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.

NOTE : In this instruction sheet, the name "HYDRASYS" is used for both semi-automated HYDRASYS and HYDRASYS 2 instruments.

PRINCIPLE OF THE TEST 6, 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

WARNING : See the safety data sheets.

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>PN 4850</th>
<th>PN 4851</th>
</tr>
</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 / A1AT / TRF (ready to use)</td>
<td>1 vial, 85 mL</td>
<td>1 vial, 85 mL</td>
</tr>
<tr>
<td>Antiserum Diluent CSF / TRF (ready to use)</td>
<td>1 vial, 6 mL</td>
<td>1 vial, 6 mL</td>
</tr>
<tr>
<td>Wash Solution CSF (ready to use)</td>
<td>1 vial, 70 mL</td>
<td>1 vial, 70 mL</td>
</tr>
<tr>
<td>Rehydrating Solution</td>
<td>1 vial, 70 mL</td>
<td>2 vials, 70 mL</td>
</tr>
<tr>
<td>TTF1 / TTF2 Solvent (ready to use)</td>
<td>1 vial, 20 mL</td>
<td>1 vial, 20 mL</td>
</tr>
<tr>
<td>TTF1 (stock solution)</td>
<td>1 vial, 0.5 mL</td>
<td>1 vial, 0.5 mL</td>
</tr>
<tr>
<td>TTF2 (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>5 packs of 10 each</td>
<td>5 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>
</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 :
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 ; buffer solution pH 9.1 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

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: buffer solution pH 9.0 ± 0.5; additives, nonhazardous at concentrations used, necessary for optimum performance.
Use
Buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

3. SAMPLE DILUENT CSF / A1AT / TRF
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.

4. ANTISERUM DILUENT CSF / TRF
Preparation
Antiserum diluent is ready to use. It contains: buffer solution pH 7.0 ± 0.5; additives nonhazardous at concentrations used, necessary for optimum performance.
Use
For diluting antisera dilution just before use.

5. CSF WASH SOLUTION
Preparation
The CSF wash solution is ready to use. It contains: 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.

6. REHYDRATING SOLUTION
Preparation
The rehydrating solution is ready to use. It contains: additives, nonhazardous at concentrations used, necessary for optimum performance.
Use
To rehydrate the agarose gel before and after the peroxidase-based visualization step.

7. 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. 8.

NOTE: During storage, buffered strips may turn yellow without any adverse effects on their performances.
8. 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:

- 2 mL of TTF1 / TTF2 solvent,
- 50 µL of TTF1,
- 50 µL of TTF2 and
- 2 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 immunofixed immunoglobulins.

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 °C, TTF1 and TTF2 stock solutions are under solid form. They easily redissolve at room temperature. Stir them prior to use.

9. APPLICATORS

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

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

10. 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.

11. 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.

12. FILTER PAPER COMBS

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

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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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
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.

2. ANTISERUM ANTI-Ig A - PER

Preparation
The antiserum vial (SEBIA, PN 4742) contains mammalian immunoglobulins directed against human Ig A, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial (SEBIA, PN 4744) contains mammalian immunoglobulins directed against human Ig M, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial (SEBIA, PN 4744) contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The ANTI-KAPPA / LAMBDA – PER antisera pack, SEBIA, PN 4745, contains 1 vial anti-Kappa – PER lyophilised antiserum and 1 vial anti-Lambda – PER lyophilised antiserum.

**4. ANTI-KAPPA / LAMBDA - PER ANTISERA PACK**

The ANTI-KAPPA / LAMBDA – PER antisera pack, SEBIA, PN 4745, contains 1 vial anti-Kappa – PER lyophilised antiserum and 1 vial anti-Lambda – PER lyophilised antiserum.

**4.1 ANTISERUM ANTI-KAPPA - PER**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted 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**

**Preparation**

The antiserum vial contains mammalian immunoglobulins directed against human bound kappa light chains, 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 (50 µL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 µL reconstituted anti-Kappa - PER and 100 µL antiserum diluent. Mix well.

**Use**

For immunofixation and visualization of the electrophoresed proteins.
4.2 ANTISERUM ANTI-LAMBDA - PER

Preparation
The antiserum vial contains mammalian immunoglobulins directed against human bound lambda light chains, 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 (50 μL per detection) just before use. For each CSF / serum pair, dilute the antiserum 11 times with antiserum diluent (1 vol. / 10 vol.), e.g., 10 μL reconstituted 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
Before reconstitution, store the lyophilised antiserum refrigerated (2 to 8 ºC) 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 ºC. 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 ºC 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 ºC or at -18 / -30 ºC, homogenize the reconstituted antiserum before the preparation of the working solution (diluted antiserum).

Before use, store the thawed antiserum at 2 – 8 ºC 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 can be kept without refrigeration (15 to 30 ºC) for 15 days without any adverse effects on performance.

5. HYDROGEN PEROXIDE : H₂O₂ 110 volumes (30 %)

Use
For the preparation of the TTF visualization solution as described in the paragraph “TTF1 AND TTF2”.

