). Although oligoclonal banding is neither diagnostic nor specific for MS, it is widely used as supportive information and considered an essential test by the 1994 consensus of the "Committee of the European Concerted Action for Multiple Sclerosis". It is confirmatory in patients with clinical MS episodes and suggestive in patients with only few episodes or inconclusive clinical symptoms.

Among others, the Committee set criteria for detection of Ig G oligoclonal banding in the CSF:

1. the most resolute and sensitive technique for the detection of oligoclonal banding is isoelectric focusing,
2. the oligoclonal Ig G must be detected by specific antiserum,
3. to confirm intrathecal Ig G synthesis, patient serum and CSF must be analyzed in parallel to demonstrate differences in Ig G distribution,
4. as an aid to interpretation, Ig G concentration in the CSF-serum sample pair should be adjusted to the same level,
5. concentrating CSF should be avoided.

The HYDRAGEL 3 & 9 CSF ISOFOCUSING test meets all the above criteria.

The assay is carried out in two stages:

• Isoelectrofocusing on agarose gel to fractionate the proteins in the CSF and serum samples.
• Immunofixation with enzyme (peroxidase) labelled anti-Ig G antiserum to detect Ig G oligoclonal bands and to demonstrate the difference, or lack of, in the distribution of Ig G in the CSF and serum.

Compared to standard immunofixation, about a 100 times increase in the sensitivity of detection is achieved with the HYDRAGEL 3 & 9 CSF ISOFOCUSING kits that use enzyme labelled antibodies. Then, with Ig G concentration at or above of only of 1 mg/dL, the Ig G can be detected and its distribution discerned without concentrating the CSF sample.

The semi-automated HYDRASYS system performs all the steps needed to obtain gels ready for interpretation. Each agarose gel in the HYDRAGEL 3 CSF ISOFOCUSING contains 6 tracks and is intended to run three CSF – serum sample pairs. Each agarose gel in the HYDRAGEL 9 CSF ISOFOCUSING contains 18 tracks and is intended to run nine CSF – serum sample pairs.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 3 CSF ISOFOCUSING AND HYDRAGEL 9 CSF ISOFOCUSING KITS

WARNING : See the safety data sheets.

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4353</th>
<th>PN 4355</th>
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</thead>
<tbody>
<tr>
<td>Agarose Gels (ready to use)</td>
<td>10 gels</td>
<td>10 gels</td>
</tr>
<tr>
<td>Ethylene glycol solution (ready to use)</td>
<td>1 vial, 3 mL</td>
<td>1 vial, 3 mL</td>
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<tr>
<td>Anodic solution (ready to use)</td>
<td>1 vial, 50 mL</td>
<td>1 vial, 50 mL</td>
</tr>
<tr>
<td>Cathodic solution (ready to use)</td>
<td>1 vial, 50 mL</td>
<td>1 vial, 50 mL</td>
</tr>
<tr>
<td>Strips</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
</tr>
<tr>
<td>Sample Diluent CSF / A1AT / TRF (ready to use)</td>
<td>1 vial, 85 mL</td>
<td>1 vial, 85 mL</td>
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<tr>
<td>Antiserum Diluent CSF ISOFOCUSING (ready to use)</td>
<td>1 vial, 7.5 mL</td>
<td>1 vial, 7.5 mL</td>
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<tr>
<td>Wash Solution CSF ISOFOCUSING (ready to use)</td>
<td>1 vial, 70 mL</td>
<td>1 vial, 70 mL</td>
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<tr>
<td>Rehydrating Solution (ready to use)</td>
<td>2 vials, 70 mL</td>
<td>2 vials, 70 mL</td>
</tr>
<tr>
<td>TTF1 / TTF2 Solvent (ready to use)</td>
<td>2 vials, 20 mL</td>
<td>2 vials, 20 mL</td>
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<tr>
<td>TTF1 (stock solution)</td>
<td>2 vials, 0.5 mL</td>
<td>2 vials, 0.5 mL</td>
</tr>
<tr>
<td>TTF2 (stock solution)</td>
<td>2 vials, 0.5 mL</td>
<td>2 vials, 0.5 mL</td>
</tr>
<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10 (6 teeth)</td>
<td>1 pack of 10 (18 teeth)</td>
</tr>
<tr>
<td>Buffer containers (ready to use)</td>
<td>1 pack of 2</td>
<td>1 pack of 2</td>
</tr>
<tr>
<td>Antiserum segments (ready to use)</td>
<td>2 packs of 5 each</td>
<td>2 packs of 5 each</td>
</tr>
<tr>
<td>Filter Papers – Thin</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
</tr>
<tr>
<td>Filter Papers – Thick</td>
<td>4 packs of 10 each</td>
<td>4 packs of 10 each</td>
</tr>
<tr>
<td>Plastic masks</td>
<td>1 pack of 10 each</td>
<td>1 pack of 10 each</td>
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</tbody>
</table>

During transportation, the kit can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.
FOR OPTIMAL RESULTS:
Only reagents from the same kit must be always used together and according to the package insert instructions. PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. AGAROSE GELS
Preparation
Agarose gels are ready to use. Each gel contains: agarose; ampholytes; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for protein isoelectrofocusing and immunofixation.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging and refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards).
Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.
DO NOT FREEZE.
Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).
NOTE: After storage at 2 - 8 °C and prior to use, it is recommended to let the gel in its protective packaging at room temperature for 5 to 10 minutes.
IMPORTANT : The unused gels must always be stored at 2 – 8 °C and should not be submitted to variation of storage temperature between 2 – 8 °C and room temperature and vice versa.

2. ETHYLENE GLYCOL SOLUTION
Preparation
The ethylene glycol solution is ready to use.

Use
To provide effective contact between the gel plastic backing and the temperature control plate of the migration module during the electrophoretic migration.

