sebia

CAPILLARYS IMMUNOTYPING

Ref. 2100

Rx only

2018/12

INTENDED USE

The CAPILLARYS IMMUNOTYPING kit is designed for the detection and the characterization of monoclonal proteins (immunotyping) in human urine and serum with the CAPILLARYS System, SEBIA, for capillary electrophoresis. It is used in conjunction with the CAPILLARYS PROTEIN(E) 6 kit, SEBIA, designed for proteins separation into 6 major fractions in alkaline buffer (pH 9.9).
The CAPILLARYS performs all procedural sequences automatically to obtain a protein profile for qualitative analysis. Each urine or serum 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 and lambda (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.

NOTE : In this instruction sheet, the name "CAPILLARYS" is used for automated CAPILLARYS, CAPILLARYS 2 and CAPILLARYS 2 FLEX-PIERCING instruments.

PRINCIPLE OF THE TEST

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening urine and serum samples for protein abnormalities [3, 5, 7, 9, 10, 18]. 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 [3, 6, 7].
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 [4, 12, 18].
In capillary electrophoresis, abnormal fractions in urine and 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 and CAPILLARYS IMMUNOTYPING URINE procedures, 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. For the immunotyping, the reference pattern (ELP 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 ELP pattern permits to visualize the disappearance and / or the decrease of a monoclonal fraction on the antiserum pattern and to indicate a gammopathy.
NOTE: In CAPILLARYS IMMUNOTYPING and CAPILLARYS IMMUNOTYPING URINE procedures, 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 urine or serum 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 four automated steps:

1. Dilution of serum or dialyzed urine samples with specific diluent preloaded in the antisera segment. This dilution is made according to the sample's immunoglobulins concentration.
2. Mixing diluted serum or urine sample with individual specific antisera. The antigen - antibody complex is formed rapidly in liquid medium without the need for extra incubation step or removal of the immune complexes.
3. Injection of prepared samples by simultaneous aspiration into 6 capillaries at the anodic end and separation of proteins by electrophoresis at high voltage. The separated proteins are detected at the cathodic end of the capillary at 200 nm.
4. Superimposition of the ELP pattern with the antisera patterns (Ig G, Ig A, Ig M, Kappa and Lambda) permits to characterize the suspected monoclonal component.

Serum and urine samples can both be analyzed with the software version 5.50 of CAPILLARYS and following versions.

REAGENTS SUPPLIED IN THE CAPILLARYS IMMUNOTYPING KIT

WARNING : See the safety data sheets.

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.

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

WARNING : Do not use marketed deionized water, such as water for ironing for example (risk of important capillaries damage). Use only water with ultrapure quality, such as injection grade water.

ANALYSIS OF SERUM SAMPLES: CAPILLARYS IMMUNOTYPING PROCEDURE

REAGENTS REQUIRED (but not supplied with the CAPILLARYS IMMUNOTYPING kit)

WARNING : See the safety data sheets.

1. CAPILLARYS PROTEIN(E) 6 KIT (SEBIA, PN 2003)

Presentation, use, storage, stability and signs of deterioration

See the instruction sheet of the kit.

WARNING: Don't use dilution segments supplied in the CAPILLARYS PROTEIN(E) 6 kit for CAPILLARYS IMMUNOTYPING procedure.

2. DISTILLED OR DEIONIZED WATER

Use

For rinsing capillaries in the CAPILLARYS System.
It is recommended to use filtered distilled or deionized water (on a filter with a porosity ≤ 0.45 µm) and with a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.
To prevent microbial proliferation, change the water every day.
For optimal operation, add CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL) in distilled or deionized water (see the instructions for use of CLEAN PROTECT) or use directly the ready to use CAPIprotect* solution (SEBIA, PN 2061 : 2 containers of 5 L of distilled water with CLEAN PROTECT).

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

** NOTE : The CAPIprotect solution can also be used to dilute the stock wash solution. Then, in that case, the diluted wash solution may show a transient more or less marked yellow colour without any adverse effects on its performance.*

3. CAPICLEAN

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2058, 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in automated system CAPILLARYS, SEBIA, for capillary electrophoresis, during the CAPICLEAN cleaning sequence.

IMPORTANT :

- When less than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence at least once a week.
- When less than 500 samples are analyzed within a day but more than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence after every 500 analyses.
- When more than 500 samples are analyzed within a day, launch a CAPICLEAN cleaning sequence once a day.

See the instruction sheets of CAPICLEAN, SEBIA.

IMPORTANT : Do not re-use the dilution segment after sample 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.
Precipitate or combined particles in suspension (floccules) may be observed in the CAPICLEAN vial without any adverse effects on its utilization.
Do not dissolve this precipitate or these particles. It is recommended to collect only the supernatant.

4. SODIUM HYPOCHLORITE SOLUTION (for sample probe cleaning)

Preparation

Prepare a sodium hypochlorite solution (2 % to 3 % chloride) by diluting 250 mL 9.6 % chloride concentrated solution 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)" and validate.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature in a closed container, it is stable for 3 months. Avoid storage in sunlight, close to heat and ignition source, and to acids and ammonia.

5. CAPILLARYS / MINICAP WASH SOLUTION

Preparation

Each vial of the stock CAPILLARYS / MINICAP Wash Solution (SEBIA, PN 2052, 2 vials, 75 mL) should be diluted up to 750 mL with distilled or deionized water.
After dilution, the wash solution contains an alkaline solution pH ≈ 12.

Use

For washing the capillaries of CAPILLARYS. This reagent is also needed when the number of tests in series is below 40.

IMPORTANT : Before filling the wash solution container, it is recommended to wash the opening of the container, the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.

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)

7. DTT DILUENT (IF / IT)

Composition

The vial of DTT Diluent (IF / IT) (SEBIA, PN 4589 : 1 vial, 13 mL) contains : phosphate buffer solution pH 7.4 ± 0.5 and additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

The DTT Diluent (IF / IT) is intended for the reconstitution of a Dithiothreitol (DTT) vial for the preparation of a DTT 0.5 M reducing solution that is used to depolymerize monoclonal proteins.
This reducing solution of Dithiothreitol can be used to replace a ß-mercaptoethanol solution.
See the instructions for use of the DTT Diluent (IF / IT), SEBIA.

Storage, stability and signs of deterioration

See the instructions for use of the DTT Diluent (IF / IT), SEBIA.

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 filter ≤ 0.45 µm) and have a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.

EQUIPMENT AND ACCESSORIES REQUIRED

1. CAPILLARYS System SEBIA : CAPILLARYS PN 1220, CAPILLARYS 2 PN 1222 or CAPILLARYS 2 FLEX-PIERCING PN 1227.
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 can be stored up to 10 days between 2 and 8 °C.
For longer storage, samples should be frozen at – 18 / – 30 °C within 8 hours of collection. Frozen sera are stable for 3 months.
Proteins of the samples stored at 2 to 8 °C or between 15 and 30 °C, degrade, particularly the C3 complement for which the degradation kinetics is very rapid at 15 - 30 °C and is clearly visible beyond 3 days.
A serum stored between 2 and 8 °C or between 15 and 30 °C has a beta-2 fraction that gradually decreases and may appear distorted (with small additional fractions appearing on the gamma side and / or beta-1 following the deterioration of C3 complement) and an alpha-2 fraction whose shape can be slightly changed.
Beyond 10 days between 2 and 8 °C or 3 days between 15 and 30 °C, the beta-1 fraction deforms by expanding, and the beta-2 fraction strongly decreases.
Depending to the samples, during their storage beyond 10 days at 2 to 8 °C or 3 days at 15 and 30 °C, the automated superposition of fractions by the software for data processing may potentially be disturbed.
NOTE : Each laboratory must ensure that the samples are transported in optimal conditions for their integrity (1).

