CAPI 3 IMMUNOTYPING

Ref. 2600

Người đại diện hợp pháp của cơ sở
Tổng Giám Đốc

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INTENDED USE

The CAPI 3 IMMUNOTYPING kit is designed for the detection and the characterization of monoclonal proteins (immunotyping) in human urine and serum with the CAPILLARYS 3 instrument, SEBIA, for capillary electrophoresis. It is used in conjunction with the CAPI 3 PROTEIN(E) 6 kit, SEBIA, designed for proteins separation into 6 major fractions in alkaline buffer (pH 9.9).
The CAPILLARYS 3 instrument 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 3" is used for the SEBIA automated instruments,

- *CAPILLARYS 3 OCTA, CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA for serum analysis,*
- *CAPILLARYS 3 OCTA and CAPILLARYS 3 TERA for urine analysis.*

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. Besides the electrophoresis techniques performed on different media, including agarose gel, the capillary electrophoresis has been developed to provide complete automation of this testing with fast separation and good resolution. It is defined as a technique of electrokinetic separation carried out in a tube of internal diameter lower than 100 µm filled with a buffer composed of electrolytes. In many aspects, the methodology can be considered as an intermediary type of technique between classical zone electrophoresis and liquid chromatography.
The CAPILLARYS 3 instrument uses the principle of capillary electrophoresis in free solution that is the most common form of capillary electrophoresis. 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 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 the CAPI 3 IMMUNOTYPING and CAPI 3 IMMUNOTYPING URINE procedures, the immunotyping is performed with specific antibodies to identify these abnormal fractions.
The CAPILLARYS 3 instrument has silica capillaries functioning in parallel allowing 6 simultaneous analyses (CAPILLARYS 3 OCTA) or 12 simultaneous analyses (CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA). For the immunotyping, a sample dilution is prepared and injected simultaneously by aspiration at the anodic end of six capillaries. The reference pattern (ELP pattern) is obtained by injection of the sample mixed with ELP solution in the 1st capillary providing a complete electrophoretic pattern of the sample's proteins. The antisera patterns are obtained by injection in the 5 following capillaries 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 : With the buffer used at alkaline pH, 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 in the pre-dilution well of the reagent cup. This dilution is made according to the sample's immunoglobulins concentration.
   NOTE : With the HYPOGAMMA dilution program of the CAPI 3 IMMUNOTYPING URINE procedure, the dialyzed urine is directly applied in 6 wells of the reagent cup without any previous dilution with the sample diluent.
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 in alkaline buffer. The separated proteins are detected at the cathodic end of the capillary at 200 nm.
4. Overlay of the ELP pattern with the antisera patterns (Ig G, Ig A, Ig M, Kappa and Lambda) allows to characterize the suspected monoclonal component.

Serum and urine samples can both be analyzed with the version 1.20 of the CAPILLARYS 3 instrument and the version 9.20 of the PHORESIS software, and following versions.

REAGENTS SUPPLIED IN THE CAPI 3 IMMUNOTYPING KIT

WARNING : See the safety data sheets.

ITEMS	PN 2600
Sample diluent (ready to use)	1 vial, 60 mL
Pierceable cap for the Sample diluent vial	1 cap
Rack with ELP solution and antiserum tubes	
ELP solution (ready to use)	1 vial, 1.2 mL
Mammalian immunoglobulins anti-human gamma heavy chains (ready to use)	1 vial, 1.2 mL
Mammalian immunoglobulins anti-human alpha heavy chains (ready to use)	1 vial, 1.2 mL
Mammalian immunoglobulins anti-human mu heavy chains (ready to use)	1 vial, 1.2 mL
Mammalian immunoglobulins anti-human kappa (free and bound) light chains (ready to use)	1 vial, 1.2 mL
Mammalian immunoglobulins anti-human lambda (free and bound) light chains (ready to use)	1 vial, 1.2 mL

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

FOR OPTIMAL MANAGEMENT OF TRACEABILITY : All reagents from the same kit must be used together.
TO OBTAIN THE EXPECTED PERFORMANCES : The package insert instructions must be observed.

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.

1. CAPI 3 IMMUNOTYPING SAMPLE DILUENT

Preparation

The sample diluent is ready to use. It contains : buffer solution pH 9.4 ± 0.5 ; additives, non-hazardous at concentrations used, necessary for optimum performance.

Use

Specific diluent for automatic dilution of samples for protein analysis in capillary electrophoresis with the CAPILLARYS 3 instrument. It contains a marker allowing an optimal overlay of the electrophoretic patterns.
Place directly the vial in the secondary compartment of the CAPILLARYS 3 instrument in position S1, S2 or S3 **after having replaced the cap of the vial by the pierceable cap from the kit** (place the side of the vial with the RFID label towards the left) (see "PHOTOS").

NOTE : The pierceable cap may stay all the time on the sample diluent vial if the vial is in a vertical position. Do not turn the vial upside down to avoid leakages.

Storage, stability and signs of deterioration

Store the sample diluent at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). Before the first use, it is stable until the expiration date indicated on the kit package or sample diluent vial labels.
The sample diluent is stable for a maximum of 2 months (accumulated) in the secondary compartment of the CAPILLARYS 3 instrument.

IMPORTANT : The accumulated time of the sample diluent placed in the CAPILLARYS 3 instrument must not exceed 2 months.

In the case of an occasional use, remove the sample diluent vial from the instrument after the end of the analyses and store it at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C) without any delay.
The sample diluent must be free of precipitate.
DO NOT FREEZE.

2. RACK WITH ELP SOLUTION AND ANTISERUM TUBES

The rack with ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera tubes is ready to use. It is shaped with a foolproof system to fit correctly in the temperature controlled part of the secondary compartment of the CAPILLARYS 3 instrument.
Place directly the rack in the secondary compartment of the CAPILLARYS 3 instrument (place the side of the rack with the RFID label towards the right).

IMPORTANT : Place the rack into the CAPILLARYS 3 instrument just before starting the analyses. After the end of the analyses, if the rack is removed from the instrument, store it refrigerated (2 - 8 °C) immediately.

WARNING : When the rack is let in the instrument, do not switch off the instrument, in order to maintain the controlled temperature from the secondary compartment.

2.1. ELP SOLUTION

Preparation

The ELP solution is ready to use. It contains : buffer solution pH 7.4 ± 0.5 ; additives, non-hazardous at concentrations used, necessary for optimum performance.
For easy identification of the ELP solution, the ELP solution is colored (yellow) with a non-hazardous dye.

Use

To obtain a reference electrophoretic pattern of the sample (ELP pattern).

Storage, stability and signs of deterioration

Store the ELP solution refrigerated (2 to 8 °C) on the rack. Before the first use, it is stable until the expiration date indicated on the kit package or the rack label.
The ELP solution vial located on the rack is stable for a maximum of **2 months** (accumulated) in the secondary compartment of the CAPILLARYS 3 instrument.

IMPORTANT : The accumulated time of the ELP solution placed in the CAPILLARYS 3 instrument must not exceed **2 months**.

In the case of an occasional use, remove the rack from the instrument after the end of the analyses and store it refrigerated (between 2 and 8 °C) without any delay.
The ELP solution must be free of precipitate.
DO NOT FREEZE.

2.2. ANTISERA

Preparation

The antisera are ready to use. Each vial contains respectively : mammalian immunoglobulins anti-human gamma heavy chains (pink), anti-human alpha heavy chains (dark blue), anti-human mu heavy chains (yellow green), anti-human kappa (free and bound) light chains (light green), anti-human lambda (free and bound) light chains (light blue) and additives, non-hazardous at concentrations used, necessary for optimum performance.
For easy identification of antisera, the antisera are colored with distinct non-hazardous dyes.

Use

For protein immunotyping on the CAPILLARYS 3 instrument by capillary electrophoresis.

IMPORTANT : The antisera are specific for the CAPI 3 IMMUNOTYPING procedure. They must not be used in any way for immunofixation procedures on agarose gels and vice versa.

Storage, stability and signs of deterioration

Store the antisera refrigerated (2 to 8 °C) on the rack. Before the first use, they are stable until the expiration date indicated on the kit package or the rack label.
The antiserum vials located on the rack are stable for a maximum of **2 months** (accumulated) in the secondary compartment of the CAPILLARYS 3 instrument.

IMPORTANT : The accumulated time of the antisera placed in the CAPILLARYS 3 instrument must not exceed **2 months**.

In the case of an occasional use, remove the rack from the instrument after the end of the analyses and store it refrigerated (between 2 and 8 °C) without any delay.
The antisera must be free of precipitate.
DO NOT FREEZE.

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

ANALYSIS OF SERUM SAMPLES : CAPI 3 IMMUNOTYPING PROCEDURE

REAGENTS REQUIRED (but not supplied with the CAPI 3 IMMUNOTYPING kit)

WARNING : See the safety data sheets.

1. CAPI 3 PROTEIN(E) 6 KIT (SEBIA, PN 2503)

Presentation, use, storage, stability and signs of deterioration

See the instruction sheet of the kit.

