INTENDED USE

The CAPILLARYS IMMUNOTYPING kit is designed for the detection and the characterization of monoclonal proteins (immunotyping) in human serum with the CAPILLARYS System, SEBIA, for capillary electrophoresis. It is used in conjunction with the CAPILLARYS PROTEIN(E) 6 or CAPILLARYS 81-82+ kits, SEBIA, designed for serum proteins separation into 6 major fractions in alkaline buffer (pH 9.9).

The CAPILLARYS System performs all procedural sequences automatically to obtain a protein profile for qualitative analysis. Each sample is mixed with individual antisera that are specific against gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and kappa (free and bound) light chains, respectively. The proteins, separated in silica capillaries, are directly detected by their absorbance at 200 nm. The electrophoregrams are evaluated visually to detect the presence of specific reactions with the suspect monoclonal proteins.

For In Vitro Diagnostic Use.

PRINCIPLE OF THE TEST

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening samples for protein abnormalities. The CAPILLARYS System, SEBIA, for capillary electrophoresis has been developed to provide complete automation of this testing with fast separation and good resolution. In many respects, the methodology can be considered as intermediary between classical zone electrophoresis and liquid chromatography.

The CAPILLARYS System uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electroosmotic flow.

In capillary electrophoresis, abnormal fractions in serum protein electrophoregrams, primarily those in the beta globulin and gamma globulin zones, are always suspect of being monoclonal proteins (M-proteins, paraproteins, monoclonal immunoglobulins) and therefore, an indication of monoclonal gammopathies. With CAPILLARYS IMMUNOTYPING procedure, the immunotyping is performed with specific antibodies to identify these abnormal fractions.

The CAPILLARYS System has 8 capillaries functioning in parallel. In this system, a sample dilution is prepared and injected simultaneously by aspiration at the anodic end of six capillaries (capillaries No. 7 and 8 are not used). The reference pattern is obtained by injection of the sample mixed with ELP solution in capillary No. 1 providing a complete electrophoretic pattern of the sample's proteins. The antisera patterns are obtained by injection in capillaries No. 2 to 6 of the previously diluted samples mixed with specific antisera against gamma (Ig G), alpha (Ig A), mu (Ig M) heavy chains, and against free and bound Kappa and Lambda light chains.

A high voltage protein separation is then performed and direct detection of the proteins is made at 200 nm at the cathodic end of the capillary. The capillaries are immediately washed with a Wash Solution and prepared for the next analysis with buffer.

The superimposition of the antisera patterns with the reference pattern (ELP) permits to visualize the disappearance and/or the decrease of a monoclonal fraction on the antisera pattern and to indicate a gammopathy.

NOTE : In CAPILLARYS IMMUNOTYPING, proteins are detected in the following order from cathode to anode : gamma globulins, beta-2 globulins, beta-1 globulins, alpha-2 globulins, alpha-1 globulins and albumin with each zone containing one or more proteins. The antigen - antibody complex (between the sample immunoglobulins and the specific antiserum) has a very anodic mobility (between alpha-1 zone and albumin or more anodic than albumin).

The immunotyping is performed in three automated steps :

1. The sample dilution is prepared with specific diluent which is preloaded in the antisera segment. This dilution is selected by the user of the CAPILLARYS System according to the sample's immunoglobulins concentration.
   - "HYPERGAMMA" if total immunoglobulins level is > 2 g/dL (hypergammaglobulinemia),
   - "HYPOGAMMA" if total immunoglobulins level is < 0.8 g/dL (hypogammaglobulinemia),
   - "STANDARD" if total immunoglobulins level is comprised between 0.8 and 2 g/dL (dilution program by default).

2. The diluted sample serum is then mixed with individual specific antisera. The antigen - antibody complex is formed rapidly in the liquid medium. The sample that has been mixed with the specific antisera in the segment is injected simultaneously by aspiration into 6 capillaries at the anodic end. The proteins are separated by electrophoresis at high voltage. The separated proteins are detected at 200 nm at the cathodic end of the capillary.

