MINICAP IMMUNOTYPING USING THE MINICAP AND THE MINICAP FLEX-PIERCING

Ref. 2300

Người đại diện hợp pháp của cơ sở
Tổng Giám Đốc

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INTENDED USE

The MINICAP IMMUNOTYPING kit is designed for the detection and the characterization of monoclonal proteins (immunotyping) in human serum with the MINICAP and the MINICAP FLEX-PIERCING instruments, SEBIA, for capillary electrophoresis. It is used in conjunction with the MINICAP PROTEIN(E) 6 kit, SEBIA, designed for proteins separation into 6 major fractions in alkaline buffer (pH 9.9).
The MINICAP and the MINICAP FLEX-PIERCING instruments perform all procedural sequences automatically to obtain a protein profile for qualitative analysis. Each 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 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 suspected monoclonal proteins.

For *In Vitro* Diagnostic Use.

PRINCIPLE OF THE TEST

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening serum samples for protein abnormalities [1, 3, 5, 6, 7, 10]. The MINICAP System, SEBIA, for capillary electrophoresis has been developed to provide complete automation of this testing with fast separation and good resolution. In many aspects, the methodology can be considered as intermediary between classical zone electrophoresis and liquid chromatography[1, 4, 5].
The MINICAP 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 [2, 9, 10].
In capillary electrophoresis, abnormal fractions in serum protein electrophoregrams, primarily those in the beta globulin and gamma globulin zones, are always suspected of being monoclonal proteins (M-proteins, paraproteins, monoclonal immunoglobulins) and therefore, an indication of monoclonal gammopathies. With MINICAP IMMUNOTYPING procedure, the immunotyping is performed with specific antibodies to identify these abnormal fractions.
The MINICAP system has 2 capillaries functioning in parallel. In this system, a sample dilution is prepared and injected simultaneously by aspiration at the anodic end of the 2 capillaries, 3 times successively. For the immunotyping, the reference pattern (ELP pattern) is obtained by injection of the sample mixed with ELP solution in a capillary providing a complete electrophoretic pattern of sample proteins. The antisera patterns are obtained with the 5 following analyses, by injection in 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 allows for visualization of the disappearance and / or the decrease of a monoclonal fraction on the antiserum pattern and to indicate a gammopathy.

NOTE : In MINICAP IMMUNOTYPING procedure, 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 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 samples with a specific diluent in a reagent cup. This dilution is made according to the concentration of immunoglobulins in the sample.
2. Mixing diluted serum sample with individual specific antisera (2 antisera per reagent cup). The antigen - antibody complex is formed rapidly in liquid medium without the need for extra incubation step or removal of the immune complexes.
3. Three following injections of prepared samples by simultaneous aspiration into 2 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.

NOTES :
- *In this instruction sheet, the name "MINICAP" is used for automated MINICAP and MINICAP FLEX-PIERCING instruments.*
- *Serum samples can be analyzed on MINICAP FLEX-PIERCING instrument with the PHORESIS software version 8.61 and following versions.*

REAGENTS SUPPLIED IN THE MINICAP IMMUNOTYPING KIT

WARNING : See the safety data sheets.

ITEMS	PN 2300
Sample diluent (ready to use)	6 vials, 4.0 mL each
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. 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 automated MINICAP system. It enables an optimal superimposition of the electrophoretic patterns.
Place directly on the MINICAP rotating sampler after having removed the cap of the vial. The bar code must be visible in the openings of the rotating sampler.

Storage, stability and signs of deterioration

Store the sample diluent at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). It is stable until the expiration date indicated on the kit package or sample diluent vial labels.
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. Place it directly on the MINICAP rotating sampler. The bar codes must be visible in the openings of the rotating sampler.

IMPORTANT : Place the rack on the MINICAP rotating sampler just before starting the analyses, and, just after the end of the analyses, store it refrigerated (2 - 8 °C) immediately.

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 and as an aid in monitoring its application, the ELP solution is colored (yellow) with a non-hazardous dye that matches the color of the vial label.

Use

For the reference electrophoretic pattern of the sample proteins (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 ELP solution vial label.
The ELP solution vial located on the rack and positioned on the MINICAP rotating sampler is stable for a maximum of **60 hours** (accumulated) at room temperature (15 to 30 °C).
After each use, the ELP solution must imperatively be stored refrigerated (between 2 and 8 °C) without any delay, it is then stable until the expiration date indicated on the ELP solution vial label.