2. DISTILLED OR DEIONIZED WATER

Use

For capillaries rinsing in automated instrument for capillary electrophoresis CAPILLARYS 3, SEBIA.
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 CAPILLARYS 3

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2060, 1 vial of 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in automated instrument for capillary electrophoresis CAPILLARYS 3, SEBIA, 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 CAPILLARYS 3 and the instruction manual of CAPILLARYS 3, SEBIA.

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 3 instrument (weekly maintenance in order to eliminate adsorbed proteins from the probe).
See the instruction manual of CAPILLARYS 3, SEBIA.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature (15 to 30 °C) 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 3 WASH SOLUTION

Preparation

The vial of the stock wash solution (SEBIA, PN 2062, 1 vial, 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 after electrophoretic separation.

IMPORTANT :

- When wash solution vial replacement, change systematically the filter. Wear clean gloves for handling and installation of the filter.
- Before placing the wash solution vial in the instrument, it is recommended to wash the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.
- Screw the filter at the connector situated at the extremity of the tube plunging in the wash solution vial.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C).
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.
After dilution and immediate installation of the vial in the instrument, the solution is stable for 3 months (if the working wash solution is stored out of the instrument before use, this time of 3 month storage must take into account the time during which the solution is stored outside the instrument).
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)

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. SEBIA CAPILLARYS 3 instrument for capillary electrophoresis : CAPILLARYS 3 OCTA PN 1245, CAPILLARYS 3 TERA PN 1246 or CAPILLARYS 3 TERA TLA PN 1248, connected to a computer equipped with the PHORESIS software for data processing.
2. Sample racks supplied with CAPILLARYS 3 instrument.
3. Container Kit supplied with CAPILLARYS 3 instrument : Rinse vial (to fill with distilled or deionized water), wash solution vial, waste container and external waste container (for CAPILLARYS 3 TERA TLA).
4. CAPILLARYS 3 & MC SWITCH RACK FOR IMMUNOTYPING (1), SEBIA, PN 1382, to launch automatically a technique change to IMMUNOTYPING procedure on the CAPILLARYS 3 instrument.
5. CAPI 3 REAGENT CUPS (24 x 14), SEBIA, PN 2582, including 24 packs of 14 CAPI 3 reagent cups : Single use cups for the preparation of biological samples to analyze with the automated instrument. To be placed on the automated loading system for cups of CAPILLARYS 3. One reagent cup is intended for the analysis of 1 sample with CAPILLARYS 3 OCTA and 2 samples with CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA, with the CAPI 3 IMMUNOTYPING procedure.

   WARNING : After use, reagent cups with biological samples have to be handled with care. When the analysis is completed, reagent cups must be discarded with biological waste products and they must NEVER be reused.

   Storage : Before use, store the reagent cups in their sealed package in a clean and dry place and at a temperature comprised between 2 and 30 °C.
6. CAPI 3 BINS FOR USED REAGENT CUPS (5), SEBIA, PN 2581 : Bins intended for automated collection of used reagent cups in CAPILLARYS 3 OCTA and CAPILLARYS 3 TERA. To place at the location intended for this purpose.

   WARNING : Bins containing used reagent cups with biological samples have to be handled with care.
7. Container for recovery of biological waste (not marketed by SEBIA, maximal capacity of 12 litres) : Container intended for automated collection of used reagent cups in CAPILLARYS 3 TERA TLA. To place at the location intended for this purpose.
8. TEST TUBES, SEBIA, PN 9214 : 200 100 mm-tubes for the hypochlorite sodium solution intended for the cleaning of the sample probe, or tubes (without cap) with equivalent dimensions (length comprised between 90 and 100 mm and diameter comprised between 13 and 16 mm).

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 3 instrument is a multiparameter instrument for serum proteins analysis on 6 parallel capillaries in the CAPI 3 IMMUNOTYPING procedure, in the following sequence :

- sample racks identification by RFID (Radio Frequency Identification),
- bar code reading of sample tubes (for up to 8 tubes),
- sample dilution from primary tubes into reagent cups,
- 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 (uncapped) sample tubes in sample racks,
- placement of the sample diluent vial (**with pierceable cap**) in the secondary compartment,
- placement of the rack with the ELP solution and antiserum tubes in the secondary compartment,
- placement of sample racks on the CAPILLARYS 3 instrument,
- removal of sample racks and sample tubes after analysis,
- removal of bins for used reagent cups.

Electrophoretic analysis on CAPILLARYS 3 instrument using the CAPI 3 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) and to select the dilution mode.

PLEASE CAREFULLY READ THE CAPILLARYS 3 INSTRUCTION MANUAL.

I. PREPARATION OF ELECTROPHORETIC ANALYSIS

1. Select samples with abnormal protein fraction by qualitative analysis of the electrophoregrams obtained with the CAPI 3 PROTEIN(E) 6 procedure.
2. Switch on CAPILLARYS 3 instrument and computer.
3. Wait until the instrument is completely initialized.
4. Start the PHORESIS software installed on the computer for data processing.
5. 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).
6. For serum samples analysis, the CAPI 3 IMMUNOTYPING kit is intended to run with "IMMUNOTYPING" analysis program from the CAPILLARYS 3 instrument and CAPILLARYS PROTEIN(E) 6 buffer. To select "IMMUNOTYPING" analysis program and place the CAPILLARYS PROTEIN(E) 6 buffer vial in the instrument, please read carefully the CAPILLARYS 3 instruction manual. If necessary, place the vial with the reconstituted wash solution in the instrument.

   NOTE : It is not necessary to change the buffer vial when switching from CAPI 3 PROTEIN(E) 6 procedure to CAPI 3 IMMUNOTYPING procedure (and vice versa).
7. Take a pack of new reagent cups by holding the handle and place it on the automated loading system for cups of CAPILLARYS 3 ; then, remove the flange (a message will be displayed when reagent cups are missing).
8. Place a new bin for used reagent cups into the CAPILLARYS 3 instrument at the location intended for this purpose.
9. The rack with ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera tubes is ready to use. Insert directly the rack in the secondary compartment at the location intended for this purpose by handling it by the two clips situated on each end ; place the side of the rack with the RFID label towards the right.

   IMPORTANT : Check that the rack is correctly inserted in the secondary compartment before starting the analysis.
10. Replace the cap of the sample diluent vial by the pierceable cap and place the vial in the secondary compartment in position S1, S2 or S3 ; place the side of the vial with the RFID label towards the left (the pierceable cap may stay on the sample diluent vial all the time).

    IMPORTANT : In the case of absence of rack with ELP solution and antisera tubes and / or sample diluent vial, the analysis cannot start and a message will be displayed.
11. The sample rack contains 8 positions for sample tubes. Position up to 8 (uncapped) sample tubes on each sample rack ; the bar code of each 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.
12. Slide the sample rack(s) into the CAPILLARYS 3 instrument through the opening in the right side of the instrument. Up to 15 sample racks can be introduced successively and continuously into the instrument.
13. Remove analyzed sample racks from the plate on the left side of the instrument.
14. In the case of an occasional use, at the end of the analyses, remove the rack with ELP solution and antisera tubes and store it refrigerated (2 – 8 °C) without any delay. Remove the sample diluent vial and store it at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C) without any delay.

    IMPORTANT : A shift of the ELP and antisera patterns may be observed between alpha-2 and beta-1 zones if the tubes are left in the instrument for a prolonged time (more than 2 months).
15. If necessary, take off carefully the bin containing used reagent cups and discard it.

    WARNING : Bins containing used reagent cups with biological samples have to be handled with care.

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Sample rack identification by RFID.
2. Bar codes are read on primary sample tubes.
3. The serum sample is diluted in the sample diluent within the pre-dilution well of the reagent cup and the diluted serum is applied in 6 wells of the cup. ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera are collected and mixed with the diluted serum within each well, the sample probe is rinsed after each collection. The selected dilution program will be performed for each sample. If not selected, the "STANDARD" dilution program will be applied by default.
4. Capillaries are washed.
5. Diluted samples with reagents are injected into capillaries.
6. Migration is carried out under constant voltage for about 4 minutes and the temperature is controlled by Peltier effect.
7. Proteins are detected directly by scanning at 200 nm and data of the obtained protein electrophoretic pattern are transmitted from the instrument to the computer equipped with the software for data processing.

II. RESULTS OF ANALYSIS

At the end of each analysis, the corresponding data are transmitted by the instrument to the software for data processing and protein patterns are then displayed on the screen. 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.
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 shut down procedure of the CAPILLARYS 3 instrument in order to store capillaries in optimal conditions.

IV. FILLING OF REAGENT CONTAINERS AND MANAGEMENT OF DISPOSABLES

The CAPILLARYS 3 instrument has an automatic control for reagents (by using RFID labels) and for disposables (reagent cups and bins for used cups).

IMPORTANT : It is necessary to respect the designed position for wash solution, rinse and waste containers.

On the screen of the CAPILLARYS 3 instrument, the "Main compartment" menu for reagents management displays information 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 3 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 and 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.

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).
- 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).
- 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 3 instrument 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 3 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.

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.

- 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.

Interference and Limitations

NOTE : The common interfering factors with the immunotyping analysis (triglycerides, bilirubin, rheumatoid factor and hemoglobin) were evaluated in studies based on the Clinical Laboratory Standards Institute (CLSI - USA) EP7-A2 guideline "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition".