3. The reference pattern (ELP) is automatically overlayed with the antisera patterns (Ig G, Ig A, Ig M, Kappa and Lambda) allowing visualization of the disappearance or decrease of the suspected monoclonal component.

REAGENTS SUPPLIED IN THE CAPILLARYS IMMUNOTYPING KIT

ANTISERA SEGMENTS

Preparation

The 60 antisera segments are ready to use ; each segment is intended to run one sample. They have 7 wells, each well contains respectively :

- a buffer for analysis (ELP solution, yellow),
- mammalian immunoglobulins anti-human gamma heavy chains (pink),
- mammalian immunoglobulins anti-human alpha heavy chains (dark blue),
- mammalian immunoglobulins anti-human mu heavy chains (yellow green),
- mammalian immunoglobulins anti-human kappa (free and bound) light chains (light green),
- mammalian immunoglobulins anti-human lambda (free and bound) light chains (light blue),
- a specific diluent for sample dilution.

Each reagent is colored with a nonhazardous dye. The antisera segments are shaped to fit on the sample racks of the CAPILLARYS System.
Use
Single use segments for protein immunotyping on the CAPILLARYS System.
The antisera segments must be placed on the sample rack after the cover (lid) is removed.

**IMPORTANT**: Before removing the cover of the antisera segment, make sure that no drop of reagents is present on the upper part of the wells; if drops are present, shake them down into the bulk liquid.

**WARNING**: The loaded antisera segments have to be handled as biological hazards.

Storage, stability and signs of deterioration
Store antisera segments refrigerated (2 – 8 °C). They are stable until the expiration date indicated on the kit box. DO NOT FREEZE.

**REAGENTS REQUIRED**

1. **CAPILLARYS PROTEIN(E) 6 KIT** (SEBIA, PN 2003) or **CAPILLARYS B1-ß2+ KIT** (SEBIA, PN 2002)

   Presentation, use, storage, stability and signs of deterioration
   See the instruction sheets of the kits.

   **WARNING**: Don’t use dilution segments supplied in CAPILLARYS PROTEIN(E) 6 and CAPILLARYS B1-ß2+ kits for CAPILLARYS IMMUNOTYPING procedure.

2. **DISTILLED OR DEIONIZED WATER**

   Use
   For rinsing capillaries in the CAPILLARYS System.
   It is recommended to filter distilled or deionized water through 0.45 µm filter before use.
   To prevent microbial contamination, change the water every day. In case of longer storage, add 3.5 µl/dL of ProClin 300.

   **IMPORTANT**: Before filling the rinse container, it is recommended to wash it with plenty of distilled or deionized water.

3. **CAPICLEAN**

   **Composition**
   The vial of CAPICLEAN concentrated solution (SEBIA, PN 2051, 12 mL) contains: proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

   **WARNING**: The CAPICLEAN solution may cause irritation or burns to skin, eyes and mucous membranes.

   Use
   For weekly capillaries and sample probe cleaning in automated system CAPILLARYS, SEBIA, for capillary electrophoresis.
   See the instruction sheets of CAPICLEAN, SEBIA.

   **IMPORTANT**: Do not re-use the dilution segment after capillaries and probe cleaning.

   Storage, stability and signs of deterioration
   Store CAPICLEAN refrigerated (2 – 8 °C). It is stable until the expiration date indicated on the vial label. DO NOT FREEZE.
   CAPICLEAN must be free of precipitate. Discard CAPICLEAN if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. **SODIUM HYPOCHLORITE SOLUTION** (for sample probe cleaning)

   **Preparation**
   Prepare a chlorinated sodium hypochlorite solution (2 % to 3 % chloride) by diluting 250 mL chlorinated concentrated solution (9.6 % chloride) to 1 liter with cold distilled or deionized water.

