HYDRAGEL 3 CSF
Ref. 4850

HYDRAGEL 6 CSF
Ref. 4851

Masque standard / Standard mask
INTENDED USE

The HYDRAGEL 3 CSF and HYDRAGEL 6 CSF kits are designed for the qualitative detection and identification of « oligoclonal » bands in the electrophoretic patterns of cerebrospinal fluid (CSF). The procedure visually compares immunofixation patterns of immunoglobulins G, A and M, and/or immunoglobulins with bound Kappa or Lambda light chains, in CSF and serum from the same patient. The analysis is typically performed on unconcentrated CSF.

Depending on the selection of detecting antisera, one to three CSF – serum sample pairs can be run on each HYDRAGEL 3 CSF gel and two to six CSF - serum sample pairs on each HYDRAGEL 6 CSF gel.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST, 11, 14, 17

Many disorders of the central nervous system are associated with increased concentration of CSF proteins either due to increase in the permeability of blood-CSF barrier or to synthesis of immunoglobulins, primarily Ig G, within the central nervous system. The latter case, the intrathecal synthesis of Ig's, is often associated with Ig heterogeneity which manifests itself as «oligoclonal banding» seen in high resolution electrophoretic migration patterns. The bands in the gamma globulin zone are not always the true oligoclonal bands, i.e., Ig's G, A or M and therefore do not have the same diagnostic significance of the oligoclonal Ig bands. Immunofixation is a choice technique since it can prove the Ig character of the oligoclonal bands and can identify the Ig involved. To confirm intrathecal Ig synthesis, patient serum and CSF must be analyzed in parallel to demonstrate differences in Ig's distribution patterns between CSF and serum. Confirmation of intrathecal Ig synthesis is an important information to suspect inflammatory disease of the central nervous system, such as caused by multiple sclerosis.

A standard immunofixation that uses unlabeled antisera needs a concentration of about 0.05 g/dL of Ig G. Since the Ig G concentration in CSF is generally between 1 and 5 mg/dL, a total volume from 2 to 5 mL of CSF is required to obtain enough of concentrate. Compared to standard immunofixation, about a 100 times increase in the sensitivity of detection is achieved with the HYDRAGEL 3 CSF or HYDRAGEL 6 CSF which use enzyme labeled antibodies. Then, with the concentration at or above the concentration limit of only of 0.5 mg/dl of the Ig of interest, the Ig can be detected and its distribution discerned without concentrating the CSF sample.

The assay is carried out in two stages:
• high resolution electrophoresis on agarose gel to fractionate the proteins in the CSF and serum samples,
• immunofixation with enzyme labeled antisera against Ig G, Ig A, Ig M and/or immunoglobulins with bound Kappa or Lambda chains to detect and identify the oligoclonal bands in CSF and to demonstrate the difference, or lack of, in the distribution of Ig's in the CSF and serum.

The semi-automated HYDRASYS system performs all the steps needed to obtain gels ready for interpretation.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 3 CSF AND HYDRAGEL 6 CSF KITS

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4850</th>
<th>PN 4851</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>Buffered Strips (ready to use)</td>
<td>10 packs of 2 each</td>
<td>10 packs of 2 each</td>
</tr>
<tr>
<td>Sample Diluent CSF (ready to use)</td>
<td>1 vial, 85 mL</td>
<td>1 vial, 85 mL</td>
</tr>
<tr>
<td>Antiserum Diluent CSF (ready to use)</td>
<td>1 vial, 6 mL</td>
<td>1 vial, 6 mL</td>
</tr>
<tr>
<td>Rehydrating Solution CSF</td>
<td>1 vial, 70 mL</td>
<td>2 vials, 70 mL</td>
</tr>
<tr>
<td>TTF3 Solvent (ready to use)</td>
<td>1 vial, 20 mL</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>TTF3 (stock solution)</td>
<td>1 vial, 0.5 mL</td>
<td>1 vial, 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 (15 teeth)</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>Filter Paper Combs</td>
<td>1 pack of 10 (6 teeth)</td>
<td>1 pack of 10 (12 teeth)</td>
</tr>
</tbody>
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FOR OPTIMAL RESULTS
All 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, 0.8 g/dL ; Tris-barbital buffer pH 8.8 ± 0.1 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: Agarose gels contain 0.31 % barbital and 0.34 % sodium barbital. Do not ingest ! If ingested, consult physician immediately !

Use
Support medium for protein electrophoresis and immunofixation.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). (The arrow on the front of the kit box must be pointing upwards). DO NOT FREEZE. Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.