The results are summarized below :
No interference with the CAPI 3 IMMUNOTYPING procedure was detected due to the serum sample's high concentration of the following interfering factors tested at levels equal to the concentrations listed below :

Interfering factor	Concentration
Triglycerides	3.59 g/dL (41 mM)
Bilirubin	20 mg/dL (342 µM)
Rheumatoid factor	981 IU/mL
Hemoglobin	0.2 g/dL

- See SAMPLES FOR ANALYSIS.
- The use of antisera other than those specific for the CAPI 3 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.
- 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.
- When a monoclonal component is detected by CAPI 3 PROTEIN(E) 6 procedure for protein analysis using the CAPILLARYS 3 instrument and not characterized by CAPI 3 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.
- 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 (Keren, 1998).

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

Repeatability

Six (6) different serum samples, including 1 normal serum sample and 5 pathological serum samples with monoclonal components were run using the CAPI 3 IMMUNOTYPING procedure on all capillaries of the same CAPILLARYS 3 instrument and with one lot of CAPI 3 IMMUNOTYPING kit.
Each sample was analyzed with each reagent (ELP solution, anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda) on all capillaries, including 2 runs per reagent.
In this study, all dilution programs were tested.
For each tested reagent, all samples gave concordant results within run and between capillaries.

Reproducibility between lots and between instruments

Six (6) different serum samples, including 1 normal serum sample and 5 pathological serum samples with monoclonal components were run using the CAPI 3 IMMUNOTYPING procedure on 3 CAPILLARYS 3 instruments and with 3 lots of CAPI 3 IMMUNOTYPING kit.
Each sample was analyzed on 18 runs over 5 working days (at 2 different times of the day).
In this study, all dilution programs were tested.
All samples gave concordant results for all runs on the 3 CAPILLARYS 3 instruments and with the 3 lots of CAPI 3 IMMUNOTYPING kit.

Concordance study

Concordance study was performed on 115 different serum samples between CAPI 3 IMMUNOTYPING procedure and a commercially available capillary electrophoresis system for immunotyping : 12 normal serum samples and 103 pathological serum samples have been run on both techniques.
This study demonstrated a 100 % agreement between the two techniques :

- For the 12 normal serum samples: complete agreement (concordance).
- For the 103 pathological serum samples: complete agreement (concordance).

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 CAPI 3 IMMUNOTYPING procedure.
The results are summarized below :

Sample No.	Monoclonal component			Detection limit (mg/dL)
	Type		Concentration (g/dL)	
1	Ig G, L	Gamma	3.96	30.9
		Lambda		30.9
2	Ig A, K	Alpha	3.40	13.3
		Kappa		13.3
3	Ig M, K	Mu	1.60	25.0
		Kappa		25.0

NOTE : 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 : CAPI 3 IMMUNOTYPING URINE PROCEDURE

REAGENTS REQUIRED (but not supplied with the CAPI 3 IMMUNOTYPING kit)

WARNING : See the safety data sheets.

1. CAPI 3 PROTEIN(E) 6 KIT (SEBIA, PN 2503)

Presentation, use, storage, stability and signs of deterioration

See the instructions for use of the kit.

2. CAPI 3 URINE KIT (SEBIA, PN 2513)

Presentation, storage, stability and signs of deterioration

See the instructions for use of the kit.

Use

For the preparation of urine samples before separation of human urine proteins by capillary electrophoresis with the CAPILLARYS 3 instrument.

3. DISTILLED OR DEIONIZED WATER

Use

For capillaries rinsing in automated instrument for capillary electrophoresis CAPILLARYS 3, SEBIA.
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 CAPILLARYS 3

Composition

The vial of CAPICLEAN concentrated solution (SEBIA, PN 2060, 1 vial of 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For sample probe cleaning in automated instrument for capillary electrophoresis CAPILLARYS 3, SEBIA, 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 instructions for use of CAPICLEAN CAPILLARYS 3 and the instruction manual of CAPILLARYS 3, SEBIA.

Storage, stability and signs of deterioration

Store CAPICLEAN refrigerated (between 2 and 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 % chlorine) by diluting 250 mL 9.6 % chlorine concentrated solution to 1 liter with cold distilled or deionized water.

Use

For the sample probe cleaning in the CAPILLARYS 3 instrument (weekly maintenance in order to eliminate adsorbed proteins from the probe).

See the instruction manual of CAPILLARYS 3, SEBIA.

Storage, stability and signs of deterioration

Store the working chlorinated solution at room temperature (15 to 30 °C) 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 3 WASH SOLUTION

Preparation

The vial of the stock wash solution (SEBIA, PN 2062, 1 vial, 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 after electrophoretic separation.

IMPORTANT :

- When wash solution vial replacement, change systematically the filter. Wear clean gloves for handling and installation of the filter.
- Before placing the wash solution vial in the instrument, it is recommended to wash the connector and the tube with plenty of distilled or deionized water to avoid salts deposit.
- Screw the filter at the connector situated at the extremity of the tube plunging in the wash solution vial.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C).
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.
After dilution and immediate installation of the vial in the instrument, the solution is stable for 3 months (if the working wash solution is stored out of the instrument before use, this time of 3 month storage must take into account the time during which the solution is stored outside the instrument).
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. SEBIA CAPILLARYS 3 instrument for capillary electrophoresis : CAPILLARYS 3 OCTA PN 1245 or CAPILLARYS 3 TERA PN 1246, connected to a computer equipped with the PHORESIS software for data processing.
2. Sample racks supplied with CAPILLARYS 3 instrument.
3. Container Kit supplied with CAPILLARYS 3 instrument : Rinse (to fill with distilled or deionized water), wash solution and waste container.
4. CAPILLARYS 3 & MC SWITCH RACK FOR IMMUNOTYPING URINE (1), SEBIA, PN 1379, to launch automatically a technique change to IMMUNOTYPING URINE procedure on the CAPILLARYS 3 instrument.
5. CAPI 3 REAGENT CUPS (24 x 14), SEBIA, PN 2582, including 24 packs of 14 CAPI 3 reagent cups : Single use cups for the preparation of biological samples to analyze with the automated instrument. To be placed on the automated loading system for cups of CAPILLARYS 3. One reagent cup is intended for the analysis of 1 sample with CAPILLARYS 3 OCTA and 2 samples with CAPILLARYS 3 TERA, with the CAPI 3 IMMUNOTYPING URINE procedure.

   WARNING : After use, reagent cups with biological samples have to be handled with care. When the analysis is completed, reagent cups must be discarded with biological waste products and they must NEVER be reused.

   Storage : Before use, store the reagent cups in their sealed package in a clean and dry place and at a temperature comprised between 2 and 30 °C.
6. CAPI 3 BINS FOR USED REAGENT CUPS (5), SEBIA, PN 2581 : Bins intended for automated collection of used reagent cups in CAPILLARYS 3 OCTA and CAPILLARYS 3 TERA. To place at the location intended for this purpose.

   WARNING : Bins containing used reagent cups with biological samples have to be handled with care.
7. TEST TUBES, SEBIA, PN 9214 : 200 100 mm-tubes for the hypochlorite sodium solution intended for the cleaning of the sample probe, or tubes (without cap) with equivalent dimensions (length comprised between 90 and 100 mm and diameter comprised between 13 and 16 mm).
8. Hemolysing tubes (75 mm high and 13 mm in diameter).

SAMPLES FOR ANALYSIS

Sample collection and storage

The analysis is carried out preferentially on urine collected over 24 hours. Urine samples must be collected following established procedures used in clinical laboratory testing.
Samples may be stored refrigerated for up to 1 week between 2 and 8 °C or for 1 month at - 70 / - 80 °C.

IMPORTANT : Do not store samples at - 18 / - 30 °C.

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

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

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

Before analysis, prepare urine samples according to the preparation procedure of dialysis and concentration described in the package insert of the CAPI 3 URINE kit (see paragraph “Reagents required but not supplied").
Use directly these prepared urine samples.

IMPORTANT : The urine analysis technique by capillary electrophoresis requires 2 steps : a dialysis step and a concentration step of urine samples (for example, with SEBIA dialysis systems, 20 mL tubes). Use only one system per sample. Then, the collected dialyzed and concentrated urine sample can be analyzed with CAPI 3 URINE and CAPI 3 IMMUNOTYPING URINE procedures.

Analyze the urine samples within a maximum of one day (24 hours) 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.
- Do not store samples in dialysis and concentration devices, some proteins may bind to the membrane.
- Do not use urine samples with turbidity or red coloration (case of hemolysis) observed after the first centrifugation.

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 3 instrument is a multiparameter instrument for urine proteins analysis on 6 parallel capillaries in the CAPI 3 IMMUNOTYPING URINE procedure, in the following sequence :

- sample racks identification by RFID (Radio Frequency Identification),
- bar code reading of urine samples to analyze (up to 8),
- sample dilution into reagent cups (for STANDARD and HYPERGAMMA dilution programs) or direct application (for HYPOGAMMA dilution program),
- mixing diluted urine samples with ELP solution / specific antisera,
- capillary washing,
- injection of samples,
- protein separation and direct detection of the separated proteins on capillaries.