   **Use**
   For the sample probe cleaning in the CAPILLARYS System (weekly maintenance in order to eliminate adsorbed proteins from the probe).
   See the SEBIA CAPILLARYS instruction manual.
   - Use the sample rack designed for the maintenance (No. 100).
   - Place a tube containing 2 mL (2 – 3 %) diluted chlorinated solution previously prepared, in position No. 1 on this sample rack.
   - Slide the sample rack No. 100 for maintenance in the CAPILLARYS System.
   - In the “MAINTENANCE” window which appears on the screen, select “Launch the probe cleaning (chlorinated sodium hypochlorite solution or CDT wash solution)” and validate.

   **Storage, stability and signs of deterioration**
   Store the working chlorinated solution at room temperature in a closed container, it is stable for 1 year. Avoid storage in sunlight, close to heat and ignition source, and to acids and ammonia.

5. **CAPILLARYS WASH SOLUTION**

   **Preparation**
   Each vial of the stock CAPILLARYS Wash Solution (SEBIA, PN 2052, 2 vials, 70 mL) should be diluted up to 700 mL with distilled or deionized water.

   **WARNING**: The wash solution contains sodium hydroxide. Corrosive solution. Causes severe burns. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Take off immediately all contaminated clothing. Wear suitable clothes and eye/face protection.

   **Use**
   For washing the capillaries of CAPILLARYS. This reagent is also needed when the number of tests in series is below 40.
   See instruction sheet for details.
Storage, stability and signs of deterioration
Store the stock and working wash solutions in closed containers at room temperature or refrigerated. The stock wash solution is stable until the expiration date indicated on the kit or wash solution vial label. Working wash solution is stable for 3 months. Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

6. BETA-MERCAPTOETHANOL (BME or 2-MERCAPTOETHANOL) (not supplied by SEBIA)

EQUIPMENT AND ACCESSORIES REQUIRED

1. CAPILLARYS System SEBIA, PN 1220 or PN 1222.
2. Sample racks supplied with CAPILLARYS.
3. Container Kit supplied with CAPILLARYS: Rinse (to fill with distilled or deionized water), wash solution and waste containers.

SAMPLES FOR ANALYSIS

Sample collection and storage
Fresh serum samples are recommended for analysis. Sera must be collected following established procedures used in clinical laboratory testing. Samples may be stored for up to 10 days between 2 and 8 °C. For longer storage, samples should be frozen within 8 hours of collection. Frozen sera are stable for one month.

NOTE: Samples should not be stored at room temperature!

Protein degradation, and in particular complement degradation, is very sample dependent for sera stored between 2 to 8 °C.

Sample preparation
Use undiluted serum samples. Upon storage at 2 to 8 °C or after freezing, some sera (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. At room temperature, these samples can be directly analyzed. Samples containing a polymerized immunoglobulin may be used without any treatment. It is advised to observe the serum features before analysis (e.g., signs of hemolysis, cryoglobulins or turbidity).

Samples to avoid
- Avoid aged, improperly stored serum samples, beta fractions would be modified.
- Avoid plasma samples. Fibrinogen migrates in beta-2 position (shoulder on beta-2).

PROCEDURE

The CAPILLARYS System is a multiparameter instrument for serum proteins analysis on 6 parallel capillaries in the CAPILLARYS IMMUNOTYPING procedure, in the following sequence:
- Bar code reading of the sample tube and of the sample-rack;
- Sample dilution from primary tube;
- Mixing diluted serum samples with ELP solution / specific antisera;
- Capillary washing;
- Injection of diluted samples;
- Protein separation and direct detection of the separated proteins on capillaries.

The manual steps include:
- Placement of the sample tube in sample-rack;
- Placement of one opened antisera segment in each sample-rack;
- Placement of racks on the CAPILLARYS instrument;
- Setting up the instrument for operation;
- Removal of sample-racks after analysis.

Electrophoretic analysis on CAPILLARYS System using CAPILLARYS PROTEIN(E) 6 or CAPILLARYS ß1-ß2+ procedure has to be first performed to select samples suspected to contain monoclonal protein(s) (e.g., with abnormal protein pattern or fraction).