Storage, stability and signs of deterioration
Store the ethylene glycol solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or ethylene glycol solution vial label.

3. ANODIC AND CATHODIC SOLUTIONS
Preparation
The anodic solution (acid solution) and cathodic solution (basic solution) are ready to use. They both contain additives, nonhazardous at concentrations used, necessary for optimum performance.
NOTE: The anodic solution is colored red as an aid in monitoring its application and for an easy identification of the prepared anodic strip.

Use
As electrolytes for preparing anodic and cathodic strips for isoelectrofocusing.
IMPORTANT: After each use, close immediately and tightly the cathodic solution vial to avoid carbonation of this solution.

Storage, stability and signs of deterioration
The anodic and acidic solutions can be stored at room temperature or refrigerated and are stable until the expiration date indicated on the kit package or solutions vial labels. Solutions must be free of precipitate.

4. STRIPS
Preparation
Saturate two sponge strips respectively with anodic and cathodic solutions 5 minutes before use:
Place the grey container for anodic strip and the blue container for the cathodic strip on a flat surface.
Dispense 5 mL of red anodic solution in the grey container and 5 mL of uncolored cathodic solution in the blue container.
Open the pack of sponges, handle the strips by the plastic ends and place them in each container.
Soak them evenly by pressing several times with the tip of the corresponding pipettes.
Use the saturated strips without any delay.
IMPORTANT: Saturate strips just before use to avoid carbonation of the cathodic solution.
NOTE: If the sponge strip is dry (hard), before soaking it with solutions, It is recommended to immerse it in hot water (around 60 °C); then wring it, rinse it with distilled or deionized water and wring the strip again. This step will improve the soaking of the sponge with anodic or cathodic solutions.
WARNING: It is recommended to manipulate carefully hot water (when at a temperature ≥ 40 °C).

Use
The strips soaked respectively with anodic and cathodic electrolyte solutions ensure contact between the gel and electrodes and determine the pH range during fociolisation.

Storage, stability and signs of deterioration
Moist sponges in the original protective packaging can be stored at room temperature or refrigerated. They must be stored horizontally (the arrow on the front of the kit box must be pointing upwards). They are stable until the expiration date indicated on the kit package or strips package label.
DO NOT FREEZE.
5. CSF / A1AT / TRF SAMPLE DILUENT

Preparation
Sample diluent is ready to use. It contains: additives nonhazardous at concentrations used, necessary for optimum performance.

Use
For CSF and serum sample dilution.

Storage, stability and signs of deterioration
Store the sample diluent refrigerated (2 to 8 ºC). It is stable until the expiration date indicated on the kit package or diluent vial label. Diluent must be free of precipitate.

6. CSF ISOFOCUSING ANTISERUM DILUENT

Preparation
Antiserum diluent is ready to use. It contains: buffer solution pH 6.0 ± 0.5; additives nonhazardous at concentrations used, necessary for optimum performance.

Use
For diluting antiserum just before use.

Storage, stability and signs of deterioration
The antiserum diluent can be stored at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or antiserum diluent vial label. Antiserum diluent must be free of precipitate.

NOTE: During storage, the antiserum diluent may turn yellow without any adverse effects on its performance.

7. CSF ISOFOCUSING WASH SOLUTION

Preparation
The CSF ISOFOCUSING wash solution is ready to use. It contains: buffer solution pH 6.1 ± 0.5; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
For washing the agarose gel after the blotting step that follows the incubation with the peroxidase-labeled antiserum.

Storage, stability and signs of deterioration
The wash solution can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the kit package or wash solution vial label. The wash solution may show mild crystallization without any adverse effects on its performance.

NOTE: During storage, wash solution may turn slightly yellow without any adverse effects on its performance.

8. REHYDRATING SOLUTION

Preparation
The rehydrating solution is ready to use. It contains components, nonhazardous at concentrations used, necessary for optimum performance.

Use
For rehydrating the agarose gel before the peroxidase-based visualization step.

Storage, stability and signs of deterioration
The rehydrating solution can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the kit package or rehydrating solution vial label. Rehydrating solution must be free of precipitate.

9. TTF1 / TTF2 SOLVENT

Preparation
The TTF1 / TTF2 solvent is ready to use. It contains components, nonhazardous at concentrations used, necessary for optimum performance.

Use
For the preparation of TTF visualization solution as described in No. 10.

Storage, stability and signs of deterioration
The TTF1 / TTF2 solvent can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the kit package or TTF1 / TTF2 solvent vial label. TTF1 / TTF2 solvent must be free of precipitate.

10. TTF1 AND TTF2

Preparation
Just before use, prepare the TTF working developing solution. Add necessarily the reagents in the following order to avoid any risk of precipitation: 4 mL of TTF1 / TTF2 solvent, 100 μL of TTF1, 100 μL of TTF2 and 4 μL hydrogen peroxide (H₂O₂) 30 %.

IMPORTANT: The TTF1 and TTF2 vials must be placed at room temperature for 30 minutes before use. Homogenize each vial before preparing the working developing solution.

Use
For visualization of the immunoglobulins separated by isofocusing via the peroxidase-labelled antiserum.

Storage, stability and signs of deterioration
Store the stock TTF1 and TTF2 vials refrigerated. They are stable until the expiration date indicated on the kit package or TTF1 and TTF2 vial labels. TTF1 and TTF2 solutions must be free of precipitation.

NOTE: After storage at 2 – 8 ºC, TTF1 and TTF2 stock solutions are under solid form. They easily redissolve at room temperature. Stir them prior to use.
11. APPLICATORS

**Use**
Precut, single use applicators for sample application onto gel.

**Storage, stability and signs of deterioration**
Store the applicators in a dry place at room temperature or refrigerated.