(1) ISO 15189 : Medical laboratories - Requirements for quality and competence.

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).

NOTE : Collection tubes for biological samples are described in the available documentation on pre-analytical phase for bio-medical analysis (data provided by the tube manufacturers, guides and recommendations on biological sample collection…). Without any indication in the instructions for use on the type of tube to use, please refer to this documentation and for the dimensions of tube to use, refer to the SEBIA document "Characteristics of tubes to use according to the instrument". The pre-analytical phase must be performed according to the state of art, the different recommendations, including those provided by the tube manufacturers, and applicable regulations.

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-rack and of the serum sample tube ;
- 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 ;
- Removal of sample-racks after analysis.

Electrophoretic analysis on CAPILLARYS System using CAPILLARYS PROTEIN(E) 6 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 procedure.
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. When analyzing the sample, if a monoclonal protein is suspected in the gamma zone, select the dilution program based on the total immunoglobulin concentration in gamma zone as outlined below. The dilution will then be automatically applied to the sample.
   - " 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. For serum samples analysis, the CAPILLARYS IMMUNOTYPING kit is intended to run with " IMMUNOTYPING 6 " analysis program from the CAPILLARYS instrument and CAPILLARYS PROTEIN(E) 6 buffer. To select " IMMUNOTYPING 6 " analysis program and place the CAPILLARYS PROTEIN(E) 6 buffer vial in the instrument, please read carefully the CAPILLARYS instruction manual.

   NOTE: It is not necessary to change the buffer vial when switching from CAPILLARYS PROTEIN(E) 6 procedure 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, 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. The sample rack will be ejected 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 rack and on sample tube.
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 sample probe 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 antiserum pattern (Ig G, Ig A, Ig M, Kappa and Lambda) is automatically overlayed to the ELP pattern. If a monoclonal component and a specific antiserum have reacted together, the corresponding fraction disappears on the antiserum pattern.
The analysis of the serum sample diluted in the diluent of the antisera segment is done during the immunotyping and provides the protein electrophoretic pattern of the neat sample ("Ref" pattern). This pattern allows to verify the concordance between the proteins analysis and the immunotyping.
These comparisons allow the identification and the characterization of monoclonal components.

IMPORTANT : The neat protein pattern ("Ref" pattern) is not overlayed, and can not be overlayed to any antiserum pattern of the immunotyping.

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.

WARNING : Do not use marketed deionized water, such as water for ironing for example (risk of important capillaries damage). Use only water with ultrapure quality, such as injection grade water.

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

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

QUALITY CONTROL

It is recommended to run an assayed control serum (such as IT / IF Control, SEBIA PN 4788) after each change of lot of a reagent.

NOTE : If necessary, the Normal Control Serum, SEBIA PN 4785, or the Hypergamma Control Serum, SEBIA PN 4787, may be used as a negative control.

RESULTS

Guidelines for pattern analysis

1. **Reference pattern (ELP pattern)**
   - First, it is recommended to examine carefully the reference pattern (ELP pattern) 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 ELP pattern, look specifically for the area of abnormalities comparing the ELP pattern with each treated frame – G, A, M, kappa & lambda.
   - Abnormalities can present as monoclonals, biclonals, triclonals, oligoclonals components, heavy chains, and as free light chains, etc…

2. **Examine each immunoglobulin treated frame comparing to the overlayed reference pattern (ELP pattern) curve. Look for the absence or reduction of an abnormal peak.**
   - Ig G: Ig G 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. Monoclonal Ig G will present with removal of a peak as compared to the ELP pattern.
   - Ig A: Normally, Ig A is in relatively small concentration compared to Ig G. Look for slight reductions in the beta-early gamma area. The ELP pattern should mirror the Ig A in normal samples.
   - Ig M: The pattern is similar to Ig A except the concentration is normally even less. Normal samples will have very little reduction without change of the symmetry of the fraction. The ELP pattern should mirror the Ig M 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 ELP pattern.
   - 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 ELP pattern.

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 Ig G, kappa monoclonal component.

Interpretation for serum samples analysis

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 antiserum. 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 track) (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 antiserum 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 the 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, 2 ME) in Fluidil (SEBIA, PN 4587, 1 vial 5 mL), (ii) the CAPILLARYS system ready waiting for rack, add 100 μL of this reducing solution to 300 μL neat serum, (iii) vortex and wait for 15 minutes maximum, then follow the standard procedure.

  IMPORTANT : After reducing treatment with beta-mercaptoethanol, the sample must be analyzed without any delay ; no introduced sample rack must be waiting for analysis in the CAPILLARYS system.

  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.
- Samples displaying monoclonal components that migrate in zones other than gamma (alpha-2, beta-1 or beta-2)

  If a strong monoclonal component migrates in a zone other than gamma (alpha-2, beta-1 or beta-2), select the dilution program based on the total concentration of immunoglobulins as seen on the profile (gamma zone + suspected monoclonal proteins in alpha-2 or beta).
- Biclonals

  Biclonals may be due to immune complexes or biclonal gammopathies, or cross reactions which are very rare (see paragraph Interference and Limitations).
- Disappearance of Ig M on anti-Kappa and Lambda antisera patterns :

  In case of a complete substraction of a peak with the anti-Ig M, anti-Kappa and anti-Lambda light chains antisera simultaneously, it is recommended to treat the sample with beta-mercaptoethanol reducing agent (see the previous paragraph) and repeat immunotyping.
- In case of multiple simultaneous reactions with anti-heavy chains G, A and M, it is recommended to analyze again the serum sample by selecting the "OPTIMIZED" dilution mode.

NOTE : The sample treatment with ß-mercaptoethanol can be replaced by a treatment with Dithiothreitol reconstituted in the DTT Diluent (IF / IT), SEBIA, PN 4589. See the paragraph "REAGENTS REQUIRED" and the instructions for use of the DTT Diluent (IF / IT) for additional information.

Interference and Limitations

Many studies have shown that the antigen – antibody reaction is different between liquid and agarose phase. Immunotyping procedures using capillary electrophoresis being entirely 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.

It should be recalled that according to international recommendations (Ludwig *et al*, 2013), the detection and characterization of a monoclonal component must be performed in serum and urine and completed by a quantification of serum free light chains. The consistency of all assays must be checked before any definitive conclusion.

If a pattern is doubtful, further testing using HYDRAGEL Immunofixation kits or sample analysis using "OPTIMIZED" dilution mode or betamercaptoethanol treatment (see § Special cases) may be necessary.

Faint shifts between the ELP pattern 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 Immunofixation kits may be necessary [10].

See SAMPLES FOR ANALYSIS.

When a monoclonal component is detected by CAPILLARYS procedures for protein analyses (such as CAPILLARYS PROTEIN(E) 6) and not characterized by CAPILLARYS IMMUNOTYPING procedure, it is recommended to repeat the immunotyping on the sample, previously treated with beta-mercaptoethanol (see the previous paragraph) and if an uncertainty persists, to confirm the result by an immunofixation technique on agarose gel.