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

I. PREPARATION OF ELECTROPHORETIC ANALYSIS

1. Select samples with abnormal protein fraction on the electrophoregrams obtained with CAPILLARYS PROTEIN(E) 6 or CAPILLARYS ß1-ß2+ procedures.
2. Switch on CAPILLARYS instrument and computer.
3. Set up the software, validate and the instrument automatically starts. After 10 minutes, the CAPILLARYS System is ready to use.
4. For each sample to analyze and according to its total immunoglobulins concentration, select the dilution program to apply automatically:
   - “HYPERGAMMA” if total immunoglobulins level is > 2 g/dL (hypergammaglobulinemia),
   - “HYPOGAMMA” if total immunoglobulins level is < 0.8 g/dL (hypogammaglobulinemia),
   - “STANDARD” if total immunoglobulins level is comprised between 0.8 and 2 g/dL (dilution program by default).
5. The CAPILLARYS IMMUNOTYPING kit is intended to run with “IMMUNOTYPING 6" analysis program from the CAPILLARYS instrument. To select “IMMUNOTYPING 6” analysis program, please read carefully the CAPILLARYS instruction manual.
   NOTE: It is not necessary to change the buffer vial when switching from CAPILLARYS PROTEIN(E) 6 or CAPILLARYS ß1-ß2+ procedures to CAPILLARYS IMMUNOTYPING procedure (and vice versa).
6. Place only one sample tube in position No. 1 on each sample rack; the bar code of the tube must be visible in the openings of the sample rack. If the sample tube placed on the sample rack is not previously selected (or if the bar code is absent), the “STANDARD” dilution program will automatically be performed.
7. For each sample to analyze, take a new antisera segment, remove its cover and place it on the same sample rack than that of the sample. A message will be displayed if the segment is missing. 

NOTE: The antisera segments are shaped in order to fit on sample racks of the CAPILLARYS System.

8. Slide the sample carrier with the sample tube and the antisera segment into the CAPILLARYS System through the opening in the middle of the instrument. Up to 13 sample racks can be introduced successively and continuously into the system.

9. Remove analyzed sample racks from the plate on the left side of the instrument.

10. Take off carefully used antisera segments from each sample rack and discard them. 

WARNING: The used antisera segments have to be handled as biological hazards.

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Bar codes are read on both sample tube and on sample rack.

2. Sample is diluted in the diluent of the antisera segment and mixed respectively with ELP solution, anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera. The dilution needle is rinsed after each sample. The selected dilution program will be performed for each sample. If not selected, the “STANDARD” dilution program will be applied by default.

3. Capillaries are washed.

4. Diluted samples with reagents are injected into capillaries.

5. Migration is carried out under constant voltage for about 4 minutes and the temperature is controlled by Peltier effect.

6. Proteins are detected directly by scanning at 200 nm and an the electrophoretic profile appears on the screen of the system.

NOTE: These steps are described for the first introduced sample rack. The electrophoretic patterns appear after 10 minutes. For the following sample rack, the two first steps (bar code reading and sample dilution) are made during analysis of the previous sample rack.

II. RESULTS OF ANALYSIS

At the end of the analysis, each antiseraum pattern (Ig G, Ig A, Ig M, Kappa and Lambda) is automatically superimposed over the reference pattern (ELP). If a monoclonal component and a specific antisera have reacted together, the corresponding fraction disappears on the antisera pattern. These comparisons allow the identification and the characterization of monoclonal components.

III. END OF ANALYSIS SEQUENCE

At the end of each analysis sequence, the operator must initiate the "stand by" or "shut down" procedure of the CAPILLARYS System in order to store capillaries in optimal conditions.

IV. FILLING OF REAGENT CONTAINERS

The CAPILLARYS System has a reagent automatic control.

IMPORTANT: Please refer to the instructions for replacement of reagent containers respecting color code for vials and connectors.

A message will be displayed when it is necessary to perform one of the following tasks:

• Place a new buffer vial and / or
• Fill the container with working wash solution and / or
• Fill the container with filtered distilled or deionized water for rinsing capillaries and / or
• Empty the waste container.