IMPORTANT : The accumulated time of the ELP solution stored at room temperature must not exceed **60 hours**.

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 and as an aid in monitoring their application, the antisera are colored with distinct non-hazardous dyes that match the color of the vial label.

Use

For protein immunotyping on the MINICAP system.

IMPORTANT : The antisera are specific for the MINICAP 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 antiserum vial labels.
The antiserum vials located on the rack and positioned on the MINICAP rotating sampler are stable for a maximum of **60 hours** (accumulated) at room temperature (15 to 30 °C).
After each use, the antisera must imperatively be stored refrigerated (between 2 and 8 °C) without any delay, they are then stable until the expiration date indicated on the antiserum vial labels.

IMPORTANT : The accumulated time of the antisera stored at room temperature (15 to 30 °C) must not exceed **60 hours**.

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 : MINICAP IMMUNOTYPING PROCEDURE

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

WARNING : See the safety data sheets.

1. MINICAP PROTEIN(E) 6 KIT (SEBIA, PN 2203)

Presentation, use, storage, stability and signs of deterioration

See the instruction sheet of the kit.

2. DISTILLED OR DEIONIZED WATER

Use

For rinsing capillaries in the MINICAP System, SEBIA, for capillary electrophoresis.
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 contamination, 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 (FOR MINICAP) OR MINICAP FLEX-PIERCING CAPICLEAN (FOR MINICAP FLEX-PIERCING)

Composition

The vial of CAPICLEAN concentrated solution (CAPICLEAN, SEBIA, PN 2058, 1 vial, 25 mL or MINICAP FLEX-PIERCING CAPICLEAN, SEBIA, PN 2251, 1 vial, 25 mL) contains : proteolytic enzymes, surfactants and additives nonhazardous at concentrations used, necessary for optimum performances.

Use

For the sample probe cleaning in automated instrument MINICAP or MINICAP FLEX-PIERCING, SEBIA, for capillary electrophoresis, during the CAPICLEAN cleaning sequence.

IMPORTANT : Launch a CAPICLEAN cleaning sequence at least once a week and at maximum once a day, or after every 500 analyses when performed within less than one week.

See the instruction sheets of CAPICLEAN or MINICAP FLEX-PIERCING CAPICLEAN, SEBIA.

IMPORTANT : For optimal use of the CAPICLEAN solution with the MINICAP and MINICAP FLEX-PIERCING instruments, it is necessary to use one bar code label intended to identify the tube which contains the diluted CAPICLEAN solution.

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.
For later use, store the tube containing the diluted solution at 2 – 8 °C. It must be used within the day.

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 automated instrument MINICAP or MINICAP FLEX-PIERCING, SEBIA, for capillary electrophoresis (weekly maintenance in order to eliminate adsorbed proteins from the probe).

See the SEBIA MINICAP or MINICAP FLEX-PIERCING instruction manual.

- For MINICAP, apply in a hemolysis tube 2 mL of diluted chlorinated solution previously prepared.
- For MINICAP FLEX-PIERCING, apply in a 100 mm tube 2 mL of diluted chlorinated solution previously prepared.
- Place the tube (identified with one bar code label specific to the sodium hypochlorite solution) on the rotating sampler of MINICAP or MINICAP FLEX-PIERCING.
- Check that new reagent cups are placed on the automated loading system for cups of MINICAP / MINICAP FLEX-PIERCING (a message will be displayed if the reagent cup is missing).
- Slide the rotating sampler into the MINICAP / MINICAP FLEX-PIERCING system.
- Close the doors of the MINICAP / MINICAP FLEX-PIERCING system, the cleaning sequence starts automatically.

IMPORTANT : For optimal use of the sodium hypochlorite solution with the MINICAP and MINICAP FLEX-PIERCING instruments, it is necessary to use one bar code label intended to identify the tube which contains the solution.

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 / 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.
For MINICAP, it is convenient to dilute only 25 mL of the stock solution to 250 mL with distilled or deionized water.
After dilution, the wash solution contains an alkaline solution pH ≈ 12.

Use
For washing the MINICAP capillaries.

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.