Storage
Hydrogen peroxide must be stored in dark bottle and refrigerated (2 to 8 ºC).

When dispensing, always use clean pipette to avoid contamination of the bottle content.

6. 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.

For rinsing the HYDRASYS staining compartment.

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.

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

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.

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

Do not add any sodium azide.

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).

When dispensing, always use clean pipette to avoid contamination of the bottle content.

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

Use
For washing the gel after enzymatic visualization and drying.

For rinsing 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.

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

Do not add any sodium azide.

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.

7. 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: HYDRASYS 2 SCAN PN 1200, HYDRASYS 2 PN 1201, HYDRASYS 2 SCAN FOCUSING PN 1202, HYDRASYS 2 FOCUSING PN 1203, HYDRASYS PN 1210 or PN 1211 or HYDRASYS FOCUSING PN 1212.
2. 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.
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.

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 \[ x (A - 1) \] 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 \[ y (B/20 - 1) \] 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, washing 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 / HYDRASYS 2 INSTRUCTION MANUAL.

IMPORTANT: Adjust the mask position for perfect alignment between electrophoretic profiles and wells of the mask (see the HYDRASYS / HYDRASYS 2 instruction manual).

NOTE: The HYDRAGEL CSF Standard mask procedure can be performed with the software version 7.01.18 of HYDRASYS instrument or with the software version 2.20 of HYDRASYS 2 instrument and the following versions.

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.

<table>
<thead>
<tr>
<th>Well No. (applicator 6 teeth)</th>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well No. (applicator 15 teeth)</th>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

IMPORTANT: For HYDRAGEL 6 CSF, the wells no. 1, 8 and 15 are not used in this test; they may be marked with a felt tip pen to avoid filling them with sample by mistake.
- 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 SM/DM TTF" migration program for HYDRAGEL 3 CSF or "6 CSF SM/DM TTF" migration program for HYDRAGEL 6 CSF from the instrument menu.

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 "START" key of the keyboard.

**IMPORTANT:** Make sure that the ventilation air inlets of the instrument are 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 message is displayed on the screen: "* AS" / "* ANTISERA" signaling to apply antisera.

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

**II. IMMUNOFIXATION**

1. After the migration, open the lid.
2. Remove the sample applicator and discard.
3. Raise both carriers and remove them.
   - Remove the buffered strips by their plastic ends and discard.
   - 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 "START" key 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 message is displayed on the screen: "* AS (SM)" / "* ANTISERA (SM)" 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 comb (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.
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: "押 PAP." / "押 THICK FILTER 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 CSF 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.
   **WARNING:** Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.
5. Close the lid of the migration module.
6. Start the blotting sequence by pressing the "START" key of the keyboard.
7. 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. The following message is displayed on the screen: "[BLOTTING]."
- A beep sounds. The following message is displayed on the screen : "押 WASH + "押 PAP. / "押 WASH, "押 THICK FILTER PAPER, "押 WASH" signaling to remove the filter paper and apply the CSF 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 template R1 for HYDRAGEL 3 CSF or ENZ 2 mL for HYDRAGEL 6 CSF (Fig. 8).
4. Apply 2 mL of CSF wash 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.
5. Take CSF wash solution without trapping any air bubbles in the pipette tip. To apply the wash solution :
   - 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 "START" key of 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 : "押 WASH + "押 PAP. / "押 WASH, "押 THICK FILTER PAPER" signaling to remove the CSF wash solution by repipetting the excess of liquid and 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. 9).
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 of the keyboard.
H YD RAGEL 3 & 6 CSF - 2013/07
Masque standard / Standard mask

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: "◊ PAP. + ◊ REHYD 1" / "◊ THICK FILTER PAPER, ◊ REHYDRATING 1" signaling to remove the filter paper to apply the first rehydrating solution.

VIII. GEL REHYDRATION
1. Open the lid of the migration module.
2. Leave the gel in place on the plate of the migration module.
3. Set up the reagent application template R1 for HYDRAGEL 3 CSF or ENZ 2 mL for HYDRAGEL 6 CSF (Fig. 8).
4. 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.
5. Take rehydrating solution without trapping any air bubbles in the pipette tip.
   • Hold the pipette vertically.
   • Carefully and progressively inject the solution without introducing air bubbles under the template.
6. Close the lid of the migration module.
7. Start the incubation procedure by pressing the "START" key 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 message is displayed on the screen: "◊ REHYD1 + ◊ PAP." / "◊ REHYDRATING 1, ◊ THICK FILTER PAPER" signaling to remove the rehydrating solution and 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.
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 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 message is displayed on the screen: "◊ PAP. + ◊ REHYD2" / "◊ THICK FILTER PAPER, ◊ REHYDRATING 2" signaling to remove the filter paper and apply the rehydrating solution.