IMPORTANT: Before filling the rinse container, it is recommended to wash it with plenty of distilled or deionized water.

Please carefully read the capillaries instruction manual.

RESULTS

Guidelines for pattern analysis

1. Reference pattern (ELP)

• First, it is recommended to examine carefully the reference pattern (ELP) for any abnormalities.
• When noting any abnormalities on the (ELP) track, take note of the migration position of the peak(s) within the curve – alpha-2, beta, beta-gamma or gamma zone. Using the reference pattern, look specifically for the area of abnormalities comparing the reference pattern (ELP) with each treated frame – G, A, M, kappa & lambda.

• Abnormalities can present as monoclonals, biclonals, triclonals, oligoclonal components, heavy chains, and as free light chains, etc...

2. Examine each immunoglobulin treated frame comparing to the overlaid reference pattern (ELP) curve. Look for the absence or reduction of an abnormal peak.

• IgG: IgG is the most abundant immunoglobulin class found in the serum and normal polyclonal removal will commonly be noted. Normal polyclonal reduction of this peak should not be mistaken for a monoclonal component. Polyclonal removal appears as a reduction of the fraction without any change of symmetry of the fraction. Monoclonal IgG will present with removal of a peak with symmetry change visible as compared to the reference pattern (ELP).
• IgA: Normally, IgA is in relatively small concentration compared to IgG. Look for slight reductions in the beta-early gamma area. The reference pattern (ELP) should mirror the IgA in normal samples.
• IgM: The pattern is similar to IgA except the concentration is normally even less. Normal samples will have very little reduction without change of the symmetry of the fraction. The reference pattern should mirror the IgM pattern in normal samples.
• Kappa: They are normally present in a ratio of 2 kappa to every 1 lambda. Normally note a 2/3 reduction in the gamma fraction. Polyclonal removal appears as a reduction of the fraction without any change of symmetry of the fraction. Monoclonal kappa component will present with removal of a peak with symmetry change visible as compared to the reference pattern (ELP).
• Lambda: Due to 2:1 ratio of kappa to lambda, the lambda track should present with a 1/3 overall reduction in the gamma fraction with normal samples. Polyclonal removal appears as a reduction of the fraction without any change of symmetry of the fraction. Monoclonal lambda component will present with removal of a peak with symmetry change visible as compared to the reference pattern (ELP).
The identification of a monoclonal component is achieved by noting the absence or removal of the abnormal peak(s) in the corresponding treated frames. For example, removal of an abnormal peak in both the treated G and kappa frames could be indicative of IgG, kappa monoclonal component.

**Interpretation**

**Absence of a monoclonal component**
A normal serum sample or a sample with hypergammaglobulinemia displays the disappearance of polyclonal immunoglobulins on antisera patterns (seen as a decrease of gamma and/or beta fractions) without any effect on other protein fractions (Fig. 1).

**Presence of a monoclonal component**

- The presence of a monoclonal protein (monoclonal gammopathy) is characterized by the disappearance of a fraction with one of the anti-heavy chain antisera (gamma, alpha or mu) and either with anti-kappa or anti-lambda light chain antisera. The detected monoclonal peak, typically sharp and demarcated in appearance, must be located at the same migration distance as the suspect monoclonal fraction seen in the reference track (ELP) (Fig. 3 and 4).
- The absence of reaction with any of the applied anti-heavy chain antisera and reaction with one of the light chain antisera might indicate:
  a) a very rare Ig D or Ig E gammopathy: confirm with anti-delta or anti-epsilon heavy chain antisera and SEBIA HYDRAGEL IF procedures,
  b) a light chain gammopathy: confirm with antisera anti-kappa or anti-lambda free light chains and SEBIA HYDRAGEL BENCE JONES or HYDRAGEL IF procedures.
- Failure to observe a positive reaction with any of the applied anti-light chain antisera, while an anti-heavy chain antisera reacts, might indicate a very rare heavy chain gammopathy (gamma, alpha or mu).