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 (2 to 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.
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. MINICAP instrument, SEBIA, PN 1230, MINICAP FLEX-PIERCING instrument, SEBIA, PN 1232.
2. Rotating sampler supplied with MINICAP.
3. Container Kit supplied with MINICAP : Rinse (to fill with distilled or deionized water) and waste container.
4. MINICAP Reagent cups / 125 (3), SEBIA, PN 2281.
5. Lids for bins for used reagent cups, SEBIA (12 units), PN 2286 : lids to close the bins containing used cups.

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 MINICAP system is a multiparameter instrument for serum proteins analysis on 2 parallel capillaries in the MINICAP IMMUNOTYPING procedure, in the following sequence :
- Bar code reading of the serum sample tubes (for up to 18), of the MINICAP IMMUNOTYPING reagent tubes and of the rotating sampler ;
- Sample dilution from primary tubes in reagent cups ;
- Mixing diluted serum samples with ELP solution and specific antisera in reagent cups ;

- Capillary washing ;
- Injection of diluted samples ;
- Protein separation and direct detection of the separated proteins on capillaries.

The manual steps include :
- Set up the opened sample tubes in rotating sampler in positions 1 to 18 ;
- Set up the sample diluent tube in rotating sampler in position 27 ;
- Set up the rack with the ELP solution and antiserum tubes in rotating sampler in positions 19 to 24 ;
- Set up the rotating sampler in the MINICAP instrument ;
- Remove the sample tubes after analysis ;
- Remove and close the bins for used cups.

Electrophoretic analysis on the MINICAP system using the MINICAP 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 MINICAP INSTRUCTION MANUAL.

I. PREPARATION OF ELECTROPHORETIC ANALYSIS

1. Select samples with abnormal protein fraction on the electrophoregrams obtained with the MINICAP PROTEIN(E) 6 procedure.
2. Switch on MINICAP instrument and computer.
3. In order to start the instrument, position at least one new reagent cup on the automated loading system for cups of MINICAP (a message will be displayed if a reagent cup is missing).
4. Set up the software, the instrument automatically starts.
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 sample analysis, the MINICAP IMMUNOTYPING kit is intended to run with "IMMUNOTYPING 6" analysis program from the MINICAP instrument and MINICAP PROTEIN(E) 6 buffer. To select "IMMUNOTYPING 6" analysis program and place the MINICAP PROTEIN(E) 6 buffer vial in position "B1" in the instrument, please read carefully the MINICAP instruction manual and follow the instructions displayed on the screen.
   NOTE : It is not necessary to change the buffer vial when switching from MINICAP PROTEIN(E) 6 procedure to MINICAP IMMUNOTYPING procedure (and vice versa).
7. Position new reagent cups on the automated loading system for cups of MINICAP (a message will be displayed if the reagent cups are missing).
8. **Position a new bin for used cups in MINICAP at the location intended for this purpose.**
9. Check the fill level of the reagent vials, add reagent if necessary and empty the waste container. In the window "Check reagent levels", update the software by moving the cursor buttons.
10. The rotating sampler contains 28 positions for sample tubes :
    - Position up to 18 opened sample tubes on the rotating sampler (positions No. 1 to 18), the bar code of each tube must be visible in the openings of the rotating sampler. If the sample tube placed on the sample rack is not previously selected, the "STANDARD" dilution program will automatically be performed.
    - 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 rotating sampler in ELP solution and antisera positions, by handling it by the two clips situated on each side, the bar code of each tube must be visible in the openings of the rotating sampler.

    IMPORTANT : Check that the rack is correctly inserted in the rotating sampler before starting the analysis.

    - Uncap the sample diluent tube and place it in position No. 27 ("Diluent / Solution" position), the bar code of the tube must be visible in the opening of the rotating sampler.

    IMPORTANT : For one analysis, the ELP solution and all the antisera must have the same lot number. Do not mix in any case the left antisera with same specificity or ELP solution between 2 following vials, even if they have the same lot number.

    IMPORTANT : If a tube is missing in positions for sample tubes, in positions No. 19 to 24 (antisera and ELP solution) and in position No. 27 (sample diluent), the analysis can not start and a message will be displayed.
11. Slide the rotating sampler into the MINICAP system.
12. Close the doors of the MINICAP system and follow the instructions displayed on the screen.
13. After the analyses, remove the rotating sampler with analyzed sample tubes. Remove the rack with ELP solution and antisera tubes and store it refrigerated (2 – 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.
14. If necessary, take off carefully the bin containing used reagent cups, close it tightly with the corresponding lid and discard it.
    WARNING : Bins containing used reagent cups with biological samples must be handled with care.