The manual steps include :

- placement of microtubes (without cap) 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 the sample diluent vial (**with pierceable cap**) in the secondary compartment,
- placement of the rack with the ELP solution and antiserum tubes in the secondary compartment,
- placement of sample racks on the CAPILLARYS 3 instrument,
- removal of sample racks and sample tubes after analysis,
- removal of bins for used reagent cups.

Electrophoretic analysis on the CAPILLARYS 3 instrument using CAPI 3 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 3 INSTRUCTION MANUAL.

I. PREPARATION OF ELECTROPHORETIC ANALYSIS

1. Select samples with abnormal protein fraction after qualitative analysis of the electrophoregrams obtained with CAPI 3 URINE procedure.
2. Switch on CAPILLARYS 3 instrument and computer.
3. Wait until the instrument is completely initialized.
4. Start the PHORESIS software installed on the computer for data processing.
5. 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 CAPI 3 URINE kit instructions for use). The "Ratio urine" is defined by the proportion of the abnormal fraction area related to the Normal Control Serum proteins total area.
6. 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 CAPI 3 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.*
7. For urine samples analysis, the CAPI 3 IMMUNOTYPING kit is intended to run with "IMMUNOTYPING URINE" analysis program from the CAPILLARYS 3 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 3 instruction manual. If necessary, place the vial with the reconstituted wash solution in the instrument.

   NOTE : It is not necessary to change the buffer vial when switching from CAPI 3 URINE procedure to CAPI 3 IMMUNOTYPING URINE procedure (and vice versa).

8. Take a pack of new reagent cups by holding the handle and place it on the automated loading system for cups of CAPILLARYS 3 ; then, remove the flange (a message will be displayed when reagent cups are missing).
9. Place a new bin for used reagent cups into the CAPILLARYS 3 instrument at the location intended for this purpose.
10. The rack with ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera tubes is ready to use.
    Insert directly the rack in the secondary compartment at the location intended for this purpose by handling it by the two clips situated on each end ; place the side of the rack with the RFID label towards the right.
    IMPORTANT : Check that the rack is correctly inserted in the secondary compartment before starting the analysis.
11. Replace the cap of the sample diluent vial by the pierceable cap and place the vial in the secondary compartment in position S1, S2 or S3 ; place the side of the vial with the RFID label towards the left (the pierceable cap may stay on the sample diluent vial all the time).
    IMPORTANT : In the case of absence of rack with ELP solution and antisera tubes and / or sample diluent vial, the analysis cannot start and a message will be displayed.
12. The sample rack contains 8 positions for sample tubes.
    - Place up to 8 empty hemolysing tubes (used as holders) on each sample rack, and then, the microtubes containing the dialyzed urines samples. Cut the cap of each microtube before using it.
    - Keep the cap of each microtube for further storage of samples, if necessary.

    IMPORTANT : It is NECESSARY to identify each hemolysing tube holding the microtube which contains the sample to analyze, with the specific sample identification bar code label corresponding to the sample. 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.
13. Slide the sample rack(s) into the CAPILLARYS 3 instrument through the opening in the right side of the instrument. Up to 15 sample racks can be introduced successively and continuously into the instrument.
14. Remove analyzed sample racks from the plate on the left side of the instrument.
15. In the case of an occasional use, at the end of the analyses, remove the rack with ELP solution and antisera tubes and store it refrigerated (2 – 8 °C) without any delay. Remove the sample diluent vial and store it at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C) without any delay.
16. If necessary, take off carefully the bin containing used reagent cups and discard it.
    WARNING : Bins containing used reagent cups with biological samples have to be handled with care.

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Sample rack identification by RFID.
2. Bar codes are read on tubes with urine samples to analyze.
3. The selected dilution program is automatically performed for each sample. If not selected, the "HYPOGAMMA" dilution program is applied by default.
   "HYPOGAMMA" dilution program : Application of the dialyzed urine sample in 6 wells of the reagent cup.
   NOTE : With this dilution program, the dialyzed urine is directly applied in the reagent cup without any previous dilution with the sample diluent. For analyses performed with the HYPOGAMMA dilution program, the sample diluent consumption is not counted for the diluent vial level. It is however necessary to place the sample diluent vial in the secondary compartment (a message will be displayed when the sample diluent vial is missing).
   "STANDARD" or "HYPERGAMMA" dilution programs : The dialyzed urine sample is diluted in the sample diluent within the pre-dilution well of the reagent cup and the diluted serum is applied in 6 wells.
   Then, for the 3 dilution programs : ELP solution and anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera are collected and mixed with the urine within each well, the sample probe is rinsed after each collection.
4. Capillaries are washed.
5. Diluted samples are injected into capillaries.
6. Migration is carried out under constant voltage, controlled by Peltier effect for about 4 minutes.
7. Proteins are detected directly by scanning at 200 nm and data of the obtained protein electrophoretic patterns are transmitted from the instrument to the computer equipped with the software for data processing.

II. RESULT ANALYSIS

At the end of each analysis, the corresponding data are transmitted by the instrument to the software for data processing and the protein profiles appear on the screen of the computer. Each antiserum pattern (Ig G, Ig A, Ig M, Kappa and Lambda) is automatically superimposed over the ELP pattern.
If a monoclonal protein 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 start the shut down procedure of the CAPILLARYS 3 instrument in order to store capillaries in optimal conditions.

IV. FILLING OF REAGENT CONTAINERS AND MANAGEMENT OF DISPOSABLES

The CAPILLARYS 3 instrument has an automatic control for reagents (by using RFID labels) and for disposables (reagent cups and bins for used cups).
IMPORTANT : It is necessary to respect the designed position for wash solution, rinse and waste containers.

On the screen of the CAPILLARYS 3 instrument, the "Main compartment" menu for reagents management displays information 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 3 INSTRUCTION MANUAL.

RESULTS

Interpretation for urine samples analysis

Absence of a monoclonal protein

- 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 protein

- 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 pattern (ELP pattern).
- 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 proteins

- 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 (drug or salts for example). When an interferent fraction is suspected, it is recommended to dialyse again the urine sample with dialysis buffer.
- Hemoglobin is commonly known to migrate in the beta zone 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 Immunofixation kits may be necessary.
- Faint shifts between the ELP pattern and the superimposed antisera patterns may be observed. They must not be considered as the result of the disappearance of a monoclonal fraction on one or more antisera pattern.
- Polymerized free light chains in the sample may give variable electrophoretic separations.
- The use of antisera other than those specific for the CAPI 3 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 i) cleaning and waste disposal, ii) labeling and safety rules applied by SEBIA, iii) packaging of biological samples for the transportation, and iv) instruments cleaning are available on the SEBIA's extranet website: www.sebia.com.

PERFORMANCE DATA

Repeatability

Four (4) different urine samples with monoclonal proteins including Bence Jones proteins were run using the CAPI 3 IMMUNOTYPING URINE procedure on all capillaries of the same CAPILLARYS 3 instrument and with one lot of CAPI 3 IMMUNOTYPING kit.

Each sample was analyzed with each reagent (ELP solution, Anti-Ig G, Anti-Ig A, Anti-Ig M, Anti-Kappa and Anti-Lambda) on all capillaries, including 2 runs per reagent.

In this study, all dilution programs were tested.

For each tested reagent, all samples gave concordant results within run and between capillaries.

Sample No.	Type	Within run	Between capillaries	Total analyses per reagent per sample
1	Ig G, Kappa	Concordant result	Concordant result	12
2	2 Lambda free	Concordant result	Concordant result	12
3	Ig M, Kappa + Kappa free	Concordant result	Concordant result	12
4	Ig A, Lambda + Lambda free	Concordant result	Concordant result	12

Reproducibility between lots and between instruments

Three (3) different urine samples with monoclonal proteins including Bence Jones proteins were run using the CAPI 3 IMMUNOTYPING URINE procedure on 3 CAPILLARYS 3 instruments and with 3 lots of CAPI 3 IMMUNOTYPING kit.

Each sample was analyzed on 18 runs over 5 working days (at 2 different times of the day).

In this study, all dilution programs were tested.

All samples gave concordant results for all runs on the 3 CAPILLARYS 3 instruments and with the 3 lots of CAPI 3 IMMUNOTYPING kit.

Sample No.	Type	Run No.	Instrument No. 1			Instrument No. 2			Instrument No. 3			Total analyses per sample
			Lot No. 1	Lot No. 2	Lot No. 3	Lot No. 1	Lot No. 2	Lot No. 3	Lot No. 1	Lot No. 2	Lot No. 3	
			Day No. 1	Day No. 1	Day No. 2	Day No. 2	Day No. 3	Day No. 3	Day No. 4	Day No. 4	Day No. 5	
1	2 Kappa free	1	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	18
		2	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	
2	Ig G, Kappa	1	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	18
		2	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	
3	Lambda free	1	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	18
		2	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	Concordant result	

Concordance study – Internal correlation

Concordance study was performed on 52 different urine samples between CAPI 3 IMMUNOTYPING URINE procedure on CAPILLARYS 3 and a commercially available capillary electrophoresis system for immunotyping : 8 urine samples without monoclonal protein and 44 urine samples with monoclonal proteins including Bence Jones proteins were run on both techniques.