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 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 SEBIA's extranet website : www.sebia.com.

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 16 normal serum samples: complete agreement (concordance).
- For the 119 pathological serum samples :
    - 112 samples demonstrated a complete agreement.
    - 4 samples with very small quantities of monoclonal on a high polyclonal background demonstrated a partial agreement.
    - 3 samples with oligoclonal profiles with multiple banding pattern demonstrated a partial agreement.

In all cases, both techniques detected and characterized the monoclonal proteins (immunotyping) in human serum with complete agreement.

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 :

	MONOCLONAL COMPONENT			
SAMPLE No.	**TYPE**		**CONCENTRATION (g/dL) (in the original serum)**	**DETECTION LIMIT (mg/dL)**
1	Ig A, K	Alpha Kappa	0.50	25 25
2	Ig G, L	Gamma Lambda	0.20	25 25
3	Ig M, K	Mu Kappa	0.39	25 25

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.

ANALYSIS OF URINE SAMPLES: CAPILLARYS IMMUNOTYPING URINE PROCEDURE

REAGENTS REQUIRED (but not supplied with the CAPILLARYS IMMUNOTYPING kit)

WARNING : See the safety data sheets.

1. CAPILLARYS PROTEIN(E) 6 KIT (SEBIA, PN 2003)

Presentation, use, storage, stability and signs of deterioration

See the instruction sheet of the kit.

WARNING: Don't use dilution segments supplied in the CAPILLARYS PROTEIN(E) 6 kit for CAPILLARYS IMMUNOTYPING URINE procedure.

2. CAPILLARYS / MINICAP URINE KIT (SEBIA, PN 2013)

Presentation, storage, stability and signs of deterioration

See the instruction sheet of the kit.

Use

For the preparation of urine samples before separation of human urine proteins by capillary electrophoresis with the CAPILLARYS System.

3. DISTILLED OR DEIONIZED WATER

Use

For rinsing capillaries in the CAPILLARYS System.
It is recommended to use filtered distilled or deionized water (on a filter with a porosity ≤ 0.45 µm) and with a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.
To prevent microbial proliferation, change the water every day.
For optimal operation, add CLEAN PROTECT (SEBIA, PN 2059, 1 vial of 5 mL) in distilled or deionized water (see the instructions for use of CLEAN PROTECT) or use directly the ready to use CAPIprotect* solution (SEBIA, PN 2061 : 2 containers of 5 L of distilled water with CLEAN PROTECT).

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

** NOTE : The CAPIprotect solution can also be used to dilute the stock wash solution. Then, in that case, the diluted wash solution may show a transient more or less marked yellow colour without any adverse effects on its performance.*

4. CAPICLEAN

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2058, 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in automated system CAPILLARYS, SEBIA, for capillary electrophoresis, during the CAPICLEAN cleaning sequence.

IMPORTANT :

- When less than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence at least once a week.
- When less than 500 samples are analyzed within a day but more than 500 samples are analyzed within a week, launch a CAPICLEAN cleaning sequence after every 500 analyses.
- When more than 500 samples are analyzed within a day, launch a CAPICLEAN cleaning sequence once a day.

See the instruction sheets of CAPICLEAN, SEBIA.

IMPORTANT : Do not re-use the dilution segment after sample 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.
Precipitate or combined particles in suspension (floccules) may be observed in the CAPICLEAN vial without any adverse effects on its utilization.
Do not dissolve this precipitate or these particles. It is recommended to collect only the supernatant.

5. SODIUM HYPOCHLORITE SOLUTION (for sample probe cleaning)

Preparation

Prepare a sodium hypochlorite solution (2 % to 3 % chloride) by diluting 250 mL 9.6 % chloride concentrated solution 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 instruction sheets of CAPILLARYS, SEBIA.

- Use the sample rack designed for the maintenance (No. 100).
- Place a tube containing 2 mL of the (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)" and validate.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature in a closed container, it is stable for 3 months. Avoid storage in sunlight, close to heat and ignition source, and to acids and ammonia.

6. CAPILLARYS / MINICAP WASH SOLUTION

Preparation

Each vial of the stock CAPILLARYS / MINICAP Wash Solution (SEBIA, PN 2052, 2 vials, 75 mL) should be diluted up to 750 mL with distilled or deionized water.
After dilution, the wash solution contains an alkaline solution pH ≈ 12.

Use

For washing the capillaries of CAPILLARYS. This reagent is also needed when the number of tests in series is below 40.

IMPORTANT : Before filling the wash solution container, it is recommended to wash the opening of the container, the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.

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.

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 filter ≤ 0.45 µm) and have a conductivity lower than 3 µS/cm, which corresponds to a resistivity higher than 0.33 MΩ.cm.

EQUIPMENT AND ACCESSORIES REQUIRED

1. CAPILLARYS System SEBIA : CAPILLARYS PN 1220, CAPILLARYS 2 PN 1222 or CAPILLARYS 2 FLEX-PIERCING PN 1227.
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.
4. Hemolysing tubes (75 mm high and 13 mm in diameter).

SAMPLES FOR ANALYSIS

Sample collection and storage

The analysis is carried out preferentially on fresh urine collected over 24 hours. Urine samples must be collected following established procedures used in clinical laboratory testing.
Urine samples may be stored for up to one week between 2 and 8 °C.
For longer storage periods, it is recommended to keep samples frozen at - 70 / - 80 °C (stable at least for one month).

IMPORTANT: Do not store samples at – 20 °C.

Thawed or improperly stored samples may show modified or additional fractions due to proteins degradation.

NOTE: Urine samples should not be stored at room temperature!

Before analysis, prepare urine samples according to the preparation procedure of dialysis and concentration described in the package insert of the CAPILLARYS / MINICAP URINE kit (see paragraph "Reagents required but not supplied").
Use directly these prepared urine samples.

Sample preparation

IMPORTANT: The urine analysis technique by capillary electrophoresis requires 2 steps: a dialysis step and a concentration step of urine samples with SEBIA dialysis systems (20 mL tubes). Use only one tube per sample. Then, the collected dialyzed and concentrated urine sample can be analyzed using CAPILLARYS URINE and CAPILLARYS IMMUNOTYPING URINE procedures.

Analyze the urine samples within a maximum of one day after their preparation. In order to limit protein adsorption onto the membrane of the dialysis and concentration device (SEBIA dialysis system), it is not recommended to store the sample in the dialysis system after centrifugation but in a microtube stored refrigerated (between 2 and 8 °C).

Samples to avoid

- Avoid aged, improperly stored urine samples, fractions would be modified due to denaturation.
- It is advised to observe the urine sample features after the first centrifugation (e.g., signs of hemolysis or turbidity).
- Do not store samples in dialysis and concentration devices, some proteins may bind to the membrane.

NOTE : Collection tubes and centrifugation parameters for biological samples are described in the available documentation on pre-analytical phase for bio-medical analysis (data provided by the tube manufacturers, guides and recommendations on biological sample collection…). Without any indication in the instructions for use on the type of tube to use or on the centrifugation, please refer to this documentation and for the dimensions of tube to use, refer to the SEBIA document "Characteristics of tubes to use according to the instrument". The pre-analytical phase must be performed according to the state of art, the different recommendations, including those provided by the tube manufacturers, and applicable regulations.