DILUTION - MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

1. Bar codes are read on sample tubes (up to 18), on MINICAP IMMUNOTYPING reagent tubes and on the rotating sampler.
2. Sample is diluted in the sample diluent in a reagent cup.
3. The 2 first reagents and the diluted sample are aspired and then mixed in a reagent cup. 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. The order of the reagents is the following : ELP solution & anti-Ig G, anti-Ig A & anti-Ig M and anti-Kappa & anti-Lambda antisera.
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 the electrophoretic profile appears on the screen of the system.

NOTE : The steps 3 to 7 are performed again for the following reagents (anti-Ig A & anti-Ig M, and anti-Kappa & anti-lambda by default).

NOTE : These steps are described for the 2 first reagents (ELP solution and anti-Ig G). For the following reagents (anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera), the step 3 is performed during the 2 previous analyses. The complete electrophoretic patterns corresponding to the immunotyping of the sample appear after about 35 minutes.

In the status window :
- The "Legend" folder shows the progress of analyses on the rotating sampler.
- The "IT counter" indicates the number of performed analyses and is displayed only with the "capped tubes" mode. After installation of the software version, the analysis counter starts at zero. It counts the analysis being run and any analysis that has been completed or stopped (after an abort).

Reset the counter when the tubes are empty (by using the "reset" button).

NOTE : A flashing warning signal is displayed in the status window when 60 analyses have been completed. This signal remains displayed but does not stop the following analyses until the analysis counter is reset.

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.
These comparisons allow the identification and the characterization of monoclonal components

III. END OF ANALYSIS SEQUENCE

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

IMPORTANT : Position at least one new reagent cup on the automated loading system for cups of MINICAP (a message will be displayed if a reagent cup is missing).

IV. FILLING OF REAGENT CONTAINERS

The MINICAP 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 MINICAP 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 patterns 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 immunoglobulins class found in the serum and normal polyclonal removal will commonly be noted. Normal polyclonal reduction of this peak should not be mistaken for a monoclonal component. Polyclonal removal appears as a reduction of the fraction without any change of symmetry of the fraction. Monoclonal Ig G will present with removal of the peak with symmetry change visible 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 position as the suspected monoclonal fraction seen on 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 MINICAP system ready waiting for rotating sampler, 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 tube must be waiting for analysis in the MINICAP 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 disappear totally 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, biclonal gammopathies or polymerizations, 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

IMPORTANT : The use of antisera other than those specific for the MINICAP IMMUNOTYPING procedure provided with the kit may affect the results.
Use only the antisera specific for the MINICAP IMMUNOTYPING procedure.
- See SAMPLES FOR ANALYSIS.
- 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.
- 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 [7].
- When a monoclonal component is detected by the MINICAP procedure for protein analyses, MINICAP PROTEIN(E) 6, and not characterized by MINICAP 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.
- No interference with MINICAP IMMUNOTYPING procedure was detected due to the serum sample's high concentration of cholesterol (≤ 8.24 mmol/L), triglycerids (≤ 11.58 mmol/L) and hemoglobin (≤ 0.40 g/dL).
- Interferences due to bilirubin have not been studied for the MINICAP IMMUNOTYPING procedure. It is advised to observe the serum sample features ; when an interferent fraction is suspected, it is recommended to perform again the analysis on the serum sample, or to use complementary studies with other techniques.

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

MINICAP IMMUNOTYPING procedure using the MINICAP instrument

Reproducibility within sequence and between runs

Reproducibility within sequence and between runs was demonstrated on three different pathological serum samples with a monoclonal component (Ig GL, Ig AK and Ig MK) and on one normal serum sample. They were analyzed on 3 MINICAP systems with the MINICAP IMMUNOTYPING procedure and the "STANDARD" dilution program, using the same lot of MINICAP IMMUNOTYPING kit. Each serum was analyzed 3 times on both capillaries of each MINICAP system with one of the 6 reagents, according to the assayed sample (ELP solution for the normal sample, anti-Ig G, anti-Ig A, anti-Ig M, anti-Kappa and anti-Lambda antisera for the pathological samples, according to the monoclonal component).
All repeats gave identical results within sequence and between runs and patterns corresponded to the type of each tested sample. With the MINICAP IMMUNOTYPING procedure, immunotyping allowed the characterization of only one monoclonal component for each pathological sample and no abnormal fraction for the normal sample.

