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 (lg'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 antiseraum.

Presence of intrathecal Ig G suggests inflammatory disease of the CNS, 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 resolutive 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

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4353</th>
<th>PN 4355</th>
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<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>
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<tr>
<td>Cathodic solution (ready to use)</td>
<td>1 vial, 50 mL</td>
<td>1 vial, 50 mL</td>
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<tr>
<td>Strips</td>
<td>10 packs de 2</td>
<td>10 packs de 2</td>
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<tr>
<td>Sample Diluent CSF (ready to use)</td>
<td>1 vial, 85 mL</td>
<td>1 vial, 85 mL</td>
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<tr>
<td>Antiserum Diluent ISOFOCUSING (ready to use)</td>
<td>1 vial, 3.75 mL</td>
<td>1 vial, 3.75 mL</td>
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<tr>
<td>Rehydrating Solution CSF (ready to use)</td>
<td>2 vials, 70 mL</td>
<td>2 vials, 70 mL</td>
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<tr>
<td>TTF3 Solvent (ready to use)</td>
<td>2 vials, 20 mL</td>
<td>2 vials, 20 mL</td>
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<tr>
<td>TTF3 (stock solution)</td>
<td>2 vials, 0.5 mL</td>
<td>2 vials, 0.5 mL</td>
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<tr>
<td>Applicators (ready to use)</td>
<td>1 pack of 10 (6 teeth)</td>
<td>1 pack of 10 (18 teeth)</td>
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<tr>
<td>Buffer containers (ready to use)</td>
<td>1 pack of 2</td>
<td>1 pack of 2</td>
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<tr>
<td>Antiserum segments (ready to use)</td>
<td>1 pack of 10</td>
<td>1 pack of 10</td>
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<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>3 packs of 10 each</td>
<td>3 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>
</tr>
</tbody>
</table>

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, 1 g/dL; 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).

2. ETHYLENE GLYCOL SOLUTION

Preparation
The ethylene glycol solution is ready to use.

WARNING: Harmful if swallowed.

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

WARNING: The cathodic solution contains sodium hydroxide. Irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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.

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

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.
Discard strips if the package is opened and the strips dry out.

5. SAMPLE DILUENT CSF

Preparation
Sample diluent is ready to use. It contains saline solution supplemented with bovine serum albumin, sodium azide and 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 °C). It is stable until the expiration date indicated on the kit package or diluent vial label. Diluent must be free of precipitate.
6. ANTISERUM DILUENT CSF ISOFOCUSING

Preparation
Antiserum diluent is ready to use. It contains 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 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.

8. TTF3 SOLVENT

Preparation
The TTF3 solvent is ready to use. It contains components, nonhazardous at concentrations used, necessary for optimum performance.

Use
For the preparation of TTF3 visualization solution as described in No. 9.

Storage, stability and signs of deterioration
The TTF3 solvent can be stored at room temperature or refrigerated and is stable until the expiration date indicated on the kit package or TTF3 solvent vial label. TTF3 solvent must be free of precipitate.

9. TTF3

Preparation
Just before use, prepare the working developing solution. Add the reagents in the following order: 4 mL of TTF3 solvent, 100 µL of TTF3 and 4 µL hydrogen peroxide (H₂O₂) 30 %.

WARNING: TTF3 contains dimethylformamide. Harmful by inhalation! In case of insufficient ventilation, wear suitable respiratory equipment. Do not ingest! If ingested, consult physician immediately! Harmful in contact with skin. Wear suitable protective clothing. Irritating to the eyes. After contact with eyes or skin, rinse immediately with plenty of water and seek medical advice. Avoid exposure, obtain special instruction before use. May cause cancer. If you feel unwell, seek medical advice immediately (show label where possible).

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

Storage, stability and signs of deterioration
Store the stock TTF3 at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or vial label. TTF3 solution must be free of precipitation.

10. 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.

11. 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.

12. 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.