PROCEDURE

The CAPILLARYS System is a multiparameter instrument for urinary proteins analysis on 6 parallel capillaries in the CAPILLARYS IMMUNOTYPING URINE procedure, in the following sequence :

- Bar code reading of the sample-rack and of the urine sample tube ;
- Sample dilution ;
- Mixing diluted urine 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 microtubes (without caps) containing the samples to analyze on holding hemolysing tubes in sample-racks; each tube being identified with the specific sample identification bar code label corresponding to the sample to analyze,
- Placement of one opened antisera segment in each sample-rack ;
- Placement of racks on the CAPILLARYS instrument ;
- Removal of sample-racks after analysis.

Electrophoretic analysis on CAPILLARYS System using CAPILLARYS URINE 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 URINE procedure.
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, determine the "Ratio urine" value of the abnormal fraction to characterize from the electrophoretic pattern obtained with the "URINE" analysis program (see the paragraph "Result analysis" of the CAPILLARYS / MINICAP URINE kit package insert). The "Ratio urine" is defined by the proportion of the abnormal fraction area related to the Normal Control Serum proteins total area.
5. Select the dilution program to apply automatically according to the "Ratio urine" value:
   - " HYPOGAMMA " if the " Ratio urine " value is < 0.55,
   - " STANDARD " if the " Ratio urine " value is comprised between 0.55 and 1.55,
   - " HYPERGAMMA " if the " Ratio urine " value is > 1.55.

   NOTE: When the "Ratio urine" value is ± 10 % from one of the threshold value (0.55 or 1.55), it is recommended to select the dilution program according to the abnormal fraction to characterize with the CAPILLARYS IMMUNOTYPING URINE procedure:
   - *to improve the detection of a weak fraction, dilute less the urine sample by selecting the " HYPOGAMMA " dilution program instead of " STANDARD " program and " STANDARD " dilution program instead of " HYPERGAMMA " program,*
   - *to improve the immunotyping of a high fraction, dilute more the urine sample by selecting the " STANDARD " dilution program instead of " HYPOGAMMA " program and " HYPERGAMMA " dilution program instead of " STANDARD " program.*
6. For urine samples analysis, the CAPILLARYS IMMUNOTYPING kit is intended to run with "IMMUNOTYPING URINE" analysis program from the CAPILLARYS instrument and CAPILLARYS PROTEIN(E) 6 buffer. To select " IMMUNOTYPING URINE" analysis program and place the CAPILLARYS PROTEIN(E) 6 buffer vial in the instrument, please read carefully the CAPILLARYS instruction manual.

   NOTE: It is not necessary to change the buffer vial when switching from CAPILLARYS URINE procedure to CAPILLARYS IMMUNOTYPING URINE procedure (and vice versa).
7. Place one empty hemolysing tube (used as holder) in position No. 1 on each sample rack, and then, the microtube containing the dialyzed urine sample. Cut the cap of each microtube before using it.
8. Keep the cap of each microtube for further storage of samples, if necessary.

   IMPORTANT: It is recommended to identify each hemolysing tube holding the microtube which contains sample to analyze, with the specific sample identification bar code label corresponding to the sample.
9. The bar code of each tube must be visible in the openings of the sample rack.
   If the urine sample placed on the sample rack is not previously selected, the " HYPOGAMMA " dilution program will automatically be performed.
10. 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 hemolysing tube and microtube containing the sample. The sample rack will be ejected if the segment is missing.

    NOTE: The antisera segments are shaped in order to fit on sample racks of the CAPILLARYS System.
11. 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.
12. Remove analyzed sample racks from the plate on the left side of the instrument.
13. 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 rack and on urine sample tube to analyse.
2. Dialyzed urine 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 sample probe is rinsed after each sample. The selected dilution program will be performed for each sample. If not selected, the "HYPOGAMMA" 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 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 dialyzed urine sample dilution) are made during analysis (step 5) of the previous sample rack.

II. RESULTS OF ANALYSIS

At the end of the analysis, each antiserum pattern (Ig G, Ig A, Ig M, Kappa and Lambda) is automatically superimposed over the ELP pattern. If a monoclonal component and a specific antiserum have reacted together, the corresponding fraction disappears on the antiserum pattern. These comparisons allow the identification and the characterization of monoclonal components.
For urine samples analysis, the different protein zone positions (Albumin, Alpha-1, Alpha-2, Beta and Gamma) are identified on the screen and on the result report.

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.

WARNING : Do not use marketed deionized water, such as water for ironing for example (risk of important capillaries damage). Use only water with ultrapure quality, such as injection grade water.

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

PLEASE CAREFULLY READ THE CAPILLARYS INSTRUCTION MANUAL.

RESULTS

Interpretation for urine samples analysis

Absence of a monoclonal component

- A normal urine sample or a sample with glomerular proteinuria and polyclonal immunoglobulins 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.

Presence of a monoclonal component

- The presence of a complete monoclonal immunoglobulin 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 antiserum. 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 track).
- 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 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 antiserum reacts, might indicate a very rare heavy chain gammopathy (gamma, alpha or mu).

Presence of two or more monoclonal components

- Polymerized immunoglobulins are characterized by the disappearance of several fractions of the same type of the light chain.

Special cases :

- If the monoclonal fraction doesn't totally disappear on the antisera patterns, repeat the procedure with a higher urine 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 in albumin and alpha-1 zones ; the monoclonal fraction(s) may not totally disappear on antisera patterns.
- The presence of a complete immunoglobulin associated to a free light chain might be characterized by the disappearance of two fractions with one of the anti-light chain antisera, and the disappearance of only one fraction with the anti-heavy chain antisera.
- It is recommended to select the dilution program according to the abnormal fraction to characterize, in order to improve the detection of a weak fraction or the immunotyping of a high fraction (see paragraph "Preparation of electrophoretic analysis").

Interference and Limitations

See SAMPLES FOR ANALYSIS.

Analyze only samples prepared with dialysis and concentration devices, such as SEBIA dialysis systems or equivalent device giving the same performances and approved for clinical assays.

A deficient sample dialysis may lead to non proteic residual fractions. When an interferent fraction is suspected, it is recommended to dialyse again the urine sample.

Interferences such as drug or salts are eliminated during the dialysis step. If this step is not carefully followed, artifactual peaks might be observed.

Hemoglobin is commonly known to co-migrate with transferrin when it is in the urine sample. It is advised to observe the urine sample features after the first centrifugation (e.g., signs of red blood cells and / or hemolysis in the urine sample).

Many studies have shown that the antigen – antibody reaction is different between liquid and agarose phase. Immunotyping procedures using capillary electrophoresis being entirely 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.

It should be recalled that according to international recommendations (Ludwig *et al*, 2013), the detection and characterization of a monoclonal component must be performed in serum and urine and completed by a quantification of serum free light chains. The consistency of all assays must be checked before any definitive conclusion.

If a pattern is doubtful, further testing using HYDRAGEL BENCE JONES Immunofixation kits may be necessary.

Faint shifts between the ELP pattern 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 URINE 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 may be difficult to discern. If small monoclonals are suspected, further testing using SEBIA HYDRAGEL Immunofixation kits may be necessary.

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 SEBIA's extranet website : www.sebia.com.