They are stable until the expiration date indicated on the kit package and the gel package labels. 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, or (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. BUFFERED STRIPS

Preparation
Buffered sponge strips are ready to use. Each contains: Tris-barbital buffer pH 9.0 ± 0.3; sodium azide; additives, nonhazardous at concentrations used, necessary for optimum performance.

WARNING: The buffer in the strips contains 1.54% barbital, 1.71% sodium barbital and 0.50% sodium azide. Harmful if swallowed! Do not ingest! If ingested, consult physician immediately! When disposing, prevent contact with acids, lead or copper, as these are known to form explosive or toxic compounds with sodium azide. After contact with skin, wash immediately with plenty of water.

Use
Buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. (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 buffered strips package label.
DO NOT FREEZE.
Discard buffered strips if the package is opened and the strips dry out.

3. 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.

4. ANTISERUM DILUENT CSF

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

Use
For diluting antisera dilution 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.

5. REHYDRATING SOLUTION

Preparation
The rehydrating solution is ready to use. It contains aqueous solution, pH 6.0, and additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
To rehydrate the agarose gel before and after 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.

6. TTF3 SOLVENT

Preparation
The TTF3 solvent is ready to use. It contains acidic solution, pH 2.5, and additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
For the preparation of TTF3 visualization solution, as described in No. 7.

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.

7. TTF3

Preparation
Just before use, prepare the working developing solution. Add the reagents in the following order: 2 mL of TTF3 solvent; 50 µL TTF3 and 2 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 immunofixed immunoglobulins.

Storage, stability and signs of deterioration
Store 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.
8. APPLICATORS
Use
Precut, single use applicators for sample application onto gel.

Storage
Store the applicators in a dry place at room temperature or refrigerated.

9. 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.

10. 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.

11. FILTER PAPER COMBS
Use
Precut, single use, thick absorbent paper combs for blotting excess of antisera off the gel surface after immunofixation step.

EQUIPMENT AND ACCESSORIES REQUIRED
1. HYDRASYS System SEBIA, PN 1210 or PN 1211.
2. Micropipettor, either manual or automated, such as HYDRAplus SEBIA, PN 1215, for an alternative way of loading the sample applicators.
3. Dry Storage Chamber for samples focusing, SEBIA, PN 1271.
4. Template Guide Bar SEBIA supplied with HYDRASYS.
5. Accessory Kit for HYDRASYS CSF SEBIA, PN 1262.
6. Pipettes: 2 µL, 20 µL, 100 µL, 200 µL and 5 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 antiserum, conjugated to peroxidase, with specificity for Ig G. For an easy identification and as an aid in monitoring its application, the immunoglobulins are colored with a non-hazardous dye that matches the color of the vial label.

Prepare working solution (50 µL per detection) just before use. Dilute the antiserum 11 times with antiserum diluent (1 vol./10 vol.), e.g., 10 µL anti-Ig G-PER and 100 µL antiserum diluent. Mix well.

Use
For immunofixation and visualization of the electrophoresed proteins.

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.

2. ANTISERUM ANTI-Ig A-PER
The antiserum vial (SEBIA PN 4742: 1 vial, 0.60 mL) contains a stock solution of a mammalian anti-human immunoglobulin antiserum, conjugated to peroxidase, with specificity for Ig A. For an easy identification and as an aid in monitoring its application, the immunoglobulins are colored with a non-hazardous dye that matches the color of the vial label.

Prepare working solution (50 µL per detection) just before use. Dilute the antiserum 11 times with antiserum diluent (1 vol./10 vol.), e.g., 10 µL anti-Ig A-PER and 100 µL antiserum diluent. Mix well.

Use
For immunofixation and visualization of the electrophoresed proteins.

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.

3. ANTISERUM ANTI-Ig M-PER
The antiserum vial (SEBIA PN 4744: 1 vial, 0.60 mL) contains a stock solution of a mammalian anti-human immunoglobulin antiserum, conjugated to peroxidase, with specificity for Ig M. For an easy identification and as an aid in monitoring its application, the immunoglobulins are colored with a non-hazardous dye that matches the color of the vial label.

Prepare working solution (50 µL per detection) just before use. Dilute the antiserum 11 times with antiserum diluent (1 vol./10 vol.), e.g., 10 µL anti-Ig M-PER and 100 µL antiserum diluent. Mix well.

Use
For immunofixation and visualization of the electrophoresed proteins.