12. BUFFER CONTAINERS

**Use**
Reusable colored containers for preparation of the anodic and cathodic strips.
The grey container is intended for the anodic strip and the blue container is intended for the cathodic strip.
After each use, wash the two containers with distilled or deionized water and dry them.

**Storage, stability and signs of deterioration**
Store the clean buffer containers on a flat surface at room temperature.

13. ANTISERUM SEGMENTS

**Use**
Single use, colored segments for antiserum application onto the gel for immunofixation with the dynamic mask.
**WARNING**: Segments loaded with antiserum have to be handled with care.

14. THIN FILTER PAPERS

**Use**
Precut, single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

**Storage**
Store the thin filter papers in a dry place at room temperature or refrigerated.

15. THICK FILTER PAPERS

**Use**
Single use, thick absorbent paper pads for blotting unprecipitated proteins off the gel after immunofixation, washing and rehydration steps and excessive visualization reagent.

**Storage**
Store the thick filter papers in a dry place at room temperature or refrigerated.

16. PLASTIC MASKS

**Use**
Plastic sheets, for single use, to shield the gel during the electrophoretic migration.

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**REAGENTS REQUIRED BUT NOT SUPPLIED**

**WARNING**: See the safety data sheets.

1. ANTISERUM ANTI-Ig G - PER

**Preparation**
The antiserum vial (SEBIA, PN 4743) contains mammalian immunoglobulins directed against human Ig G, conjugated to peroxidase. The antiserum is in a stabilized lyophilised form.

For an easy identification and as an aid in monitoring its application, the antiserum is coloured with a non-hazardous dye that matches the colour of the vial label.

Reconstitute the lyophilised antiserum vial with 0.7 mL of distilled or deionized water. Allow to stand for 5 minutes and mix gently (avoid formation of foam).

Then, prepare working solution just before use.
**For HYDRAGEL 3 CSF ISOFOCUSING**: 25 μL reconstituted anti-Ig G – PER and 210 μL antiserum diluent. Mix well.
**For HYDRAGEL 9 CSF ISOFOCUSING**: 40 μL reconstituted anti-Ig G – PER and 420 μL antiserum diluent. Mix well.

**Use**
For immunofixation and visualization of the isoelectrofocused Ig G's.

**Storage, stability and signs of deterioration**
Before reconstitution, store the lyophilised antiserum refrigerated (2 to 8 °C) away from light. It is stable until the expiration date indicated on the antiserum vial or box labels.

Store the reconstituted antiserum away from light at 2 - 8 °C. Due to the risk of microbial contamination and denaturation, use it within one week.

The reconstituted antiserum may also be frozen (in aliquots) and stored at - 18 / - 30 °C for 6 months maximum ; it is recommended to freeze it into microtubes with screw caps in order to avoid evaporation. This evaporation causes a concentration that leads to a too high intensity of staining during the enzymatic visualization step.

**IMPORTANT**: After storage at 2 - 8 °C or at - 18 / - 30 °C, homogenize the reconstituted antiserum before the preparation of the working solution (diluted antiserum).

Before use, store the thawed antiserum at 2 – 8 °C and use it within the day. Do not freeze and thaw the antiserum more than twice.
Discard antiserum if any change in appearance, e.g., cloudiness due to microbial contamination is observed.

**NOTE**: Antisera may originate from different animal species. Don’t mix two different antisera vials, even with the same specificity, and ALWAYS change the tip of the pipette when changing antiserum vials.

During transportation, the antiserum may be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.
2. HYDROGEN PEROXIDE: \( \text{H}_2\text{O}_2, 30\% \) (110 Vol.)

Hydrogen peroxide must be stored in dark bottle and refrigerated (2 to 8 °C). When dispensing, always use clean pipette to avoid contamination of the bottle content.

3. DESTAINING SOLUTION

**Preparation**

Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials 100 mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container.

After dilution, the working destaining solution contains an acidic solution pH = 2.

**Use**

For washing the gel after enzymatic visualization and drying and for rinsing of the HYDRASYS staining compartment.

To neutralize the acidity of the destaining solution for disposal, pour 15 mL of a 50 % solution of sodium hydroxide, into the empty waste container.

**Storage, stability and signs of deterioration**

Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels.

Working destaining solution is stable for one week at room temperature in a closed bottle.

To prevent microbial proliferation in the diluted destaining solution to be stored more than one week, add 5 μL/dL of ProClin 300 or CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL).

See the CLEAN PROTECT package insert for directions to use.

Working destaining solution added with ProClin or CLEAN PROTECT is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

Do not add any sodium azide.

Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. HYDRASYS WASH SOLUTION

**Preparation**

Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water. After dilution, the working wash solution contains: buffer solution pH 8.7 ± 0.5.

**Use**

The HYDRASYS wash solution is designed for cleaning of the HYDRASYS staining compartment. Use periodically, e.g., if the instrument is used daily, wash the staining compartment weekly.

See the package insert for directions to use.

**Storage, stability and signs of deterioration**

Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.

Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

**NOTES:**

The assays that were performed for the validation of reagents demonstrated that, for the different solutions and using an adapted equipment for the reconstitution volume, a variation of ± 5 % on the final volume has no adverse effect on the analysis.

The distilled or deionized water used to reconstitute solutions, must be free of bacterial proliferation and mold (use a 0.22 µm filter) and have a resistivity higher than 10 Megohms x cm.

**EQUIPMENT AND ACCESSORIES REQUIRED**

1. HYDRASYS System SEBIA, PN 1211 with OPTION FOCUSING HYDRASYS SEBIA, PN 1235 or HYDRASYS FOCUSING System SEBIA, PN 1212.