Reproducibility between runs and between systems

Reproducibility between runs and between systems was demonstrated on three different pathological serum samples with one monoclonal component (Ig GK, Ig GL and Ig MK) and with different immunoglobulin concentrations. One sample (with total Ig level < 0.8 g/dL) was analyzed with the MINICAP IMMUNOTYPING procedure and the "HYPOGAMMA" dilution program, one sample (with total Ig level comprised between 0.8 and 2 g/dL) with the "STANDARD" dilution program and one sample (with total Ig level > 2 g/dL) with the "HYPERGAMMA" dilution program. These samples were analyzed successively 3 times on each of the 3 MINICAP systems, using the same lot of MINICAP IMMUNOTYPING kit.
All repeats gave identical results between runs and between systems and patterns correctly identified the monoclonal components. With the MINICAP IMMUNOTYPING procedure, immunotyping allowed the characterization of the expected monoclonal components for the three pathological samples.

Reproducibility between runs and between lots

Reproducibility between runs and between lots was demonstrated on two different pathological serum samples with one monoclonal component (respectively Ig GK and Ig MK) and on one serum sample with two monoclonal components (Ig AL), all samples had Ig level comprised between 0.8 and 2 g/dL. These samples were analyzed successively 3 times, using three different lots of MINICAP IMMUNOTYPING kits and the "STANDARD" dilution mode.
All repeats gave identical results between runs and between lots and patterns correctly identified the monoclonal components. With the MINICAP IMMUNOTYPING procedure, immunotyping allowed the characterization of the expected monoclonal components for the three pathological samples.

Concordance Study

Concordance study was performed on 69 serum samples between the MINICAP IMMUNOTYPING procedure and a commercially available capillary electrophoresis system for immunotyping: 57 different pathological serum samples and 12 normal 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 57 pathological serum samples: complete agreement (concordance).

In all cases, both techniques detected and charaterized 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 MINICAP IMMUNOTYPING procedure.
The results are summarized below :

SAMPLE No.	MONOCLONAL COMPONENT TYPE		CONCENTRATION (g/dL) (in the original serum)	DETECTION LIMIT (mg/dL)
1	Ig A, L	Alpha	2.7	25
		Lambda		25
2	Ig G, K	Gamma	2.9	25
		Kappa		25
3	Ig M, K	Mu	1.7	25
		Kappa		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.

MINICAP IMMUNOTYPING procedure using the MINICAP FLEX-PIERCING instrument

Concordance Study

Concordance study was performed on 48 serum samples between the MINICAP IMMUNOTYPING procedure and a commercially available capillary electrophoresis system: 38 different pathological serum samples and 10 normal serum samples have been run on both techniques.
This study demonstrated a 100 % agreement between the two techniques:

- For the 10 normal serum samples: complete agreement (concordance).
- For the 38 pathological serum samples: complete agreement (concordance).

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

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PHOTO

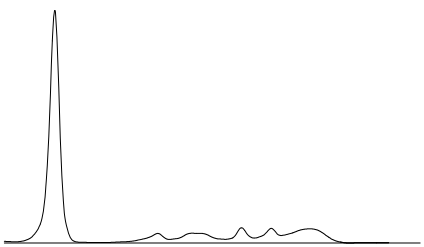
INSERTION DU SUPPORT SUR LE CARROUSEL - INSERTION OF THE RACK IN THE ROTATING SAMPLER

SCHÉMAS / FIGURES

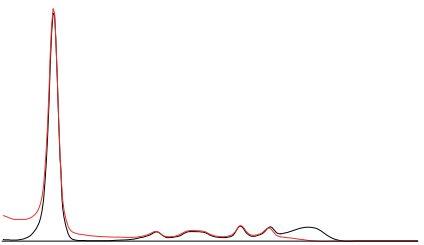
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Normal / Normal serum