XI. GEL REHYDRATION
1. Open the lid of the migration module.
2. Leave the gel in place on the plate of the migration module.
3. Set up the reagent application template R1 for HYDRAGEL 3 CSF or ENZ 2 mL for HYDRAGEL 6 CSF (Fig. 8).
4. 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.
5. Take rehydrating solution without trapping any air bubbles in the pipette tip. Apply the rehydrating solution as described in § V.
6. Close the lid of the migration module.
7. Start the rehydration procedure by pressing the "START" key 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 and the temperature of the plate increases from 20 to 30 °C. The following message is displayed on the screen: "◊ REHYD2 + ◊ TTF" / "◊ REHYDRATING 2, ◊ TTF" signaling to remove the rehydrating solution and 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. Apply 1 mL of TTF visualization solution for HYDRAGEL 3 CSF or 2 mL for HYDRAGEL 6 CSF, prepared just before use, into the space underneath the template.
   Ensure that solution under the template is uniformly spread in the rectangular surface centered on the hole of the template.
   Take TTF visualization solution without trapping any air bubbles in the pipette tip. Apply the visualization solution as described in § V.
3. Close the lid of the migration module.
4. Start immediately the incubation procedure by pressing the "START" key 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 message is displayed on the screen: "◊ TTF + ◊ PAP." / "◊ TTF, ◊ THICK FILTER PAPER" signaling to remove visualization solution and 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 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. Close the lid of the migration module.
4. Start the blotting sequence by pressing the "START" key of the keyboard.
5. 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.

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: "θ PAP. + θ REHYD3" / "θ THICK FILTER PAPER, θ REHYDRATING 3" signaling to remove the filter paper and apply the rehydrating solution.

XVI. GEL REHYDRATION
1. Open the lid of the migration module.
2. Leave the gel in place on the plate of the migration module.
3. Set up the reagent application template R1 for HYDRAGEL 3 CSF or ENZ 2 mL for HYDRAGEL 6 CSF (Fig. 8).
4. 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.
   - Take rehydrating solution without trapping any air bubbles in the pipette tip.
5. Close the lid of the migration module.
6. Start the incubation procedure by pressing the "START" key of the keyboard.

GEL REHYDRATION - DESCRIPTION OF THE AUTOMATED STEPS
• Incubation at 30 °C controlled by Peltier effect, for 5 minutes.
• The following message is displayed on the screen: "θ REHYD3 + θ PAP." / "θ THICK FILTER PAPER, θ REHYDRATING 3" signaling to remove the rehydrating solution and apply one thick filter paper.

XVII. REHYDRATING SOLUTION ELIMINATION
1. Open the lid of the migration module.
2. Remove the rehydrating solution as previously described in § VI.
3. Grasp the reagent application template by the flap, lift it and remove it.

XVIII. BLOTTING 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. Close the lid of the migration module.
4. Start the blotting sequence by pressing the "START" key of the keyboard.
5. 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 ENZ 2 mL after visualization step with TTF. Rinse well with water.

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: "θ PAP." / "θ THICK FILTER PAPER" signaling to remove the filter paper.

XIX. 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 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.
5. Open the cover.
6. When the gel is dried, remove it immediately for further processing.

NOTES:
- 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.
XX. WASH AND FINAL PROCESSING OF THE GEL

After drying, the gel must be washed without any delay in the 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. 10).

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. Select "WASH ISOENZ/GEL" washing program from the instrument menu and start the run by pressing the "START" key of the keyboard. During washing and drying steps, the compartment remains locked.

4. 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.

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 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 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 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 procedure on gels from the same lot and incubated with the peroxidase labelled anti-Ig G antiserum. Each CSF / serum pair was run 8 times on a single gel and the CSF Control was run in 12 tracks of a single gel.
**Gel-to-gel reproducibility**

Four CSF / serum pairs (2 pairs with intrathecal synthesis and 2 pairs without intrathecal synthesis), 1 pathological serum sample with monoclonal components and the SEBIA CSF Control were analyzed with the HYDRAGEL CSF procedure and incubated with the peroxidase labelled anti-Ig G antiserum on 10 gels from the same lot, including one analysis of each CSF / serum pair per gel and 2 analyses of the pathological serum sample and of the CSF Control.

**Results**

Results obtained with the HYDRAGEL CSF procedure indicate a very good within gel and gel-to-gel reproducibility with peroxidase labelled anti-Ig G antiserum.

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**

Samples from 77 patients, including 28 CSF / serum pairs and 49 pathological serum samples with monoclonal components, were analyzed using the HYDRAGEL CSF procedure with immunofixation using peroxidase labelled anti-Ig G, anti-Ig A or anti-Ig M and anti-Kappa or Lambda light chains antisera, in parallel with a commercially available immunofixation agarose gel electrophoresis procedure intended for the detection of oligoclonal Ig banding. The electrophoregrams were both evaluated visually for the presence of oligoclonal Ig banding.

This study demonstrated a 100 % agreement between the 2 techniques:

For the 28 CSF / serum pairs (including 18 normal pairs and 10 pathological pairs): complete agreement (concordance).

For the 49 pathological serum samples: complete agreement (concordance).

**Sensitivity**

The sensitivity of the HYDRAGEL 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.


Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6