PERFORMANCE DATA

Reproducibility within run

Reproducibility within run was demonstrated on five different pathological samples containing one or two monoclonal components and on one normal sample. Each sample was analyzed 6 times in the same run with CAPILLARYS IMMUNOTYPING URINE procedure and "HYPOGAMMA" dilution program with 2 different lots of reagents for antisera segments, each segment containing the same reagent in every well according to the tested sample (ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa or anti-Lambda antisera).
All repeats gave concordant results within run and within the two lots of reagents contained in the antisera segments. Patterns corresponded to the type of each tested sample.

Reproducibility between runs and lot-to-lot

Reproducibility between runs was demonstrated on seven different pathological samples with monoclonal or biclonal components and on one normal sample, exhibiting different protein concentrations. Four samples (with a ratio urine < 0.55) were analyzed with CAPILLARYS IMMUNOTYPING URINE procedure and "HYPOGAMMA" dilution program, 2 samples (with a ratio urine comprised between 0.55 and 1.55) with "STANDARD" dilution program and 2 samples (with a ratio urine > 1.55) 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.
The analysis of the normal sample showed a polyclonal pattern without any abnormal component.

Concordance Study

Concordance study for paraproteins and free light chains characterization was performed on 33 different pathological urine samples (with paraproteins or Kappa or Lambda free light chains), on 22 samples with polyclonal pattern and on 6 normal samples, between CAPILLARYS IMMUNOTYPING URINE procedure and immunofixation on agarose gels, HYDRAGEL 9 BENCE JONES, SEBIA (reference procedure). All 61 samples have been run on both techniques.
This study demonstrated a 100 % agreement between the 2 techniques:
For the 6 normal urine samples: complete agreement (concordance).
For the 22 urine samples with polyclonal pattern: complete agreement (concordance).
For the 33 pathological urine samples: complete agreement (concordance).
For the analyzed pathological urine samples, the results obtained with the individual procedures were in agreement and identical bands were detected on all pathological samples with each system.

Sensitivity

Serial dilutions were prepared with typical urine samples exhibiting Bence Jones proteins and were analyzed with CAPILLARYS IMMUNOTYPING URINE technique. The minimal detection limit for free light chains was about 2.5 mg/dL with the analyzed urine samples and with anti-Kappa and anti-Lambda antisera contained in the CAPILLARYS IMMUNOTYPING kit.

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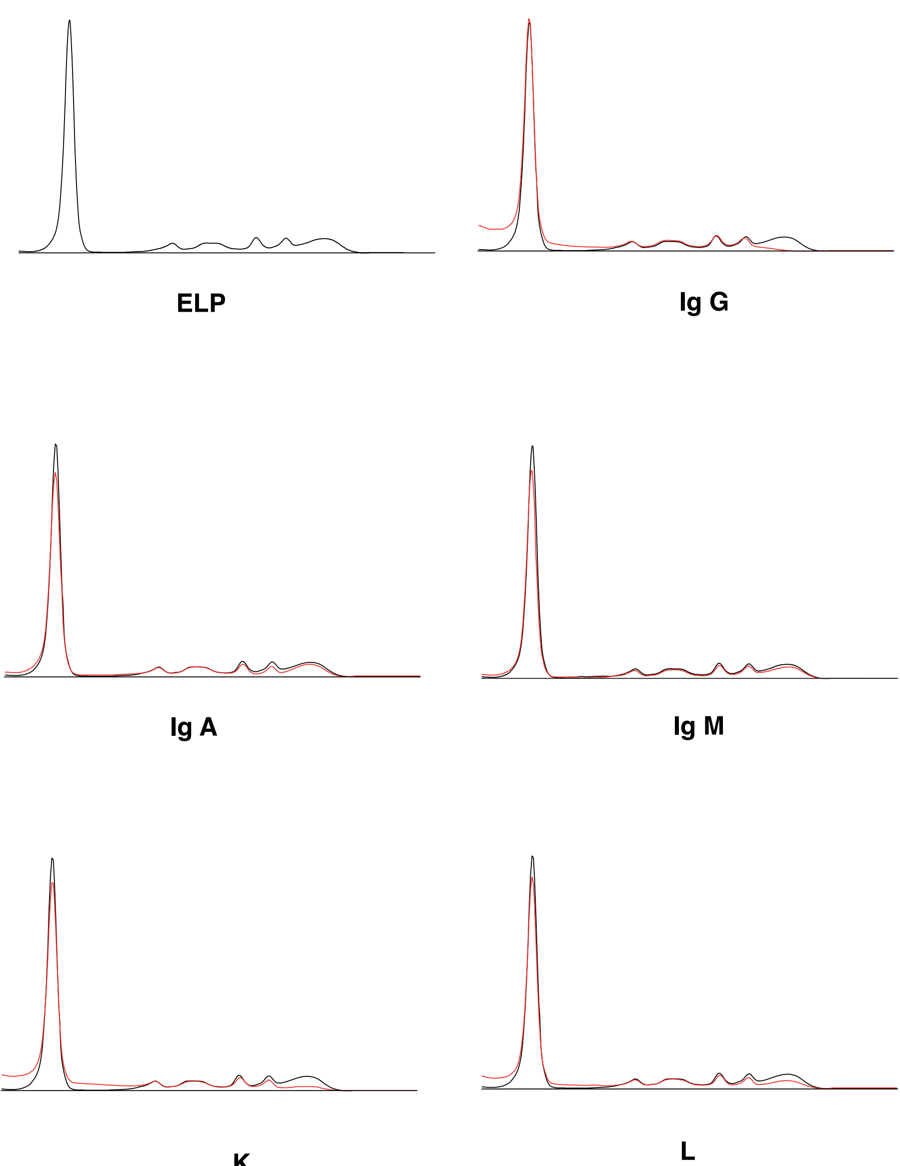
SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Normal / Normal serum