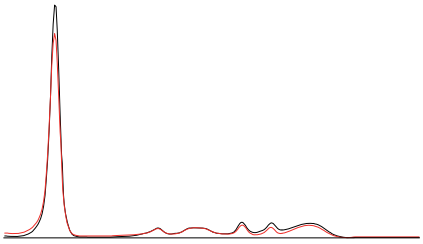
Figure 1



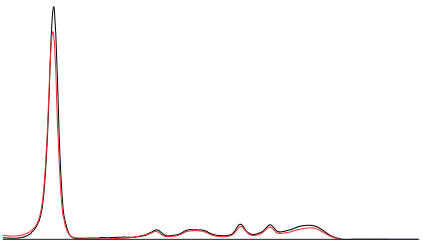
ELP



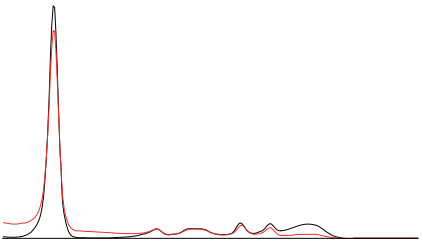
Ig G



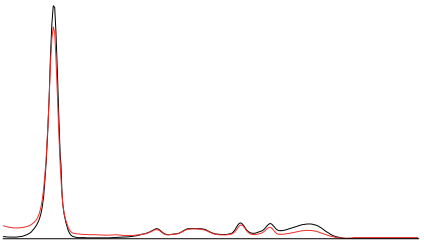
Ig A



Ig M



K



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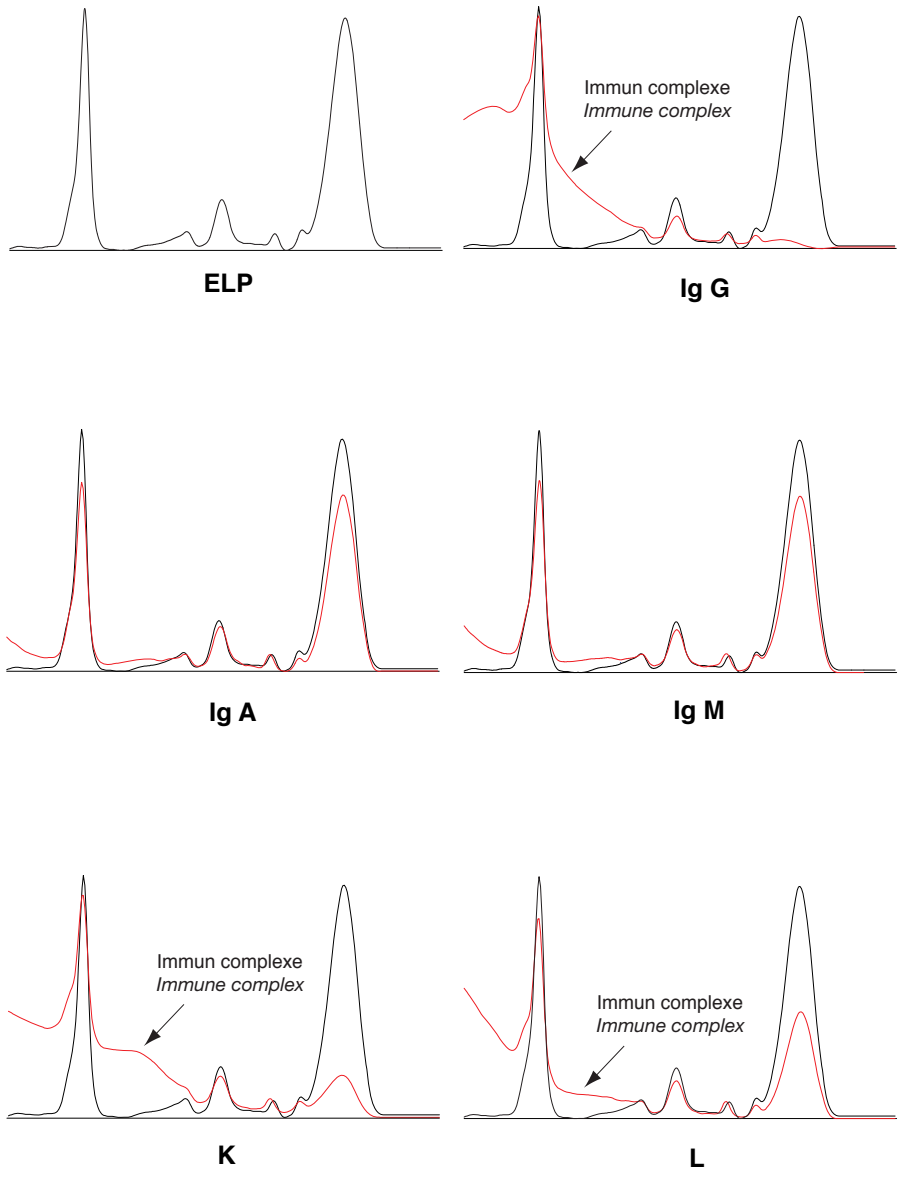
SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Sérum Hypergamma / Hypergamma serum