This study demonstrated a 100 % agreement between the two procedures with a 100.0 % sensibility and a 100.0 % specificity of the CAPI 3 IMMUNOTYPING URINE procedure on CAPILLARYS 3 instrument compared to the reference procedure, calculated using the recommended method (Wendling, 1986) :

- For 8 urine samples without monoclonal protein : complete agreement (concordance).
- For the 44 urine samples with monoclonal proteins including Bence Jones proteins : complete agreement (concordance). In all cases, both techniques detected and characterized the monoclonal proteins (immunotyping) in human urine with complete agreement.

Sensitivity

Serial dilutions were prepared in normal urines with three urine samples with monoclonal proteins including Bence Jones proteins and analyzed using the CAPI 3 IMMUNOTYPING URINE procedure on CAPILLARYS 3.
The results are summarized below :

Sample	Monoclonal protein		Monoclonal protein concentration		Detection limit	
	Type		g/L	mg/dL	g/L	mg/dL
A	Lambda free	Lambda	2.432	243.2	0.010	1.0
C	Kappa free	Kappa	0.946	94.6	0.020	2.0
D	Ig G, Lambda	Gamma	0.118	11.8	0.002	0.2
		Lambda			0.002	0.2

NOTE : According to the position of the monoclonal protein and polyclonal background in the gamma and beta zones, the detection limit may vary.

PHOTOS

REPOSITIONNEMENT DU SEPTUM DANS LE BOUCHON À VIS - REPLACING THE SEPTUM INTO THE SCREW CAP

ATTENTION : Le bouchon perçable est constitué de 2 éléments : un bouchon à vis et un septum. Dans le cas où le septum est désolidarisé du bouchon à vis, le repositionner dans le bouchon de la façon suivante et vérifier qu'il est bien enfoncé avant de mettre le bouchon perçable sur le flacon de réactif.

WARNING : The pierceable cap is composed of 2 parts : a screw cap and a septum. When the septum is separated from the screw cap, replace it in the cap as follows and check that it is correctly inserted before placing the pierceable cap on the reagent vial.

Photo 1

Bouchon perçable
Pierceable cap

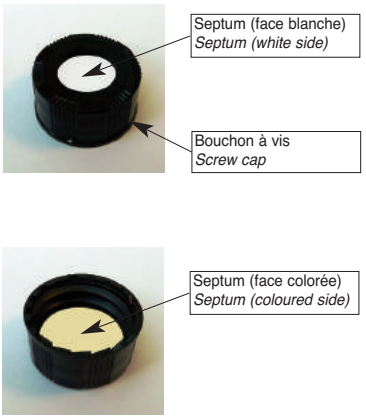


Photo 2

Bouchon à vis et septum désolidarisés
Screw cap and septum separated

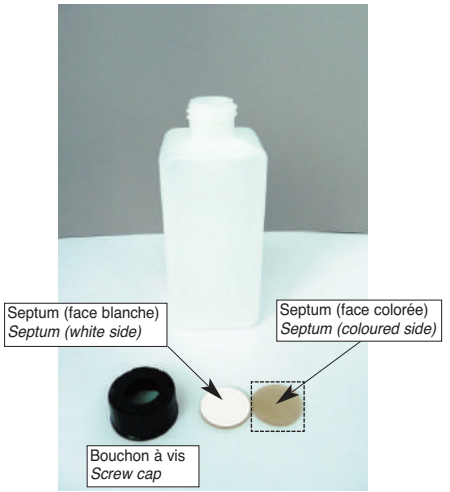


Photo 3

Repositionner le septum dans le bouchon à vis
Replace the septum into the screw cap

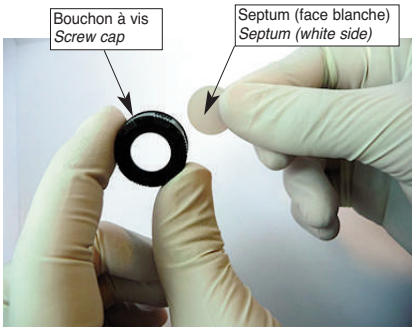
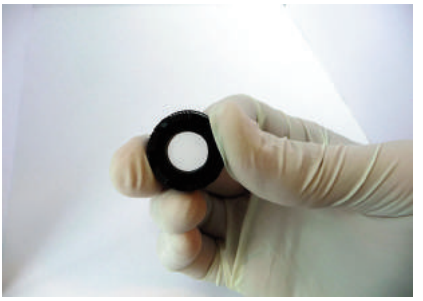


Photo 4

Vérifier que le septum est bien enfoncé dans le bouchon à vis
Check that the septum is correctly inserted into the screw cap



PHOTOS

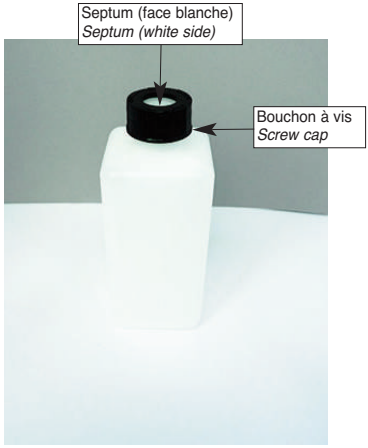
REPOSITIONNEMENT DU SEPTUM DANS LE BOUCHON À VIS - REPLACING THE SEPTUM INTO THE SCREW CAP

Photo 5

Positionner le bouchon perçable sur le flacon
Place the pierceable cap on the vial

Photo 6

Bouchon perçable installé sur le flacon
Pierceable cap installed on the vial



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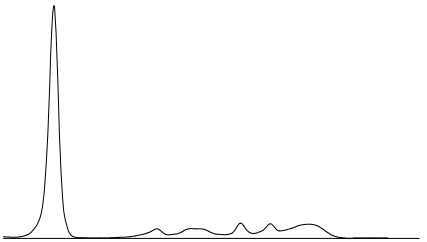
SCHÉMAS / FIGURES

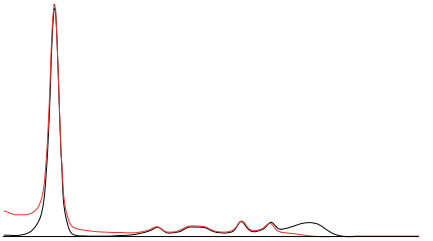
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Normal / Normal serum