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.

4. ANTI-KAPPA / LAMBDA – PER ANTISERA PACK
The ANTI-KAPPA / LAMBDA – PER antisera pack, SEBIA, PN 4745 contains 1 vial anti-Kappa – PER antiserum (0.60 mL) and 1 vial anti-Lambda – PER antiserum (0.60 mL).
4.1 ANTISERUM ANTI-KAPPA – PER
The antiserum vial contains a stock solution of a mammalian anti-human immunoglobulin antiserum, conjugated to peroxidase, with specificity for bound Kappa light chains. For an easy identification and as an aid in monitoring its application, the immunoglobulins are colored with a non-hazardous dye that matches the color of the vial label. Prepare working solution (50 µL per detection) just before use. Dilute the antiserum 11 times with antiserum diluent (1 vol./10 vol.), e.g., 10 µL anti-Kappa – PER and 100 µL antiserum diluent. Mix well.

Use
For immunofixation and visualization of the electrophoresed proteins.

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.

4.2 ANTISERUM ANTI-LAMBDA – PER
The antiserum vial contains a stock solution of a mammalian anti-human immunoglobulin antiserum, conjugated to peroxidase, with specificity for bound Lambda light chains. For an easy identification and as an aid in monitoring its application, the immunoglobulins are colored with a non-hazardous dye that matches the color of the vial label. Prepare working solution (50 µL per detection) just before use. Dilute the antiserum 11 times with antiserum diluent (1 vol./10 vol.), e.g., 10 µL anti-Lambda – PER and 100 µL antiserum diluent. Mix well.

Use
For immunofixation and visualization of the electrophoresed proteins.

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 antiserum vials, even with the same specificity, and ALWAYS change the tip of the pipette when changing antiserum vials.

During transportation, the antisera can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

5. HYDROGEN PEROXIDE: \( \text{H}_2\text{O}_2 \), 30 %.

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 concentrations using appropriate procedures.
• The concentration of any particular Ig in the CSF and serum samples to be compared must always be adjusted to the same level in both samples. The adjustment depends on the original CSF’s Ig concentration ; use Sample Diluent for all dilutions:
  1st case: The concentration of the Ig of interest is over 1 mg/dL.
  Dilute CSF and serum with the sample diluent to obtain an Ig concentration of 1 mg/dL for Ig of interest.
  2nd case: The CSF concentration of the Ig of interest is between 0.5 and 1 mg/dL.
  Use neat CSF. Dilute serum to obtain the same concentration of Ig as is in the CSF sample.
  3rd case: The CSF concentration of the Ig of interest is below 0.5 mg/dL.
  Concentrate CSF with any appropriate device to obtain a concentration of Ig of interest between 0.5 and 1 mg/dL. Dilute serum to obtain the same Ig concentration as is in the CSF sample.

Example of dilutions (only for samples with concentration of the Ig of interest over 1.0 mg/dL):

For CSF:
A (mg/dL) = concentration of Ig of interest over 1.0 mg/dL.
Collect x µL CSF and add \( x \times (A -1) \) sample diluent (suggested value for x = 10 µL).
If A is below 1.0 mg/dL, use neat CSF.

For serum:
B (mg/dL) = concentration of Ig of interest.
 - Dilute serum 20 times with sample diluent, e.g., 5 µL serum and 95 µL sample diluent.
 - Collect y µL diluted serum and add \( y \times (B/20 -1) \) sample diluent (suggested value for y = 2 µL).

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, incubation with 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.

I. MIGRATION SET UP
1. Switch on HYDRASYS instrument.
2. Place one applicator 6 teeth for HYDRAGEL 3 CSF or one applicator 15 teeth for HYDRAGEL 6 CSF on a flat surface with the well numbers in the right-side-up position.
   - Apply 15 µL sample in each well (Fig. 1). Load the applicator within 2 minutes. The following example shows analysis of three or six CSF/serum pairs on one gel with one kind of antibody (e.g., anti-Ig G); other desired combination can be similarly performed, e.g., two CSF/serum pairs can be analyzed for Ig G, Ig A and Ig M.
- Apply 15 µL sample diluent into wells No. 1, 8 and 15 of the applicator 15 teeth.
- Grasp the applicator by the plastic tooth protection frame and place it with the teeth up into the dry storage chamber, previously reactivated.

*See dry storage chamber package insert for further details.*

- Close the cover of the dry storage chamber.
- Let the applicator in the dry storage chamber at room temperature for 15 minutes. The sample proteins will then focus into the tip of the applicator teeth and concentrate by partial evaporation.