Or

2. HYDRASYS 2 System SEBIA, PN 1200, PN 1201 with OPTION FOCUSING HYDRASYS 2 SEBIA, PN 1207 or HYDRASYS 2 FOCUSING System SEBIA, PN 1202, PN 1203.

3. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216, HYDRAPLUS 2 SEBIA, PN 1217 or ASSIST SEBIA, PN 1218, for an alternative way of loading the sample applicators.

4. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.

5. Template Guide Bar SEBIA supplied with HYDRASYS.

6. Accessory Kit for HYDRASYS CSF ISOFOCUSING SEBIA, PN 1258. It contains: the reagent application masks R3 and ENZ 4 mL, the single trough segment holder and the dynamic mask guide with the length-half reducing device, specific for the HYDRAGEL CSF ISOFOCUSING procedure.

7. Container Kit supplied with HYDRASYS.

8. Pipettes: 2 µL, 20 µL, 100 µL, 200 µL and 5 mL (or graduated pipettes/pipettors: 0-100 µL, 100-500 µL and 1-10 mL).

**SAMPLES FOR ANALYSIS**

**Sample collection and storage**

Serum and CSF from the same patient must be collected at the same time according to conventional procedures used in clinical laboratory testing. It is recommended to carry out analyses on fresh sera and CSF. The samples may be stored for up to one week refrigerated (2 to 8 °C). For longer storage periods, freeze the samples. Frozen serum and CSF are stable at least for one month.

**Sample preparation**

- Measure the CSF and serum Ig G concentrations using appropriate procedures.
- The concentration of Ig G in the corresponding CSF and serum samples should be adjusted to the same level.
The adjustment depends on the original CSF’s Ig concentration; use Sample Diluent for all dilutions:

1st case: the concentration of Ig G is over 2 mg/dL  
Dilute CSF and serum with the sample diluent to obtain Ig G concentration of 2 mg/dL.

2nd case: the CSF concentration of Ig G is between 1 and 2 mg/dL  
Use neat CSF. Dilute serum to obtain the same Ig G concentration as is in the CSF sample.

3rd case: The CSF concentration of Ig G is below 1 mg/dL  
Concentrate CSF with any appropriate device to obtain Ig G concentration between 1 and 2 mg/dL. Dilute serum to the same Ig G concentration as is in the concentrated CSF sample.

Example of dilutions (only for samples with Ig G concentration over 2.0 mg/dL):

For CSF:

A = Ig G concentration in mg/dL.  
Collect 20 µL CSF and mix well with 10(A - 2) µL of sample diluent.

For serum:

B = Ig G concentration in mg/dL.  
- Dilute serum 10 times with sample diluent, e.g., 10 µL serum and 90 µL sample diluent.  
- Collect y µL diluted serum and add y [(B/20) - 1] µL sample diluent (for suggested value of y = 2 µL, add [(B/10) - 2] µL of sample diluent).

When the Ig G concentration is unknown  
Use neat CSF and dilute serum 300 – 400 times.  
NOTE: Failure to adjust CSF and serum samples to the same Ig G concentration may negatively affect interpretation of the patterns – see “INTERPRETATION”.

PROCEDURE

I. MIGRATION SET UP

1. Switch on HYDRASYS / HYDRASYS 2 instrument.
2. With the HYDRASYS instrument, in order to obtain the high voltage required for the isoelectric focusing, press the green high voltage switch to high voltage mode; then, the switch becomes red. With the HYDRASYS 2 instrument, the high voltage mode is integrated into the specific migration program for the HYDRAGEL CSF ISOFOCUSING procedure; just follow the technique after having selected the corresponding program.

NOTE: The high voltage switch displays migration information. During migration, it shows volt.hours (Vh) and current (mA) values. When pressed during migration, it shows the voltage (V) and power (W) values. If pressed again, the display returns to Vh and mA values.

When the HYDRASYS is in the high voltage mode, the display window shows the real current value and 1/10 of other values such as voltage, volt.hours and power.
3. Place one applicator 6 teeth for HYDRAGEL 3 CSF ISOFOCUSING or one applicator 18 teeth for HYDRAGEL 9 CSF ISOFOCUSING, on a flat surface with the well numbers in the right-side-up position.
4. Apply 10 µL sample in each well of the applicator (Fig. 1). Load the applicator within 2 minutes.

The following example shows analysis of three CSF-serum pairs on one HYDRAGEL 3 CSF ISOFOCUSING gel and nine CSF-serum pairs on one HYDRAGEL 9 CSF ISOFOCUSING gel.
- Place immediately the applicator into the wet storage chamber with the teeth up (handle it by the plastic tooth protection frame).

See wet chamber package insert for further details.

Before sample application onto the gel surface, a pre-migration step until 75 Vh have accumulated must be carried out, according to the following instructions:

5. Open the lid of the migration module and raise the electrode and applicator carriers.

**WARNING:** Never close the lid while the carriers are raised!

**IMPORTANT:** The migration program required for HYDRAGEL CSF ISOFOCUSING procedure on HYDRASYS instrument contains 2 parts, "3/9 CSF FOCUS 1/2" and "3/9 CSF FOCUS 2/2", follow the indications displayed on the screen of HYDRASYS instrument.

6. Select "3/9 CSF FOCUS 1/2" migration program from the HYDRASYS instrument menu or "3 & 9 CSF FOCUSING TTF" migration program from the HYDRASYS 2 instrument menu.

7. Prepare electrode strips with cathodic and anodic solutions: see No. 4 under REAGENTS AND MATERIALS SUPPLIED. Remove strips from the anodic and cathodic containers; handle them by the plastic ends.

8. On the raised carrier, engage the punched ends of the strip's plastic backing to the pins on the electrode carrier, the strip's plastic backing must face the carrier (Fig. 2). The red anodic strip is at the bottom (anode), the uncolored cathodic strip is at the top (cathode).