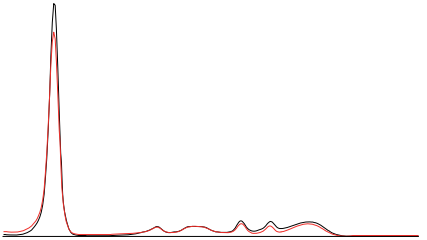
Figure 1



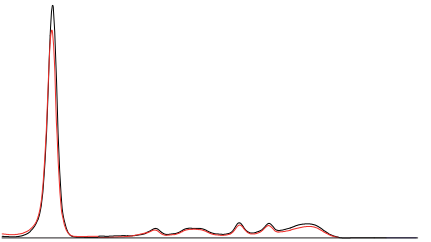
ELP



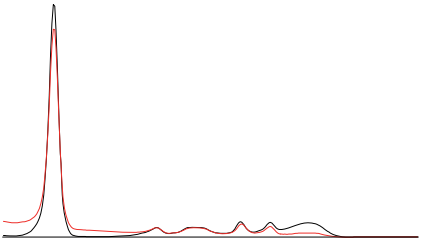
Ig G



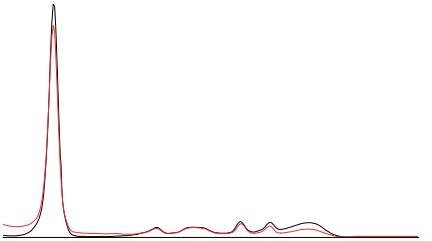
Ig A



Ig M



K



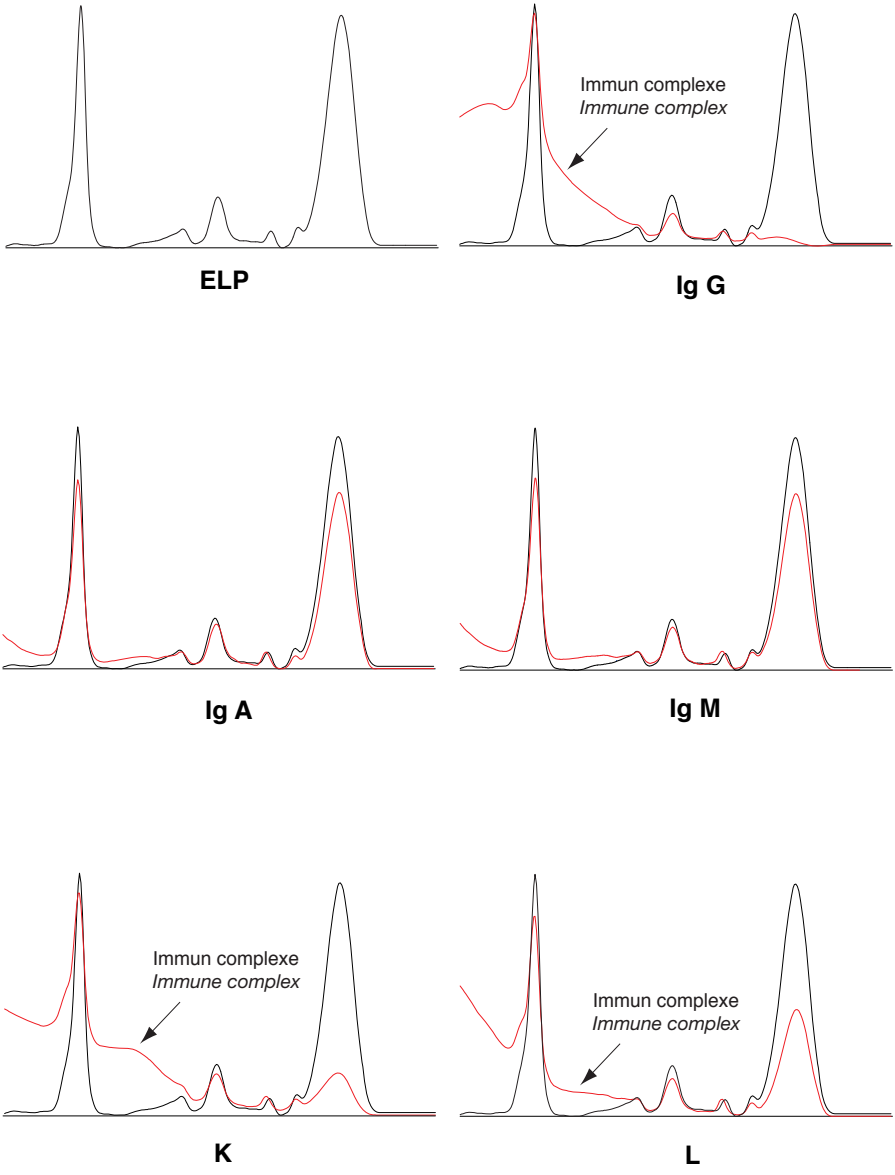
L

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Hypergamma / Hypergamma serum

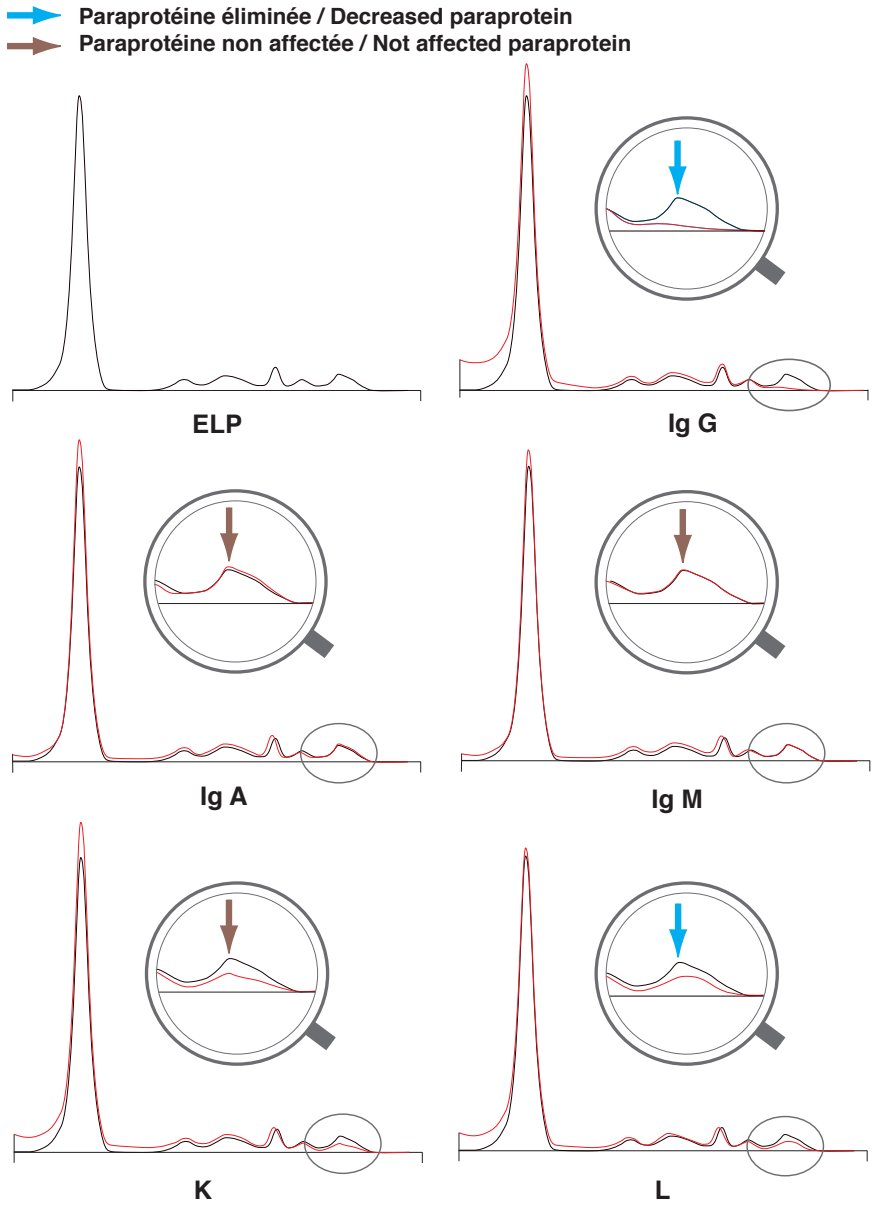
Figure 2



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



Interpretation : Ig G, Lambda

SCHÉMAS / FIGURES

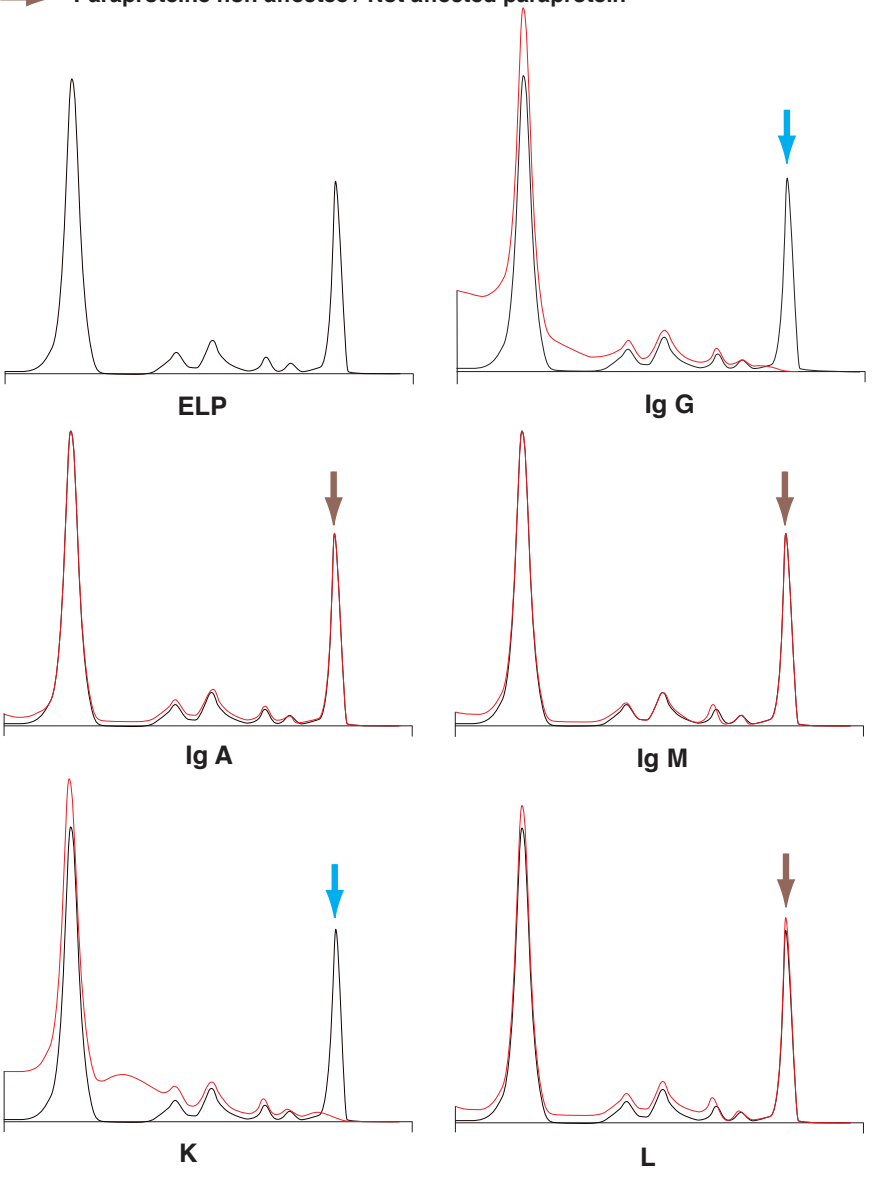
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