Figure 1

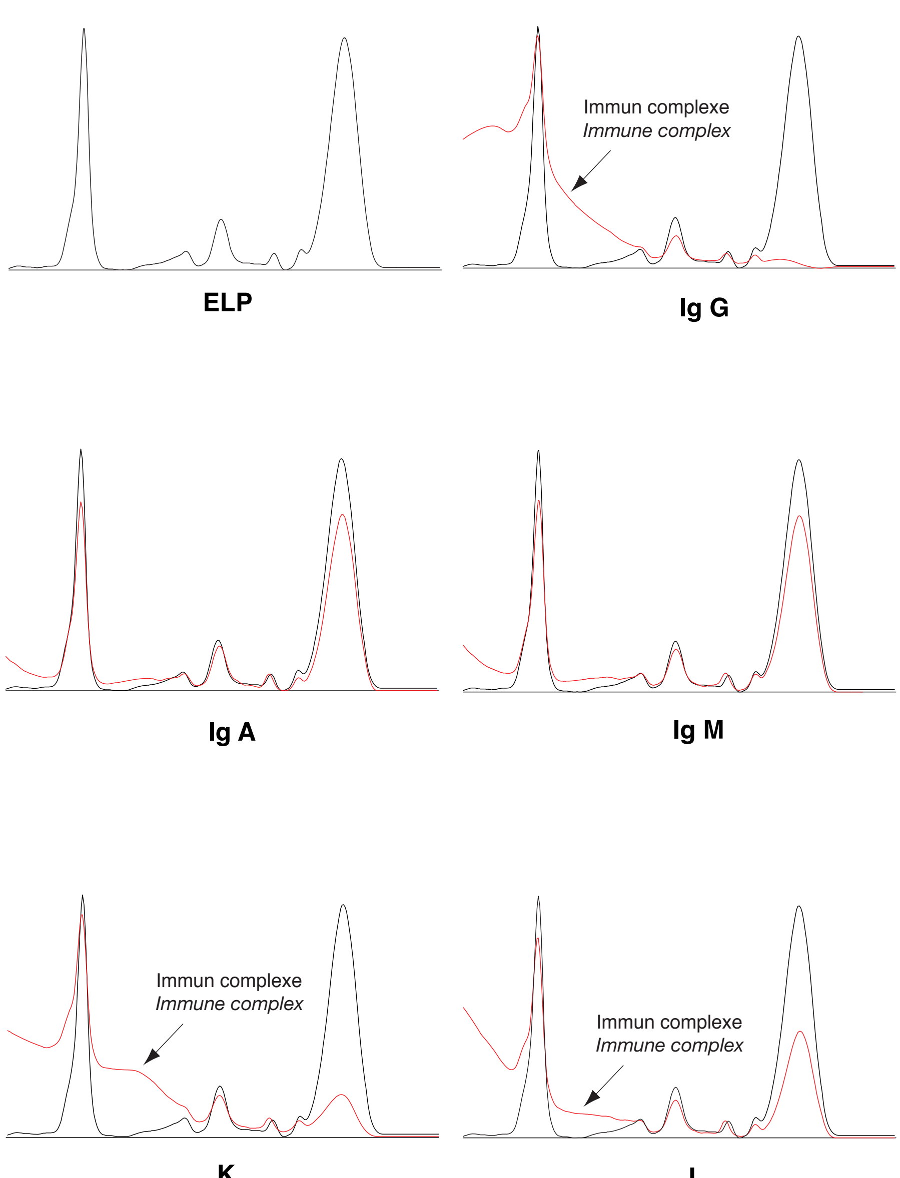


SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Hypergamma / Hypergamma serum

Figure 2



SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Protéine monoclonale 0,5 g/L / Monoclonal component 0.5 g/L

Figure 3

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein

ELP

Ig G

Ig A

Ig M

K

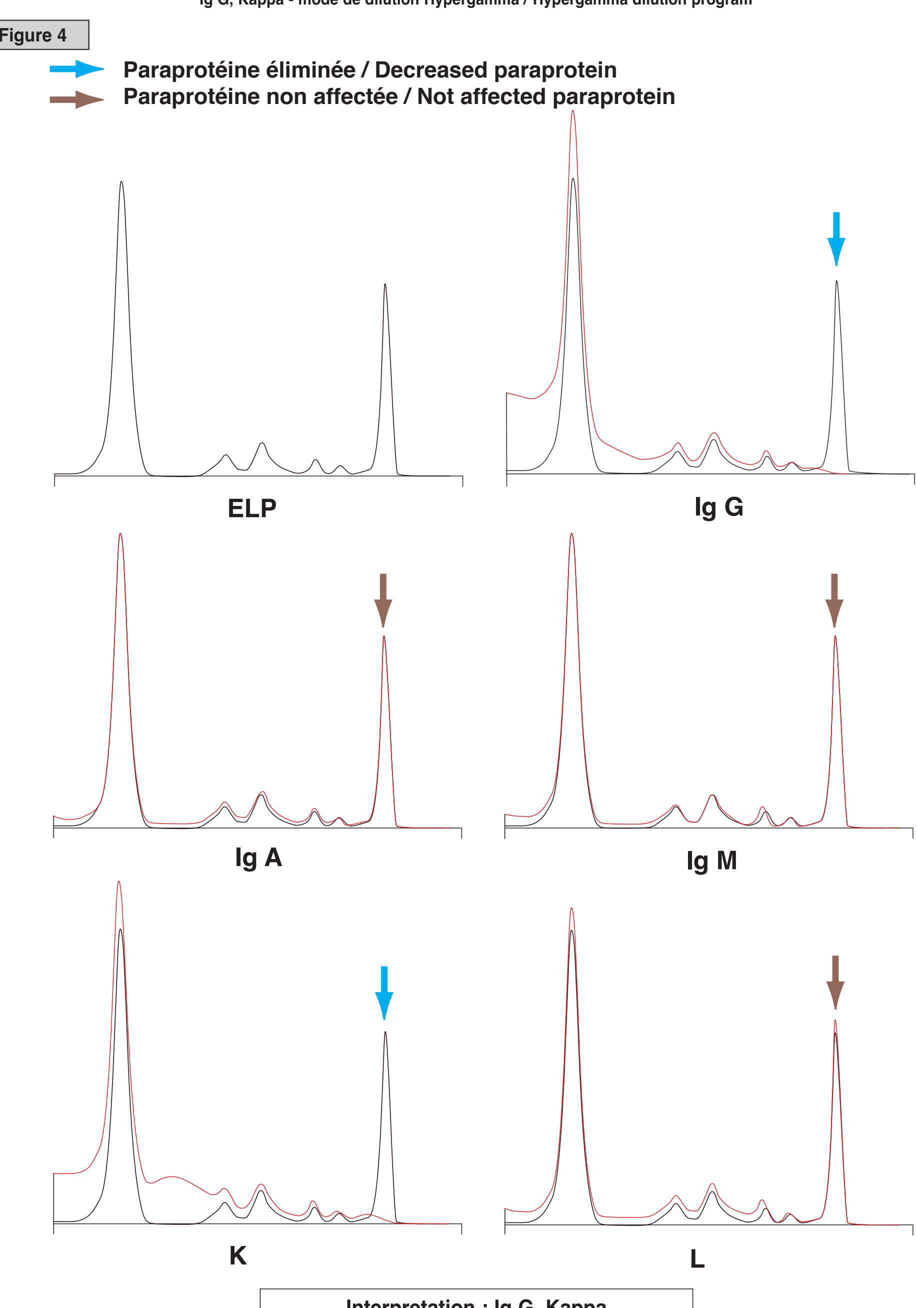
L

Interpretation : Ig G, Lambda

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Ig G, Kappa - mode de dilution Hypergamma / Hypergamma dilution program



SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Profil biclonal / Biclonal pattern : Ig G, Kappa + Ig M, Kappa

Figure 5

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein

ELP

Ig G

Ig A

Ig M

K

L

Interpretation : Ig G, Kappa + Ig M, Kappa

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Profil biclonal / Biclonal pattern : Ig G, Kappa + Ig G, Lambda

Figure 6

Paraprotéine éliminée / Decreased paraprotein

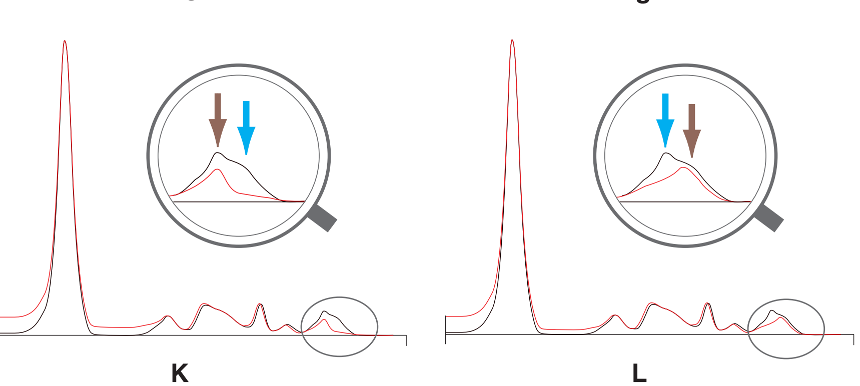
Paraprotéine non affectée / Not affected paraprotein