**Presence of two or more monoclonal components**

The same interpretation may be performed for samples with two or more monoclonal components. In rare cases, several clones of B-cells proliferate as indicated by several monoclonal bands revealed by immunotyping:

- A biclonal gammopathy is characterized by the disappearance of two fractions of heavy chain (identical or different) and two fractions of light chains (identical or different) (Fig. 5).
- Polymerized immunoglobulins are characterized by the disappearance of several fractions of the same type of heavy chain and of the same type of light chain. To confirm the presence of a single monoclonal abnormality, it is necessary to depolymerize with beta-mercaptoethanol and repeat immunotyping.
  - In this case (i) prepare 1% beta-mercaptoethanol (BME, or 2-mercaptoethanol) in Fluidil (SEBIA, PN 4587, 1 vial 5 mL), (ii) add 100 µL of this reducing solution to 300 µL neat serum, (iii) vortex and wait at least 15 minutes minimum (maximum 30 minutes) and then follow the standard procedure. After treatment with beta-mercaptoethanol, the sample presents only one monoclonal component if a single clone is present in the sample. The reducing treatment of the sample induces a C3 complement degradation (with high distortion of the beta zone): a wide fraction between alpha-1 zone and albumin may appear.
- An oligoclonal gammopathy is characterized by the disappearance of multiple, usually small peaks or deflections with one or more types of heavy chains and the two types of light chains (Fig. 7).

**Special cases**

- If the monoclonal fraction doesn't totally disappear on the antisera patterns, repeat the procedure with a higher sample dilution. Select "STANDARD" dilution program instead of "HYPOGAMMA" program or "HYPERGAMMA" dilution program instead of "STANDARD" program.
- Samples with monoclonal components at high total immunoglobulins level ("HYPERGAMMA" dilution program)
  - In this case, the antigen - antibody complex is a large and wide fraction located between albumin and alpha-1 zone; the monoclonal fraction(s) may not totally disappear on antisera patterns (Fig. 2).
- Samples with polymerized monoclonal components
  - In this case, the antigen - antibody complex is a large and wide fraction located between albumin and beta-1 zone.
  - If a monoclonal component is not detected in the gamma zone, select the dilution program adapted to the Ig's concentration of the sample. If another monoclonal component is suspected in the gamma zone, select the dilution program adapted to this protein.
- Biclonals
  - Biclonals may be due to immune complexes or biclonal gammopathies, or cross reactions which are very rare (see paragraph Interference and Limitations).

**Interference and Limitations**

Many studies have shown that the antigen – antibody reaction is different between liquid and agarose phase. CAPILLARYS IMMUNOTYPING procedure being totally performed in a liquid medium, some antisera may sometimes cross-react with monoclonal components present in the sample. There is no risk of false negative results such as failing to detect a gammopathy, but this cross-reaction, which occurs very rarely, may lead to a biclonal gammopathy conclusion instead of a real monoclonal gammopathy. According to the literature, the clinical treatment is not different between a biclonal gammopathy and a monoclonal gammopathy (Kyle et al. 1981).

- Faint shifts between the reference and the superimposed antisera patterns may be observed (especially in beta-1 zone). They must not be considered as the result of the disappearance of a monoclonal fraction on one or more antisera pattern.
- The use of antisera other than those specific for the CAPILLARYS IMMUNOTYPING procedure 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.
- As with any electrophoretic method, small monoclonal proteins which comigrate with other normal serum proteins may be difficult to discern. If small monoclonals are suspected, further testing using SEBIA HYDRAGEL Immuno fixation kits may be necessary.

**Troubleshooting**

- Call SEBIA when the test fails to perform even thought when the instructions for the preparation, storage of materials, and for the procedure were carefully followed.
- Kit reagent Safety Data Sheets and information on elimination of waste products are available from SEBIA.
PERFORMANCE DATA

Reproducibility within run
Reproducibility within run was demonstrated on five different pathological serum samples containing a monoclonal component and on one normal sample. They were analyzed with CAPILLARYS IMMUNOTYPING procedure and "STANDARD" dilution program. The five pathological and one normal sample were each run six times in the same run. Each sample was run with antisera: Reference pattern (ELP) solution, anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda.