Figure 4

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



Interpretation : Ig G, Kappa

SCHÉMAS / FIGURES

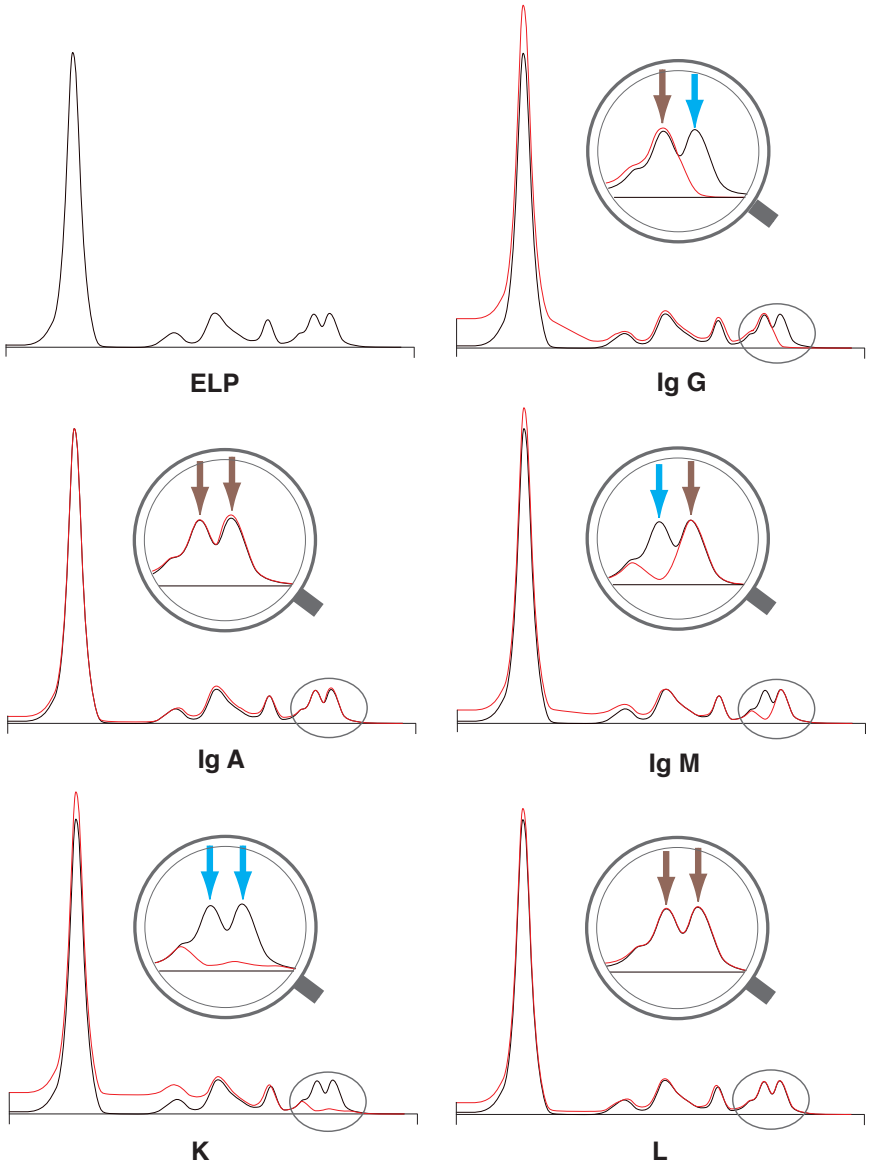
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

Figure 5

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



Interpretation : Ig G, Kappa + Ig M, Kappa

SCHÉMAS / FIGURES

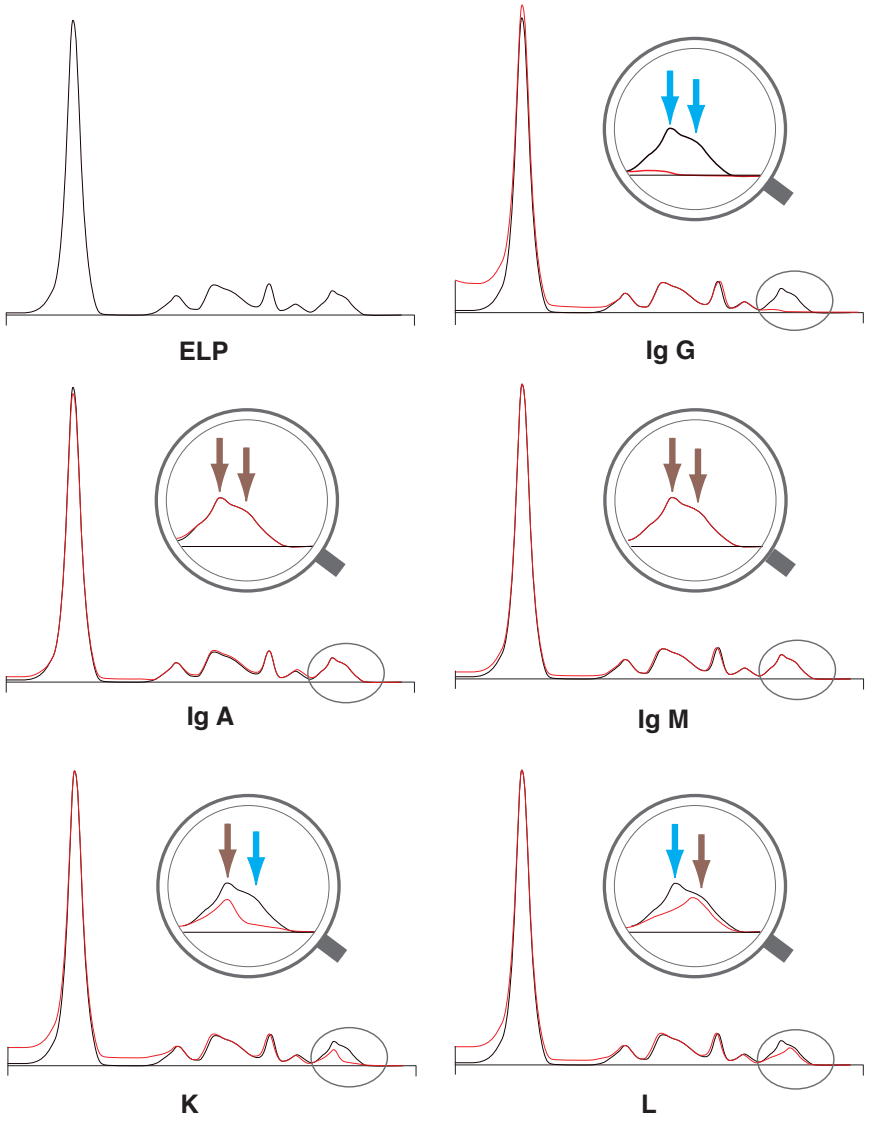
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

Figure 6

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



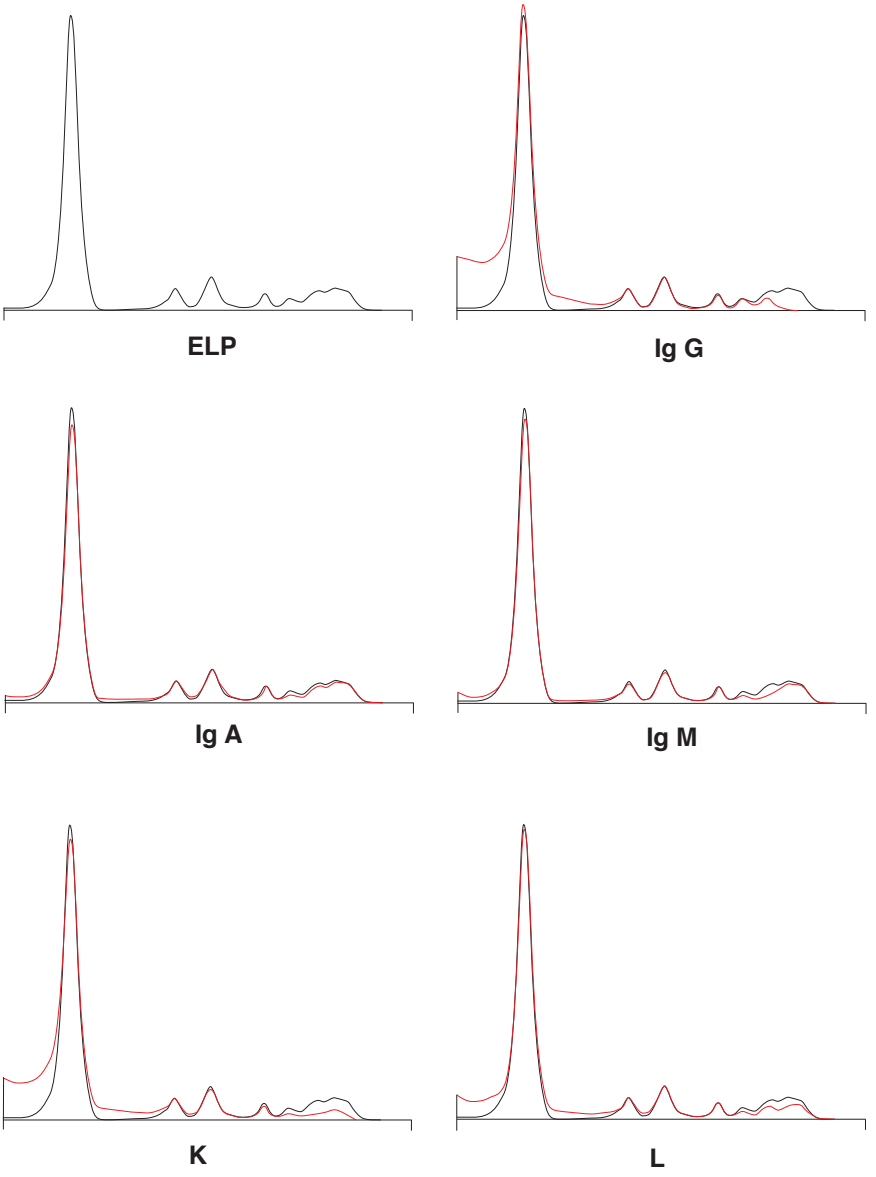
Interpretation : Ig G, Kappa + Ig G, Lambda