ELP

Ig G

Ig A

Ig M



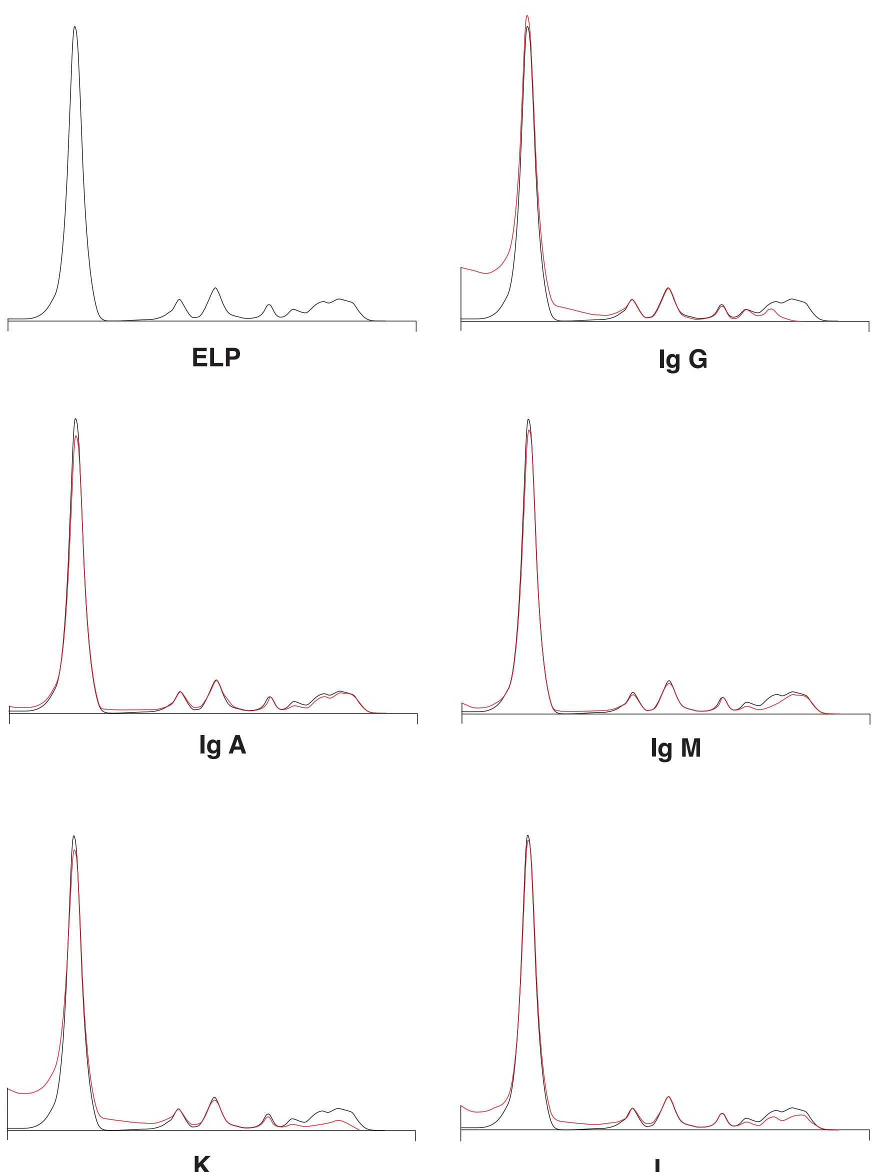
Interpretation : Ig G, Kappa + Ig G, Lambda

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Profil oligoclonal / Oligoclonal pattern

Figure 7



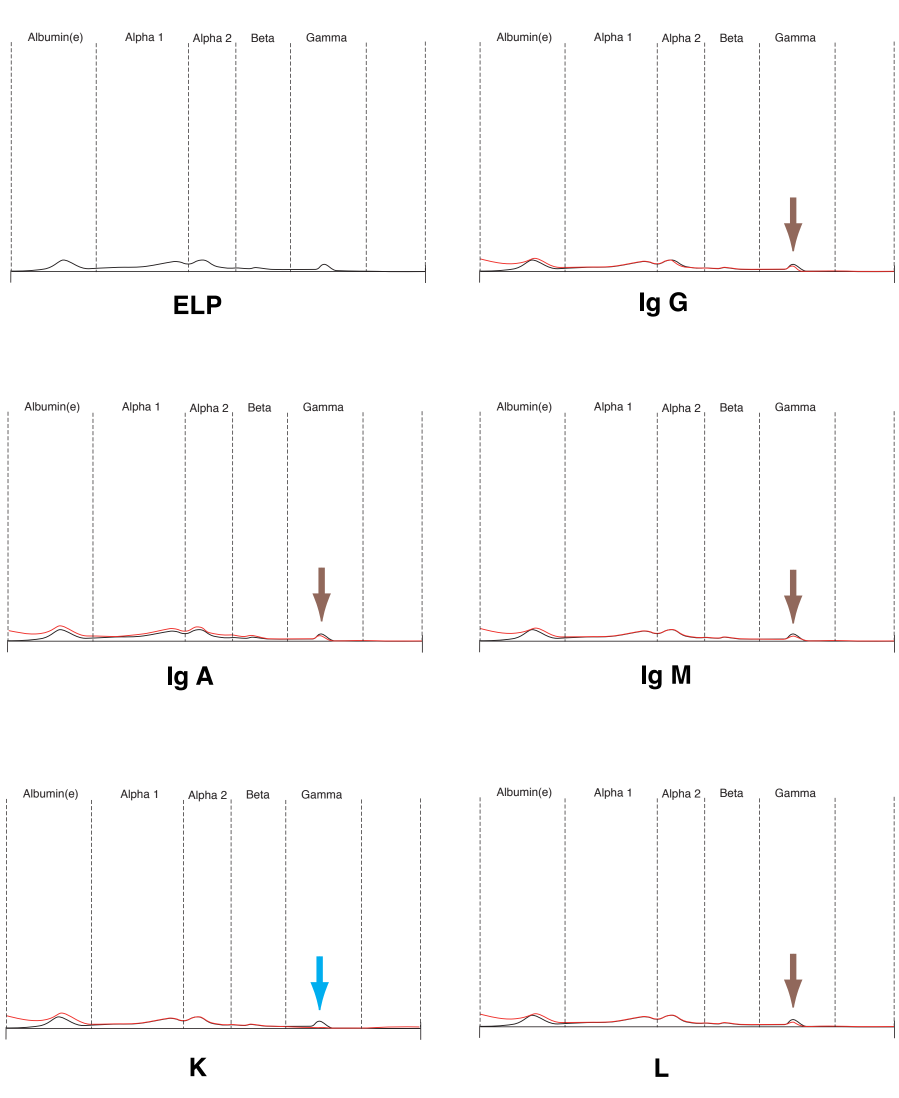
SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

Proteine monoclonale / Monoclonal component – mode de dilution Hypogamma / Hypogamma dilution program