13. 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.
14. THICK FILTER PAPERS

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

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

15. PLASTIC MASKS

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

EQUIPMENT AND ACCESSORIES REQUIRED

1. HYDRASYS System SEBIA, PN 1211 with OPTION FOCUSING HYDRASYS SEBIA, PN 1235.
2. HYDRASYS FOCUSING System SEBIA, PN 1212.
3. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216 or HYDRAPLUS 2 SEBIA, PN 1217, for an alternative way of loading the sample applicators or antisera segments.
4. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.
5. Template Guide Bar SEBIA supplied with HYDRASYS.
6. Dynamic mask, SEBIA, PN 1255.
7. Accessory Kit for HYDRASYS CSF ISOFOCUSING SEBIA, PN 1258. It contains: the reagent application masks R3, ENZ 4 mL and the half length reducing device.
8. Container Kit supplied with HYDRASYS.
9. 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).

REAGENTS REQUIRED BUT NOT SUPPLIED

1. ANTISERUM ANTI-Ig G - PER
The antiserum vial (SEBIA PN 4743: 1 vial, 0.60 mL) contains a stock solution of a mammalian anti-human immunoglobulin antisera with specificity for Ig G, conjugated to peroxidase. For an easy identification and as an aid in monitoring its application, the antiserum is colored with a non-hazardous dye that matches the color of the vial label.
Prepare working solution just before use:
For HYDRAGEL 3 CSF ISOFOCUSING: 25 µL anti–Ig G - PER and 175 µL antiserum diluent.
For HYDRAGEL 9 CSF ISOFOCUSING: 40 µL anti–Ig G - PER and 300 µL antiserum diluent.
Mix well.
Use
For immunofixation and visualization of the isoelectrofocused Ig G’s.

Storage, stability and signs of deterioration
Store the antiserum refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the antiserum vial label.
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 antisera 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: H₂O₂, 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: citric acid, 50 mg/dL.
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.
Working destaining solution added with ProClin 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: alkaline buffer pH 8.8 ± 0.3 ; sodium azide.

WARNING: The stock wash solution contains 0.625 % sodium azide. Do not ingest! If ingested, consult physician immediately! Sodium azide may lead to formation of explosive or toxic compounds when in contact with acids, lead or copper. Always flush with a large quantity of water when disposing.

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.

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

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: pre-migration, sample application, isoelectric focusing migration, incubation with enzyme-labelled antisera, incubation with enzyme substrate, stopping the enzymatic reaction, blotting and final drying of the gel. The manual steps include handling samples and gels, application of reagents and setting up the instrument for operation.

READ CAREFULLY HYDRASYS INSTRUCTION MANUAL.

IMPORTANT: Verify the dynamic mask has been properly positioned for perfect alignment between electrophoretic profiles and wells of the mask.

I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. In order to obtain the high voltage required for the isoelectricfocusing, press the green high voltage switch to high voltage mode; then, the switch becomes red.

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.

<table>
<thead>
<tr>
<th>PATIENT No.</th>
<th>WELL No. (applicator 6 teeth)</th>
<th>WELL No. (applicator 18 teeth)</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
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<td>9</td>
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<td>17</td>
</tr>
</tbody>
</table>

- 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!

6. Select "3/9 CSF FOCUSING" migration program from the instrument menu (left side of the keyboard).

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.

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

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.
   - 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 green arrow "START" key on the left side of the keyboard.

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

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

- The electrode carrier 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 flashing message is displayed on the screen: "POS : 1" 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 green arrow "START" key on the left side of 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 700 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 flashing message is displayed on the screen: "§ AS" signalling to apply antisera.

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, a dynamic mask guide and a length-half reducing device (Fig. 6).

1. Place the dynamic mask guide on a flat surface.
2. For HYDRAGEL 3 & 9 CSF ISOFOCUSING, position a length-half reducing device on the far end of dynamic mask guide.
3. 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.

4. Set up the holder with the segment on the dynamic mask guide with length-half reducing device already in place (Fig. 8).

Apply reagents as follows:
- For HYDRAGEL 3 CSF ISOFOCUSING, apply 20 µL/well of antiserum anti-Ig G - PER in wells 4 to 12.
- For HYDRAGEL 9 CSF ISOFOCUSING, apply 20 µL/well of antiserum anti-Ig G - PER in each of the 15 wells.

Aspirate reagents avoiding any air bubbles in the pipette tip.