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Profil oligoclonal / Oligoclonal pattern

Figure 7



SCHÉMAS / FIGURES

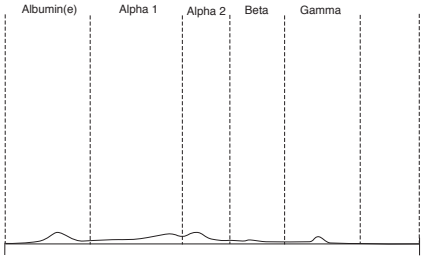
PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

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

Figure 8

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



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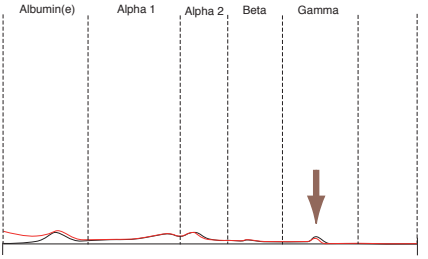
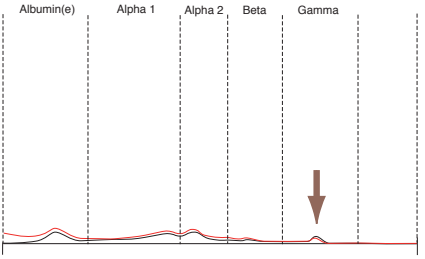
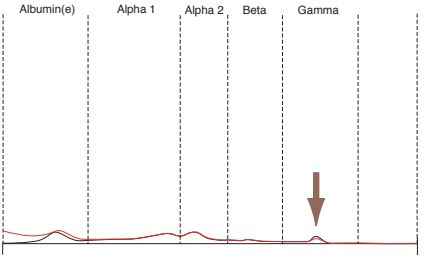


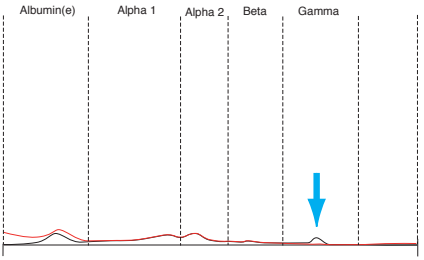
Ig G



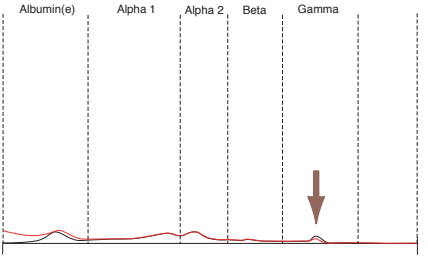
Ig A



Ig M



K



L

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

SCHÉMAS / FIGURES

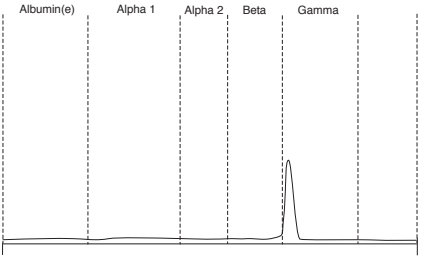
PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

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

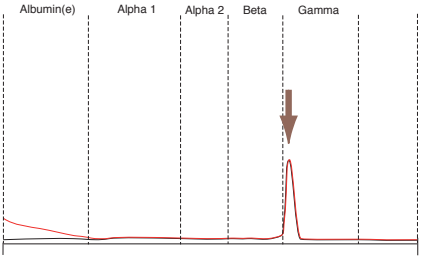
Figure 9

Paraprotéine éliminée / Decreased paraprotein

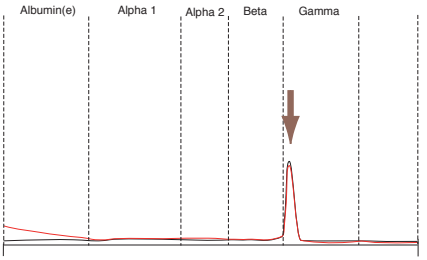
Paraprotéine non affectée / Not affected paraprotein



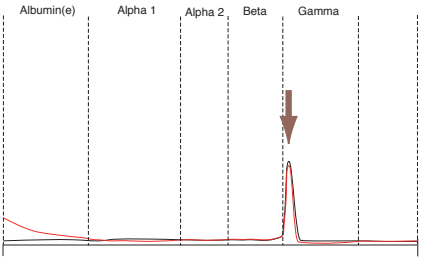
ELP



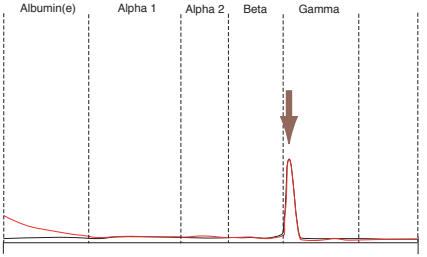
Ig G



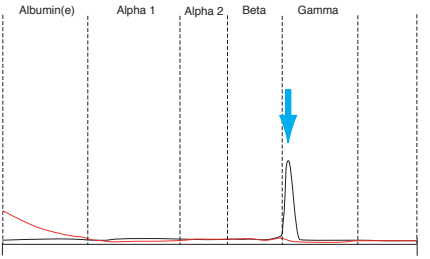
Ig A



Ig M



K



L

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

SCHÉMAS / FIGURES

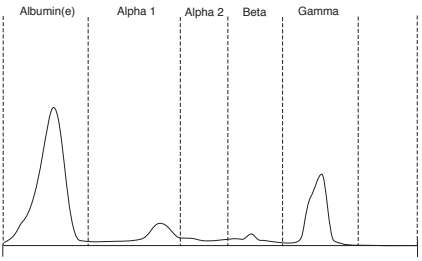
PROFILS ÉLECTROPHORÉTIQUES D'URINES - ELECTROPHORETIC PATTERNS OF URINE SAMPLES

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

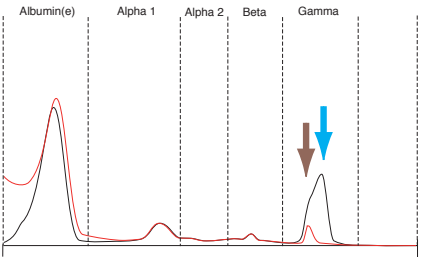
Figure 10

Paraprotéine éliminée / Decreased paraprotein

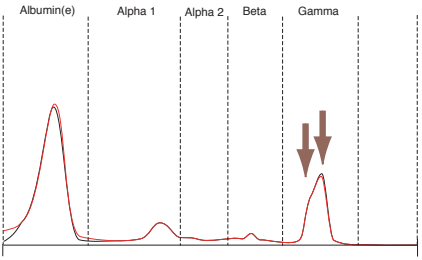
Paraprotéine non affectée / Not affected paraprotein



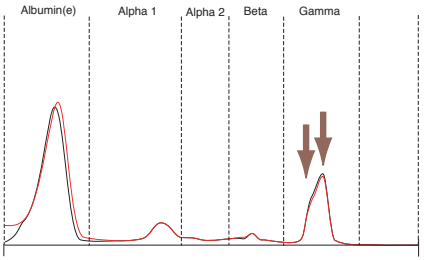
ELP



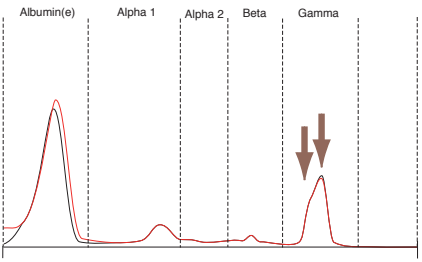
Ig G



Ig A



Ig M



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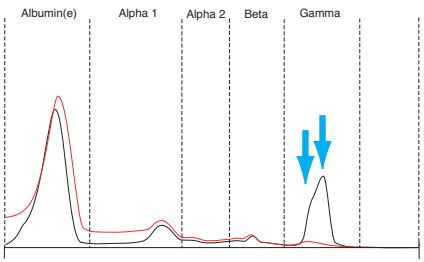

L

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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