All six samples (five pathological and one normal) were also run using two different lots of antisera segments. All repeats gave concordant results within run and within the two antisera lots. Patterns corresponded to the type of each tested sample.

Reproducibility between runs and lot-to-lot
Reproducibility between runs was demonstrated on nine different pathological serum samples containing a monoclonal component run at different immunoglobulin concentrations (3- HYPOGAMMA dilution, 3- STANDARD dilution, 3-HYPERGAMMA dilution).

Three samples (with total Ig level < 0.8 g/dL) were analyzed with CAPILLARYS IMMUNOTYPING procedure with "HYPOGAMMA" dilution program, three samples (with total Ig level comprised between 0.8 and 2 g/dL) with "STANDARD" dilution program and three samples (with total Ig level > 2 g/dL) with "HYPERGAMMA" dilution program. These samples were analyzed 4 times using 3 lots of antisera segments.

All repeats gave concordant results between runs and lot-to-lot. Patterns correctly identified the monoclonal components.

Concordance Study
Concordance study was performed on 135 serum samples between CAPILLARYS IMMUNOTYPING and HYDRAGEL 9 IF kits: 119 different pathological serum samples and 16 normal serum samples have been run on both techniques. This study demonstrated a 95 % agreement between the two techniques:

- For the 119 pathological serum samples : complete agreement (concordance).
- For the 16 normal serum samples : complete agreement (concordance).

Sensitivity
Serial dilutions were prepared in normal serum with three pathological serum samples all exhibiting monoclonal components and analyzed using the CAPILLARYS IMMUNOTYPING procedure.

The results are summarized below:

<table>
<thead>
<tr>
<th>SAMPLE No.</th>
<th>TYPE</th>
<th>MONOCLOAL COMPONENT</th>
<th>CONCENTRATION (g/dL) (in the original serum)</th>
<th>DETECTION LIMIT (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ig A, K</td>
<td>Alpha Kappa</td>
<td>0.50</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Ig G, L</td>
<td>Gamma Lambda</td>
<td>0.20</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Ig M, K</td>
<td>Mu Kappa</td>
<td>0.39</td>
<td>25</td>
</tr>
</tbody>
</table>

The detection limit of a monoclonal component is about 25 mg/dL.

NOTE: The detection limit may vary depending on the proximity and the magnitude of the interfering protein. The sensitivity tends to be higher for a monoclonal migrating at the cathodic end of the gamma zone and lower in the middle of the polyclonal hypergammaglobulinemia zone. According to the position of the monoclonal component and polyclonal background in the gamma and beta zones, the detection limit may vary.

BIBLIOGRAPHY

Figure 1

Sérum Normal / Normal serum

ELP

Ig G

Ig A

Ig M

K

L
Figure 2

**Sérum Hypergamma / Hypergamma serum**

**ELP**

**Ig G**

**Ig A**

**Ig M**

**K**

**L**

*Immun complexe* (Immune complex)
Paraprotéine éliminée / Decreased paraprotein
Paraprotéine non affectée / Not affected paraprotein

Figure 3

Interpretation : Ig G, Lambda
Paraprotéine éliminée / Decreased paraprotein
Paraprotéine non affectée / Not affected paraprotein

Figure 4

Interpretation : Ig G, Kappa
Figure 5

Interpretation: Ig G, Kappa + Ig M, Kappa

Paraprotéine éliminée / Decreased paraprotein
Paraprotéine non affectée / Not affected paraprotein
Paraprotéine éliminée / Decreased paraprotein
Paraprotéine non affectée / Not affected paraprotein

Interpretation : Ig G, Kappa + Ig G, Lambda
Figure 7

Profils oligoclonal / Oligoclonal pattern

ELP

Ig G

Ig A

Ig M

K

L