9. Unpack the HYDRAGEL agarose gel plate put back to room temperature.

10. Place its plastic side down on a tissue or filter paper to remove water droplets.

**WARNING:** Do not leave the filter paper on the gel for more than 3 seconds to avoid bubbles on the surface of the gel.

11. Roll one thin filter paper onto the gel surface and leave it there for 3 seconds to absorb the excess of liquid.

12. Streak 150 µL ethylene glycol (EG) for HYDRAGEL 3 CSF ISOFOCUSING or 300 µL for HYDRAGEL 9 CSF ISOFOCUSING across the lower third of the frame printed on the Temperature Control Plate of the migration module.

13. Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 3).

Bend the gel and ease it slowly down onto the EG streak. Ensure that no air bubbles are trapped, EG is spread underneath the entire gel plate and the gel is lined up with the printed frame.

14. Position a plastic mask on the gel:

- Take one plastic mask.
- Align the lower side of the plastic mask with the two lateral marks printed at 1.5 cm from the bottom of the plastic gel support (cathodic side) (Fig. 4).
- Roll the mask onto the gel surface. Avoid trapping air bubbles between the gel and the mask.

**NOTE:** The size of the plastic mask has been adjusted to the width of the gel. When positioning the plastic mask, it must be perfectly aligned on the two lateral marks printed on the plastic support and centered in order to cover the whole width of the gel to avoid any distortion.

- When air bubbles are trapped, remove without delay the mask from one side to eliminate the bubble and roll it again slowly on the gel.
- Avoid moving the gel on the migration plate during this manipulation.

**WARNING:** The plastic mask must not be placed under the two lateral marks printed on the plastic gel support.

15. Lower both carriers down. In this position, the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.

16. Close the lid of the migration module.

17. Start the procedure immediately by pressing the "START" key on the keyboard (and confirm with HYDRASYS 2 instrument).

**IMPORTANT:** Make sure that the ventilation air inlet on the instrument is not blocked.

**PRE-MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS**

- The electrode carriers is lowered so that buffered strips contact the gel surface.
- Migration is carried out at 20 °C controlled by Peltier effect, until 75 Vh have accumulated; the voltage gradually raises to its maximum value (1000 V), required for the procedure.
- The electrode carrier rises to disconnect the electrodes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. The following message is displayed on the screen: "POS : 1/" followed by the applicator position number signalling to place immediately the applicator on the carrier.

**NOTE:** The migration module lid remains closed during all migration steps.

18. Open the lid of the migration module.

19. Remove the applicator from the wet chamber. Handle it by the protection frame.

- Snap off the applicator teeth's protection frame.
- Place the applicator into position No. 1 on the carrier.

**IMPORTANT:** The numbers printed on the applicator must face the operator (Fig. 5).

20. Close the lid of the migration module.

21. Start the procedure immediately by pressing the "START" key on the keyboard.

**MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS**

- The two carriers are lowered so that buffered strips and applicator contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out for about 45 minutes at 20 °C controlled by Peltier effect, until 75 Vh have accumulated; the voltage gradually raises to its maximum value (1000 V), required for the procedure.
- The electrode carrier rises to disconnect the electrodes.
- A beep sounds and the cover unlocks. This signal remains until the operator intervenes. With HYDRASYS instrument, the following message is displayed on the screen: "3/9 CSF FOCUS 2/2" signalling to carry on with the second part of the migration program. Press the "STOP" key to return to the main menu in order to select "3/9 CSF FOCUS 2/2" migration program. With HYDRASYS 2 instrument, the following message is displayed on the screen: "° ANTISERUM" signalling to apply the peroxydase-labeled antiserum.

**NOTE:** The migration module lid remains closed during all migration steps.
II. IMMUNOFIXATION SET UP
During the migration, assemble the dynamic mask which contains an antiserum segment, a segment holder and a dynamic mask guide with the length-half reducing device (Fig. 6).
1. Place the dynamic mask guide containing the length-half reducing device specific for the HYDRAGEL CSF ISOFOCUSING, on a flat surface.
2. Set up an antiserum segment on the segment holder (Fig. 7):
   - Tilt the antiserum segment at a 45° angle and position it against the plastic springs of the segment holder.
   - Pull the segment and pivot it until it snaps into the notches of the segment holder.
   
   **WARNING:** Be sure the segment is correctly positioned on the holder: the pins at the ends of the segment must be locked into the notches of the holder.
3. Place the holder with the segment on the dynamic mask guide containing the length-half reducing device (Fig. 8).

III. IMMUNOFIXATION
1. After the migration, open the lid.
2. Remove the sample applicator and discard.
3. Raise both carriers and remove them.
4. Remove the buffered strips by their plastic ends and discard.
5. Clean the electrodes by wiping them carefully with a soft wet tissue.
6. Leave the dynamic mask in the HYDRASYS chamber with the antiserum segment.
7. Set up the dynamic mask for antiserum application onto the gel as follows (Fig. 9):
   - Position the mask guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the dynamic mask by the tab and position it into the guide with the notches aligned with the marks.
   - Lower the dynamic mask onto the plate of HYDRASYS.
   - Make sure the segment holder is at the lowest point on the mask guide, facing the operator.
8. With HYDRASYS 2 instrument, follow the indications displayed on the screen.
9. Set up the dynamic mask for antiserum application onto the gel as follows (Fig. 9):
   - Position the mask guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the dynamic mask by the tab and position it into the guide with the notches aligned with the marks.
   - Lower the dynamic mask onto the plate of HYDRASYS.
   - Make sure the segment holder is at the lowest point on the mask guide, facing the operator.
10. Apply carefully and gradually in a single shot the anti-Ig G – PER antiserum, previously diluted, in the space between the segment and the gel through the central well (well No. 8): 235 μL of anti-Ig G – PER for HYDRAGEL 3 CSF ISOFOCUSING or 460 μL of anti-Ig G – PER for HYDRAGEL 9 CSF ISOFOCUSING.
    The antiserum is in contact with the gel and remains by capillarity by the antiserum segment. Aspirate reagent avoiding any air bubbles in the pipette tip.