**IMPORTANT:** When 15 minutes have passed, proceed immediately to step No. 8 - samples application onto gel.

While the samples are being focused/concentrated, prepare the migration.

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

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

4. Select «3 CSF» migration program for HYDRAGEL 3 CSF or «6 CSF» migration program for HYDRAGEL 6 CSF from the instrument menu (left side of the keyboard).

5. Remove buffered strips from the package; handle them by the plastic ends. 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).

6. Unpack the HYDRAGEL agarose gel plate.

- Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately.

**WARNING:** *Do not leave the filter paper for a too long contact with the gel to avoid its dehydration.*

- Pool 120 µL distilled water for HYDRAGEL 3 CSF, or 200 µL for HYDRAGEL 6 CSF, on the lower third of the frame printed on the Temperature Control Plate of the migration module.
- 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 down onto the water pool (Fig. 3). Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame.

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

8. After the samples have been focused (concentrated) on the applicator:

- Snap off the applicator teeth's protection frame.
- Place the applicator into position No. 8 on the carrier.

**IMPORTANT:** The numbers printed on the applicator must face the operator (Fig. 4).

9. Close the lid of the migration module.

10. 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.

**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 under 10 W constant for HYDRAGEL 3 CSF, or 20 W constant for HYDRAGEL 6 CSF, at 20 °C controlled by Peltier effect until 80 Vh accumulated (for about 17 minutes).
- 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 APPLICATION < UPPORTED SIGNALING TO APPLY ANTI- SERA.**

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

**II. 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.
   - Remove both carriers.
   - Clean the electrodes by wiping them carefully with a soft wet tissue.
   - Leave the gel in place in the migration module.
4. Set up the reagent application template 3 CSF or 6 CSF as follows (Fig. 5):
   - Position the application template guide on the anchoring clips (the guide can be left on the HYDRASYS at all times).
   - Hold the flap on the template and put the notches in the guide marks.
   - Lower the template onto the gel.
5. For HYDRAGEL 3 CSF, apply 40 µL of diluted antiserum into each trough of the reagent application template 3 CSF and for HYDRAGEL 6 CSF, apply 50 µL of diluted antiserum into each trough of the reagent application template 6 CSF. Use antisera with the specificity for the immunoglobulins of interest and apply the same antiserum to corresponding CSF and serum samples.
   - Take reagents without trapping any air bubbles in the pipette tip.
   - To apply the reagent (Fig. 6):
     - Hold pipette vertically and rest its tip lightly at the bottom of the well.
     - Carefully and progressively, inject reagent so it spreads through the trough without trapping any bubbles.
6. Close the HYDRASYS cover.
7. 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.
• A beep sounds. The following flashing message is displayed on the screen: «REMOVE <ophil> AS» signaling to remove antisera.

III. ANTISERA REMOVAL
1. Open the lid of the migration module.
2. Remove the excess of reagents with the filter paper (Fig. 7).
   - Insert each comb at a 30° angle into the slots at the lower end of the template troughs so that the teeth touch the vertical side away from the operator.
   - Allow the teeth to contact delicately the liquid by tilting each comb to a 45° angle enabling the teeth to wick off the liquid.
   **IMPORTANT:** Each comb must stay inclined (45 °). If it is straightened up, it could damage the gel.
3. Start the run by pressing the «START» key (green arrow on the left side of the keyboard). The flashing message stops.
4. Remove the remaining antiserum solution during the 15 seconds countdown.

ANTISERA REMOVAL - DESCRIPTION OF THE AUTOMATED STEPS
• The reagents are allowed to wick off the troughs for 15 seconds at 20 °C (controlled by Peltier effect).
• An audible beep sounds. The following message is displayed on the screen: «phil PAPER» signaling to apply a blotting paper.

IV. GEL BLOTTING
1. Remove the filter paper comb.
2. Check that the reagents are well absorbed as indicated by:
   - the absence of reagents on the gel.
   - the full lengths of the teeth are stained.
   If the reagent absorption is incomplete, insert the same filter paper comb again (in the same position) and repeat manually the removal procedure.
3. Grasp the reagent application template by the flap, lift it and remove it.
4. Apply one thick filter paper 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.
   - Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
5. Start the blotting sequence by pressing the «START» key (green arrow on the left side of the keyboard).
6. Clean the reagent application template CSF under water with a small brush (e.g., toothbrush). Ensure the template is completely dry before re-use; remove water droplets from the wells by tapping it on soft paper. DO NOT USE ALCOHOL OR OTHER SOLVENT TO CLEAN REAGENT APPLICATION TEMPLATE CSF.