5. Apply the reagents (Fig. 9):
   - Hold pipette at an angle and rest its tip lightly at the side of the well, without touching the bottom of the well.
   - Inject the volume of reagent into the well.

III. IMMUNOFIXATION

1. After the migration, open the lid (the message stops flashing).
2. Remove the sample applicator and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
4. Remove both carriers.
5. Clean the electrodes by wiping them carefully with a soft wet tissue.
6. Leave the gel in place in the migration module and remove the plastic mask by a corner and discard.

WARNING: Don’t move the gel on the printed frame during this step.

7. Set up the dynamic mask for antiserum application onto the gel as follows (Fig. 10):
   - 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.
   - Hold the segment holder by the handle situated on its right and press on the central pressure point such that the antiserum segment contacts the gel.
   - Release the pressure; then, reagent will spread under the entire segment (Fig. 11).
   - Immediately, using the segment holder handle, move the segment slowly but steadily up and down the entire length of the gel to apply the reagent. Repeat this step twice; application should take approximately 5 seconds for each passage (Fig. 12).

8. Leave the dynamic mask in the HYDRASYS chamber with the antiserum segment at the lowest point on the mask guide.
9. Close the lid of the migration module.
10. Start immediately the incubation procedure by pressing the green arrow “START” key on the left side of the keyboard.

The following message is displayed on the screen: "[INCUBATION]."

IMMUNOFIXATION - DESCRIPTION OF THE AUTOMATED STEPS

- Incubation at 20 °C controlled by Peltier effect, for 10 minutes.
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: "§ PAP."

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 have 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 (green arrow on the left side of the keyboard).

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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 flashing message is displayed on the screen: " PAP. + REHYD 1" signalling to remove the filter paper to apply the first rehydrating solution.

V. 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. 13).
4. Take rehydrating solution for HYDRAGEL CSF FOCUSING without trapping any air bubbles in the pipette tip.
5. 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 14). Ensure the solution is evenly spread in the rectangular space under the template.
   • Hold the pipette vertically.
   • Lightly press the tip of the pipette into the hole of the template.
   • 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 green arrow “START” key on the left side of 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 flashing message is displayed on the screen: " REHYD 1 + PAP." signalling to remove the first rehydrating solution by repipetting the excess of liquid to apply a thick filter paper.

VI. REHYDRATING SOLUTION ELIMINATION
1. Open the lid of the migration module.
2. Remove the rehydrating solution.
3. Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig 14).
4. Carefully and progressively withdraw the reagent.
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 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 (green arrow on the left side of the keyboard).

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS
• Blotting at 20 °C controlled by Peltier effect, for 3 minutes.
  • A beep sounds. The following flashing message is displayed on the screen: " PAP. + REHYD 2" signalling to remove the filter paper to apply the second 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 ISOFOCUSING or mask ENZ 4 mL for HYDRAGEL 9 CSF ISOFOCUSING (Fig. 13).
4. Take rehydrating solution for HYDRAGEL CSF FOCUSING 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 14). Ensure the solution is evenly spread in the rectangular space under the template.
   • Hold the pipette vertically.
   • Lightly press the tip of the pipette into the hole of the template.
   • Carefully and progressively inject the solution under the template without introducing air bubbles.
5. Close the lid of the migration module.
9. Start immediately the incubation procedure by pressing the green arrow "START" key on the left side of 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 flashing message is displayed on the screen: " REHYD 2 + TTF3" signalling to remove the rehydrating solution to apply the visualization solution.

IX. 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.

X. VISUALIZATION
1. Deliver TTF3 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 TTF3 visualization solution without trapping any air bubbles in the pipette tip.
3. Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
4. Close the lid of the migration module.
4. Start immediately the incubation procedure by pressing the green arrow "START" key on the left side of the keyboard.
INCUBATION - DESCRIPTION OF THE AUTOMATED STEPS

- Incubation at 30 °C controlled by Peltier effect, for 15 minutes.
- A beep sounds. The following flashing message is displayed on the screen: " TTF3 / PAP" signalling to remove visualization solution to apply one thick filter paper.