Figure 8

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

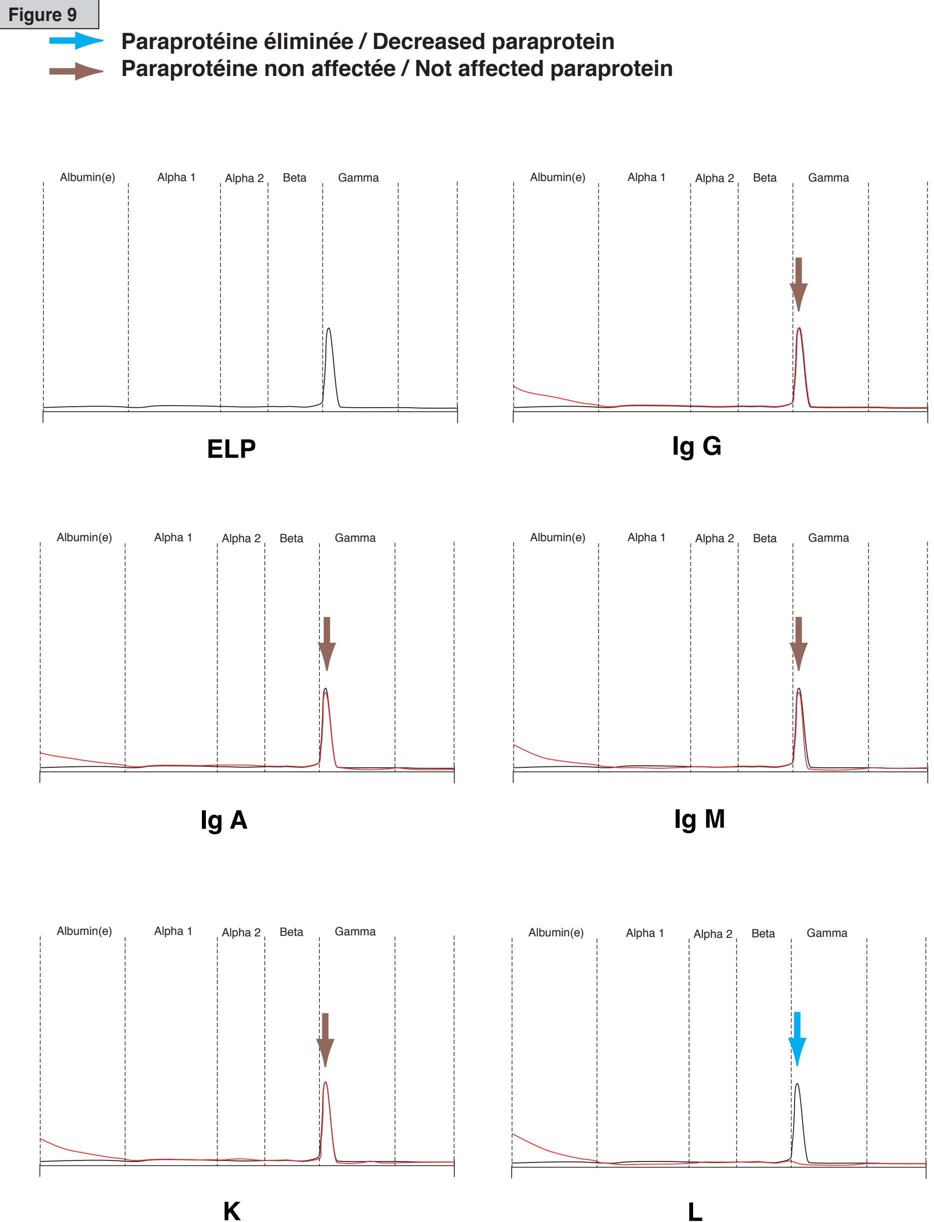


Interpretation :	Suspicion de chaîne légère libre Kappa (à confirmer) Kappa free light chain suspected (to confirm)

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

Proteine monoclonale / Monoclonal component – mode de dilution Hypergamma / Hypergamma dilution program



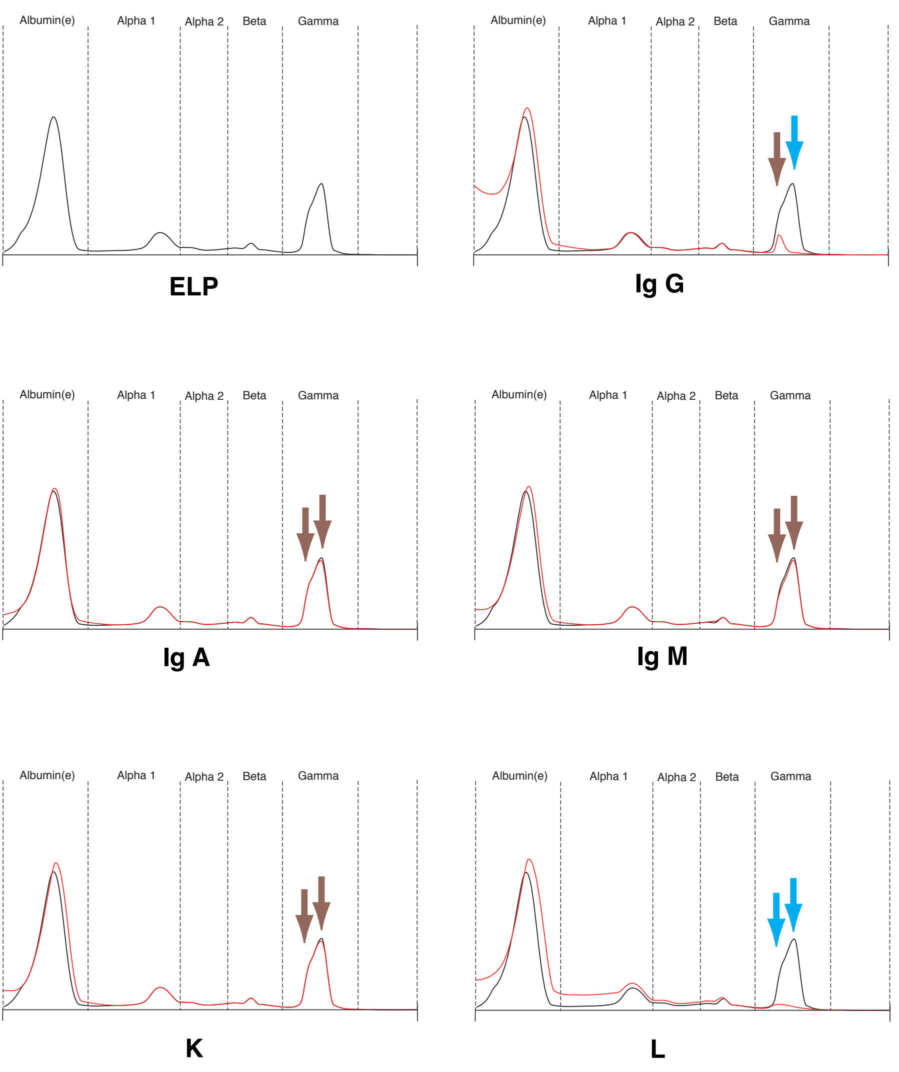
Interpretation : **Suspicion de chaîne légère libre Lambda (à confirmer)**
Lambda free light chain suspected (to confirm)

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

Profil biclonal / Biclonal pattern – mode de dilution Hypogamma / Hypogamma dilution program

Figure 10



Interpretation : Ig G, Lambda + suspicion de chaîne légère libre Lambda (à confirmer)
Ig G, Lambda + Lambda free light chain suspected (to confirm)

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