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: «phil PAPER, phil REHYD 1» signaling to apply a blotting paper.

V. GEL REHYDRATION
1. Leave the gel in place on the plate of the migration module.
2. Set up the reagent application template R1 for HYDRAGEL 3 CSF or 2 for HYDRAGEL 6 CSF (Fig. 8).
3. Apply 2 mL of rehydrating solution for HYDRAGEL 3 CSF or 4 mL for HYDRAGEL 6 CSF through the template hole into the space underneath (Fig. 9). Ensure that solution under the template is uniformly spread in the rectangular surface, centered on the hole of the template.
   Take rehydrating solution without trapping any air bubbles in the pipette tip.
   - Hold the pipette vertically.
   - Lightly press the tip of the pipette into the hole of the template.
   - Carefully and progressively inject the solution without introducing air bubbles under the template.
4. 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: «phil REHYD 1 / phil PAP» signaling to remove the rehydrating solution and apply a thick filter paper.

VI. REHYDRATING SOLUTION ELIMINATION
1. Remove the rehydrating solution:
   - Hold the pipette vertically and lightly press the tip of the pipette into the well (Fig. 9).
   - Carefully and progressively withdraw the reagent.
2. 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.
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. 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» signaling to remove the filter paper and apply the rehydrating solution.

VIII. GEL REHYDRATION
1. Leave the gel in place on the plate of the migration module.
2. Set up the reagent application template R1 for HYDRAGEL 3 CSF or 2 for HYDRAGEL 6 CSF (Fig. 8).
3. Apply 2 mL of rehydrating solution for HYDRAGEL 3 CSF or 4 mL for HYDRAGEL 6 CSF into the space underneath the template (Fig. 9).
   - Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
4. Take rehydrating solution without trapping any air bubbles in the pipette tip.
5. 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 30 minutes.
• After incubation time, a beep sounds and the temperature of the plate increases from 20 to 30 °C.
• The following flashing message is displayed on the screen: «❉ REHYD 2 / ❉ TTF» signaling to remove the rehydrating solution and apply the visualization solution.

IX. REHYDRATING SOLUTION ELIMINATION
1. Remove the rehydrating solution as previously described in § VI.
2. Leave the template in place.

X. VISUALIZATION
1. Apply 1 mL of TTF3 visualization solution for HYDRAGEL 3 CSF or 2 mL for HYDRAGEL 6 CSF, prepared just before use, into the space underneath the template.
2. Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
   - Take TTF3 visualization solution without trapping any air bubbles in the pipette tip.
3. 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: «❉ TTF / ❉ PAPER» signaling to remove visualization solution and apply one thick filter paper.

XI. VISUALIZATION SOLUTION REMOVAL
1. Remove the visualization solution as previously described in § VI.
2. Grasp the reagent application template by the flap, lift it and remove it.

XII. BLOTTING OF THE GEL
1. Apply one thick filter paper on the revealed area of the gel, as described in § IV.
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. Start the blotting sequence by pressing the «START» key (green arrow on the left side of the keyboard).
4. Rinse the template with distilled water or alcohol 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 R1 or 2 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 / ❉ REHYD 3» signaling to remove the filter paper and apply the rehydrating solution.

XIII. GEL REHYDRATION
1. Leave the gel in place on the plate of the migration module.
2. Set up the reagent application template R1 for HYDRAGEL 3 CSF or 2 for HYDRAGEL 6 CSF (Fig. 8).
3. Apply 2 mL of rehydrating solution for HYDRAGEL 3 CSF or 4 mL for HYDRAGEL 6 CSF into the space underneath the template (Fig. 9).
   - Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
4. Start immediately the incubation procedure by pressing the green arrow «START» key on the left side of the keyboard.

GEL REHYDRATATION - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 30 °C controlled by Peltier effect, for 5 minutes.
• The following flashing message is displayed on the screen: «❉ REHYD 3 / ❉ PAP» signaling to remove the rehydrating solution and apply one thick filter paper.

XIV. REHYDRATING SOLUTION ELIMINATION
1. Remove the rehydrating solution as previously described in § VI.
2. Grasp the reagent application template by the flap, lift it and remove it.