**IMPORTANT:** For this step, use a 1 mL pipette tip, ensure that the tip does not become lodged in the segment well.
11. To apply the reagent (Fig. 10):
   - Hold the pipette upright and rest its tip lightly on the bottom of the well (avoid pressing down on segment).
   - Carefully and gradually, inject the antiserum for about 5 seconds; reagent will spread under the entire segment.
12. Immediately, using the segment holder handle, move the segment four times slowly but steadily up and down the entire length of the gel to apply the reagent; application should take approximately 5 seconds for each passage (Fig. 11).
   During this step, hold the mask only by the segment holder handle. Avoid touching the guide.
13. Leave the dynamic mask in the HYDRASYS chamber with the antiserum segment at the lowest point on the mask guide.
14. Close the lid of the migration module.
15. Start immediately the incubation procedure by pressing the "START" key on the keyboard.

The following message is displayed on the screen: 

**IMMUNOFIXATION - DESCRIPTION OF THE AUTOMATED STEPS**

- Incubation at 20 °C controlled by Peltier effect, for 15 minutes.
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: 
  "\( \bigcirc \ PAP/ \bigcirc \ THICK \ FILTER \ PAPER \)."
  
  **NOTE:** The migration module lid remains locked during incubation.

IV. GEL BLOTTING
1. Open the lid of the migration module.
2. Remove the dynamic mask assembly:
   - Remove the segment holder using its handle.
   - Remove the antiserum segment from the holder and discard.
   
   **WARNING:** Segment with antiserum has to be handled with care.
3. Apply a thick filter paper, the smooth side down, on the gel:
   - Slope the filter paper at about 45°. Align the lower side of the filter paper with the edge of the gel.
   - Lower the filter paper onto the gel.
   
   **WARNING:** Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.
4. Close the lid of the migration module.
5. Start the blotting sequence by pressing the "START" key on the keyboard.

**BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS**

- Blotting at 20 °C controlled by Peltier effect, for 3 minutes. The following message is displayed on the screen: "[BLOTTING]."
- A beep sounds. The following message is displayed on the screen: 
  "\( \bigcirc \ PAP + \bigcirc \ WASH \)."
  "\( \bigcirc \ THICK \ FILTER \ PAPER, \bigcirc \ WASH \) signalling to remove the filter paper and to apply the CSF ISOFOCUSING wash solution."
V. GEL WASHING
1. Open the lid of the migration module.
2. Remove the filter paper and leave the gel in place on the plate of the migration module.
3. Set up the reagent application mask R3 for HYDRAGEL 3 CSF ISOFOCUSBING or mask ENZ 4 mL for HYDRAGEL 9 CSF ISOFOCUSBING (Fig. 12).
4. Take wash solution for HYDRAGEL CSF ISOFOCUSBING without trapping any air bubbles in the pipette tip.
5. Apply 4.5 mL for HYDRAGEL 3 CSF ISOFOCUSBING or 7 mL for HYDRAGEL 9 CSF ISOFOCUSBING of the wash solution through the template hole (Fig. 13). Ensure the solution is evenly spread in the rectangular space under the template.
   - Hold the pipette vertically.
   - Carefully and progressively inject the solution under the template without introducing air bubbles.
6. Close the lid of the migration module.
7. Start immediately the incubation procedure by pressing the "START" key on the keyboard.

GEL WASHING - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 20 °C controlled by Peltier effect, for 5 minutes.
• A beep sounds. The following message is displayed on the screen: "ewish + δ PAP." / "ewish, δ THICK FILTER PAPER" signalling to remove the CSF ISOFOCUSBING wash solution by repipetting the excess of liquid to apply a thick filter paper.

VI. WASH SOLUTION ELIMINATION
1. Open the lid of the migration module.
2. Remove the wash solution.
3. Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig. 13).
4. Carefully and progressively withdraw the wash solution.
5. Grasp the reagent application template by the flap, lift it and remove it. The gel area must be rehydrated.

VII. GEL BLOTTING
1. Apply one thick filter paper on the gel as described in § IV (the smooth side down, on the gel).
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. Close the lid of the migration module.
4. Start the blotting sequence by pressing the "START" key on the keyboard.

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS
• Blotting at 20 °C controlled by Peltier effect, for 3 minutes. The following message is displayed on the screen: "[BLOTTING]".
• A beep sounds. The following message is displayed on the screen: "δ PAP, + δ REHYD 1" / "δ THICK FILTER PAPER, δ REHYDRATING 1" signalling to remove the filter paper to apply the first rehydrating solution.

VIII. GEL REHYDRATION
1. Open the lid of the migration module.
2. Remove the filter paper and leave the gel in place on the plate of the migration module.
3. Set up the reagent application mask R3 for HYDRAGEL 3 CSF ISOFOCUSBING or mask ENZ 4 mL for HYDRAGEL 9 CSF ISOFOCUSBING (Fig. 12).
4. Take rehydrating solution for HYDRAGEL CSF ISOFOCUSBING without trapping any air bubbles in the pipette tip.
5. Apply 4.5 mL for HYDRAGEL 3 CSF ISOFOCUSBING or 7 mL for HYDRAGEL 9 CSF ISOFOCUSBING of the solution through the template hole (Fig. 13). Ensure the solution is evenly spread in the rectangular space under the template.
   - Hold the pipette vertically.
   - Carefully and progressively inject the solution under the template without introducing air bubbles.
6. Close the lid of the migration module.
7. Start the incubation procedure by pressing the "START" key on the keyboard.