XI. 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.

XII. BLOTTING OF THE GEL

1. Open the lid of the migration module.
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. Rinse the template with distilled water and dry it thoroughly with soft absorbent paper. Prior to re-use, ensure the template is completely dry.

NOTE: Alcohol may be used to clean application templates R3 or ENZ 4 mL after visualization step with TTF3.

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS

- Blotting at 30 °C controlled by Peltier effect, for 3 minutes.
- A beep sounds. The following flashing message is displayed on the screen: " PAP" signalling to remove the filter paper.

XIII. 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 (green arrow on the left side of the keyboard).

NOTE: 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.

XIV. WASH AND FINAL PROCESSING OF THE GEL

After drying, the gel is washed in staining compartment using the "WASH ISOENZ/GEL" program. If the chamber has been previously used to stain protein gel, clean the chamber with the "WASH CHAMBER" program.

1. Open the gel holder. Lay it flat and position the gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder (Fig. 15).
2. Place the gel holder into the gel processing / staining module.

IMPORTANT: Before starting the gel processing / staining program, check the following:
- the destaining solution container contains at least 400 mL of destaining solution;
- the waste container is empty.

3. For reagent line connection: Refer to the information displayed on the screen of the instrument (select key: REAGENT LINES).

IMPORTANT: Do not forget to block up the unused lines.
4. Select "WASH ISOENZ/GEL" washing program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

During washing and drying steps, the compartment remains locked.
After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).
5. Remove the gel holder from the compartment; open the clips and remove the dried gel. If needed, clean the backside (the plastic support side) of the dry film with a wet tissue paper. The gel is ready for evaluation.

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. 16). 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 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. Due to the resolution and sensitivity limits of electrophoresis, it is possible that some oligoclonal components may not be detected with this method.

Troubleshooting
Call 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 waste product elimination are also available from the Technical Service of the supplier.

PERFORMANCE DATA

Reproducibility
Within gel reproducibility
CSF and serum samples from four patients were each applied in all 18 tracks of HYDRAGEL 9 CSF ISOFOCUSING gels from two lots (two pairs with intrathecal synthesis and two pairs without intrathecal synthesis).

Gel-to-gel and lot-to-lot reproducibility
Eight CSF – serum pairs were applied on each of the ten gels HYDRAGEL 9 CSF ISOFOCUSING from the same lot and on two gels from another lot (six pairs with intrathecal synthesis and two pairs without intrathecal synthesis).

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 samples from patients with various diseases of the central nervous system (n = 108) were analyzed using the standard HYDRAGEL 9 CSF ISOFOCUSING kit, materials and procedure in parallel with a commercially available immunofixation electrophoresis system intended for the detection of Ig G oligoclonal banding. The electrophoregrams were evaluated visually for the presence of Ig G oligoclonal banding. There was a general agreement in the detection of oligoclonal banding between the two tests. The few differences observed were due to a greater resolution of the HYDRAGEL 9 CSF ISOFOCUSING procedure. The results were consistent with clinical diagnosis, indication of intrathecal synthesis and blood-brain barrier damage.

Sensitivity
The sensitivity of the HYDRAGEL 9 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.

BIBLIOGRAPHY


**Figure 6**

*Barrette antisérum*
*Antiserum Segment*

*Support barrette*
*Segment Holder*

*Demi réducteur de course*
*IEF Length Reducing Device*

*Guide du masque dynamique*
*Dynamic Mask Guide*
PROFILS ÉLECTROPHORÉTIQUES / MIGRATION PATTERNS

1. CSF: Polyclonal, Serum: Polyclonal, Same

2. CSF: Oligoclonal, Serum: Polyclonal, Different

3. CSF: Oligoclonal, Serum: Oligoclonal, Different

4. CSF: Oligoclonal, Serum: Oligoclonal, Same

5. CSF: Monoclonal, Serum: Monoclonal, Same

Type 1: Profil normal
Type 2: Synthèse intrathécale d’Ig G (ex: Sclérose en Plaques)
Type 3: Synthèse intrathécale d’Ig G dans les maladies systémiques
Type 4: Inflammation systémique (profil en miroir avec bandes oligoclonales)
Type 5: Gammapathie monoclonale (profil en miroir avec bandes monoclonales)

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)