XV. BLOTTING AND DRYING OF THE GEL
1. Apply one thick filter paper on the gel.
2. Press on the whole surface of the filter paper to ensure perfect adherence to the gel.
3. 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 30 °C controlled by Peltier effect, for 3 minutes.
• A beep sounds. The following flashing message is displayed on the screen: « PAPER » signaling to remove the filter paper.

XVI. DRYING OF THE GEL
1. Remove the filter paper and leave the gel in place.
2. Close the cover of HYDRASYS.
3. Start the drying step by pressing the « START » key (green arrow on the left side of 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 signaling to open the cover.
4. Open the cover.
5. Remove the dried gel.
6. If needed, take off any lint from the gel side with soft paper. Clean the back side (the plastic support side) of the dried film with a damp soft paper.
   - 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.

INTERPRETATION 1, 2, 11, 16

The intrathecal synthesis, within the central nervous system, is indicated by the following comparative observations:
• Different immunofixation pattern of CSF compared to serum from the same patient.
• Oligoclonal bands are present in most but not all cases.
• A stronger band, an additional band, even a single faint immunofixation band observed in the CSF pattern, but not in the serum pattern, are all indicative of intrathecal Ig synthesis.
• Infrequently, no oligoclonal or monoclonal Ig bands are observed yet the clinical observations suggest multiple sclerosis. In such cases, the polyclonal type of immunoglobulin band in the CSF pattern might be more cathodic than in the serum pattern.
• In most cases, the intrathecal immunoglobulins are of the Ig G class; very infrequently, they are Ig A or Ig M.
• Intrathecal synthesis of Ig A is usually manifested as a more cathodic migration of CSF’s Ig A compared to serum’s Ig A.

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 of the samples which might alter the concentration of immunoglobulins must be avoided.
• The concentrations of CSF and serum immunoglobulins must be determined accurately so that after adjustment equal quantities in CSF and serum of the Ig of interest are applied to the gel.

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 and other proteins.

Confirmation of intrathecal Ig synthesis is an important information to suspect inflammatory disease of the central nervous system. The oligoclonal profile or other indication of the intrathecal synthesis of Ig’s can be found in different diseases of the central nervous system, such as:
• 70 to 85 % of multiple sclerosis
• 100 % of non treated neurosyphilis
• 100 % of subacute scleroting leucoencephalitis.

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 and microbiological testing.

Limitations
Antisera: The use of antisera other than those available from the kit supplier may affect the results.
Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some monoclonal components may not be detected with this method.

Troubleshooting
Call Technical Service of the supplier when the test fails to perform while the instructions for the preparation and storage of materials, and for the procedure were carefully followed.
Kit reagent Safety Data Sheets and informations on waste products elimination are available from the Technical Service of the supplier.

PERFORMANCE DATA

Reproducibility
Within gel reproducibility
CSF and serum samples from two patients were each applied in all 12 tracks of a single gel.
Within lot and gel-to-gel reproducibility
Six CSF - serum sample pairs were applied on each of the six gels from the same lot.
Lot-to lot and gel-to-gel reproducibility
Six CSF - serum sample pairs were applied on each gel from three lots.
Results:
Upon visual examination, in all reproducibility studies the oligoclonal banding was correctly identified in each sample and on all gels, 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 = 72) were analyzed using the HYDRAGEL 6 CSF kit and procedure, and a commercially available high resolution electrophoresis system (HRE). The CSF and serum samples were prepared following the respective procedures, e.g., the CSF and serum samples for the Sebia test were diluted to contain 1 mg/dL of Ig G; for HRE, the CSF samples were concentrated to contain about 400 mg/dL of total protein and the serum samples were diluted 1:10. The electrophoretograms were evaluated visually for the presence of oligoclonal Ig banding (SEBIA test) or banding in the gamma and beta-gamma zones (HRE). There was a general agreement in detection of oligoclonal banding between the two tests. The few differences observed were due to a greater sensitivity of the HYDRAGEL immunofixation procedure and/or its ability to detect the true oligoclonal Ig banding. The results were consistent with clinical diagnosis, and indication of intrathecal synthesis (Tibblink-Link index) and blood-brain barrier damage (the albumin ratio index).

Sensitivity
The sensitivity of the HYDRAGEL 6 CSF procedure is the following: the detection limit of a monoclonal band of Ig G type is comprised between 3.0 and 12.5 µg/dL, between 6.0 and 15.0 µg/dL for an Ig A or Ig M type, and about 3.1 and 6.2 µg/dL for paraproteins with respectively bound Kappa or Lambda light chains.

BIBLIOGRAPHY