GEL REHYDRATION - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 20 °C controlled by Peltier effect, for 5 minutes.
• A beep sounds. The following message is displayed on the screen: "δ REHYD 1 + δ PAP." / "δ THICK FILTER PAPER, δ REHYDRATING 1" signalling to remove the first rehydrating solution by repipetting the excess of liquid to apply a thick filter paper.

IX. REHYDRATING SOLUTION ELIMINATION
1. Open the lid of the migration module.
2. Remove the rehydrating solution as described in § VI.
3. Grasp the reagent application template by the flap, lift it and remove it. The gel area must be rehydrated.

X. GEL BLOTting
1. Apply one thick filter paper on the rehydrated area of the gel as described in § IV (the smooth side down, on the gel).
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. Close the lid of the migration module.
4. Start the blotting sequence by pressing the "START" key on the keyboard.

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS
• Blotting at 20 °C controlled by Peltier effect, for 3 minutes.
• A beep sounds. The following message is displayed on the screen: "δ PAP, + δ REHYD 2" / "δ THICK FILTER PAPER, δ REHYDRATING 2" signalling to remove the filter paper to apply the second rehydrating solution.
XI. GEL REHYDRATION
1. Open the lid of the migration module.
2. Remove the filter paper and leave the gel in place on the plate of the migration module.
3. Set up the reagent application mask R3 for HYDRAGEL 3 CSF ISOFOCUSING or mask ENZ 4 mL for HYDRAGEL 9 CSF ISOFOCUSING (Fig. 12).
4. Take rehydrating solution for HYDRAGEL CSF ISOFOCUSING without trapping any air bubbles in the pipette tip. Apply 4.5 mL for HYDRAGEL 3 CSF ISOFOCUSING or 7 mL for HYDRAGEL 9 CSF ISOFOCUSING of the solution through the template hole (Fig 13). Ensure the solution is evenly spread in the rectangular space under the template.
5. Hold the pipette vertically.
6. Lightly press the tip of the pipette into the hole of the template.
7. Carefully and progressively inject the solution under the template without introducing air bubbles.
8. Close the lid of the migration module.
9. Start the incubation procedure by pressing the “START” key on the keyboard.

GEL REHYDRATION - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 20 °C controlled by Peltier effect, for 5 minutes.
• After incubation time, a beep sounds, the following message is displayed on the screen: "\( \text{REHYD 2 + \& TTF} \) / "\( \text{REHYDRATING 2, \& TTF} \)" signalling to remove the rehydrating solution to apply the visualization solution.

XII. REHYDRATING SOLUTION ELIMINATION
1. Open the lid of the migration module.
2. Remove the rehydrating solution as previously described in § VI.
3. Leave the template in place.

XIII. VISUALIZATION
1. Deliver TTF visualization solution prepared just before use into the space underneath the template : 3 mL for HYDRAGEL 3 CSF ISOFOCUSING or 3.5 mL for HYDRAGEL 9 CSF ISOFOCUSING. Follow the same precautions as previously described.
2. Take TTF visualization solution without trapping any air bubbles in the pipette tip. Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
3. Close the lid of the migration module.
4. Start immediately the incubation procedure by pressing the "START" key on the keyboard.

INCUBATION - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 30 °C controlled by Peltier effect, for 15 minutes.
• A beep sounds. The following message is displayed on the screen: "\( \text{TTF + \& PAP} \) / "\( \text{TTF, \& THICK FILTER PAPER} \)" signalling to remove visualization solution to apply one thick filter paper.

XIV. VISUALIZATION SOLUTION REMOVAL
1. Open the lid of the migration module.
2. Remove the visualization solution as previously described in § VI.
3. Grasp the reagent application template by the flap, lift it and remove it.

XV. BLOTTING OF THE GEL
1. Apply one thick filter paper on the developed area of the gel, as described in § IV (the smooth side down, on the gel).
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. Close the lid of the migration module.
4. Start the blotting sequence by pressing the "START" key on the keyboard.
5. Rinse the template with distilled water and dry it thoroughly with soft absorbent paper. Prior to re-use, ensure the template is completely dry ; remove droplets from the wells by tapping it on soft paper.

NOTE: Alcohol may be used to clean application templates R3 or ENZ 4 mL after visualization step with TTF.

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS
• Blotting at 30 °C controlled by Peltier effect, for 3 minutes.
• A beep sounds. The following message is displayed on the screen: "\( \text{PAP} \) / "\( \text{THICK FILTER PAPER} \)" signalling to remove the filter paper.

XVI. DRYING OF THE GEL
1. Open the lid of the migration module.
2. Remove the filter paper and leave the gel in place.
3. Close the cover of HYDRASYS.
4. Start the drying step by pressing the "START" key on the keyboard. The following message is displayed on the screen: "[DRYING]."

DRYING - DESCRIPTION OF THE AUTOMATED STEPS
• Drying of the gel at 50 °C, for 3 minutes.
• A beep sounds signalling to open the cover.
• Open the cover.
• When the gel is dried, remove it immediately for further processing.

NOTE:
- When the cycle is completed, the temperature of the plate decreases to 20 °C in less than 5 minutes.
- When 20 °C is reached, a new migration run can be started.
- Position the sample applicator and electrode carriers in place.
- Wipe the temperature control plate with a soft wet tissue.
**QUALITY CONTROL**

It is advised to include into each series of analysis a control serum (such as CSF Control, SEBIA, PN 4794).

* US customers : Follow federal, state and local guidelines for quality control.

**INTERPRETATION**

The intrathecal synthesis, within the central nervous system (CNS), is indicated by the presence of Ig G bands in the immunofixation pattern of CSF that are not in the serum pattern from the same patient (Fig. 15). Very faint bands are always present in serum that may or may not be at the same migration level as those observed in the CSF. Such bands should be disregarded for the pattern interpretation. They represent heterogeneity of the serum Ig G's and could be seen only with high resolution and high sensitivity techniques. In theory, a single Ig G band in CSF that is absent in serum is indicative yet chancy sign of intrathecal synthesis. In practice, two or more bands are taken as dependable indication of it. Two or more bands also serve as supportive evidence of multiple sclerosis although four or more Ig G oligoclonal bands generally present. Therefore, some recommend four bands as supportive of MS although this may change as more data is being collected. It should be noted that the number of bands in the oligoclonal patterns does not correlate with the severity and prognosis in confirmed MS cases. For these reasons, the number of bands should not be reported to avoid misinterpretations of the number.

To assure correct comparative interpretation, it is imperative to observe the following:

- The CSF and serum samples must be collected at the same time, from the same patient. Any treatment which might alter the concentration of immunoglobulins must be avoided.
- The concentrations of CSF and serum Ig G must be determined accurately so that after adjustment equal quantities of Ig G in CSF and serum are applied to the gel.
- If the Ig G concentration is unknown, serum should be run at 300 – 400 x dilution and the CSF neat. Then the inequality of Ig G concentrations must be considered for its possible adverse effects on the pattern interpretation.

The detection of intrathecal Ig synthesis by sensitive immunofixation is more specific and more sensitive indicator than the information given by the various ratios calculated from the total concentrations of CSF and serum immunoglobulins, albumin and other proteins.

Confirmation of intrathecal Ig synthesis is important information to suspect inflammatory disease of the CNS. The oligoclonal profile or other indication of the intrathecal synthesis of Ig G an be found in different diseases of the central nervous system, such as:

- 95 % of multiple sclerosis,
- 100 % of untreated neurosyphilis,
- 100 % of subacute sclerosing leucoencephalitis.

Uncommonly, the indication of intrathecal synthesis of Ig G can be found in many diseases of the CNS, usually associated with inflammation, such as infections, peripheral neuropathies, neoplasms, cerebrovascular accidents, etc.

The diagnosis must not be based solely on the immunofixation findings. These findings must be considered together with the clinical observations and history, and complemented by biochemical, microbiological and cytology testing.

**Interference and Limitations**

See SAMPLES FOR ANALYSIS.

The use of antiserum other than that designed for the HYDRAGEL 3 & 9 CSF ISOFOCUSING procedures with the dynamic mask may affect the results. To manufacture its reagents, SEBIA rigorously selects all raw materials. However, according to manufactured gel lots, the electrophoretic pattern may be differently centered without any adverse effects on performance. A distortion of the track, due to a gel swelling by liquid accumulation during the electrophoretic migration, may be observed on the cathodic side of the gel without any adverse effects on performance. This distortion, with variable intensity, appears more particularly in samples with proteins at low concentration migrating in this zone.

**Troubleshooting**

Call SEBIA Technical Service of the supplier when the test fails to perform while the instruction for the preparation and storage of materials, and for the procedure were carefully followed.

Kit reagent Safety Data Sheets and information on cleaning and waste disposal, labeling and safety rules applied by SEBIA, packaging for the transportation of biological samples, and instruments cleaning are available on the "INSTRUCTIONS & SAFETY DATA SHEETS" DVD.
PERFORMANCE DATA

Reproducibility

Within gel reproducibility
CSF and serum sample pairs from two patients (one pair with intrathecal synthesis and one pair without intrathecal synthesis) and the SEBIA CSF Control were each analyzed with the HYDRAGEL CSF ISOFOCUSING procedure on gels from the same lot. Each CSF / serum pair was run 9 times on a single gel and the CSF Control was run in 18 tracks of a single gel.

Gel-to-gel reproducibility
Nine CSF / serum pairs (3 pairs with intrathecal synthesis and 6 pairs without intrathecal synthesis) were analyzed with the HYDRAGEL CSF ISOFOCUSING procedure on 10 gels from the same lot, including one analysis of each CSF / serum pair per gel.

Results:
Upon visual examination, in all reproducibility studies the presence / absence of oligoclonal banding was correctly detected in each sample and on all gels with anti-Ig G – PER antiserum, there were no false positives / negatives and no differences were observed among the repeats.

Accuracy - Detection and Identification of oligoclonal banding
CSF and serum sample pairs from 79 patients, including 44 normal CSF / serum pairs and 35 pathological CSF / serum pairs, were analyzed using the HYDRAGEL CSF ISOFOCUSING procedure in parallel with a commercially available immunofixation electrophoresis procedure intended for the detection of Ig G oligoclonal banding. The electrophoregrams were evaluated visually for the presence of Ig G oligoclonal banding.
This study demonstrated a 100 % agreement between the 2 techniques:
For the 44 normal sample pairs: complete agreement (concordance).
For the 35 pathological sample pairs: complete agreement (concordance).

Sensitivity
The sensitivity of the HYDRAGEL CSF ISOFOCUSING procedure has been determined by serial dilution of a monoclonal Ig G protein, 2 mg/dL. The detection limit of a Ig G monoclonal band was determined 0.031 mg/dL.
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Figure 6

Barrette antisérum

*Antiserum Segment*

Support barrette

*Segment Holder*

Guide du masque dynamique + Demi réducteur de course

*Dynamic Mask Guide + Length-half Reducing Device*
Type 1: Normal pattern
Type 2: Intrathecal Ig G synthesis (ex: Multiple Sclerosis)
Type 3: Intrathecal Ig G synthesis in systemic disease
Type 4: Systemic inflammation (mirror pattern with oligoclonal pattern)
Type 5: Monoclonal gammopathy (mirror pattern with monoclonal bands)