Figure 2



SCHÉMAS / FIGURES

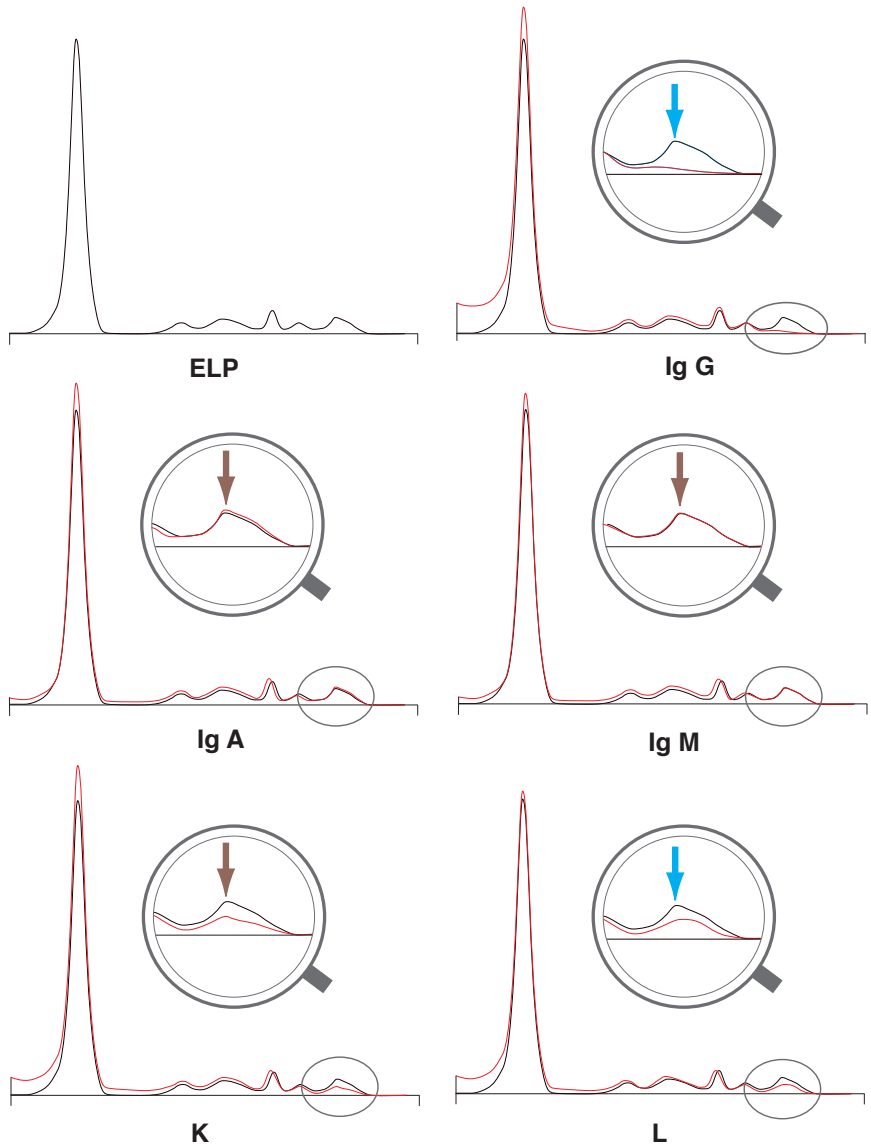
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

Figure 3

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



Interpretation : Ig G, Lambda

SCHÉMAS / FIGURES

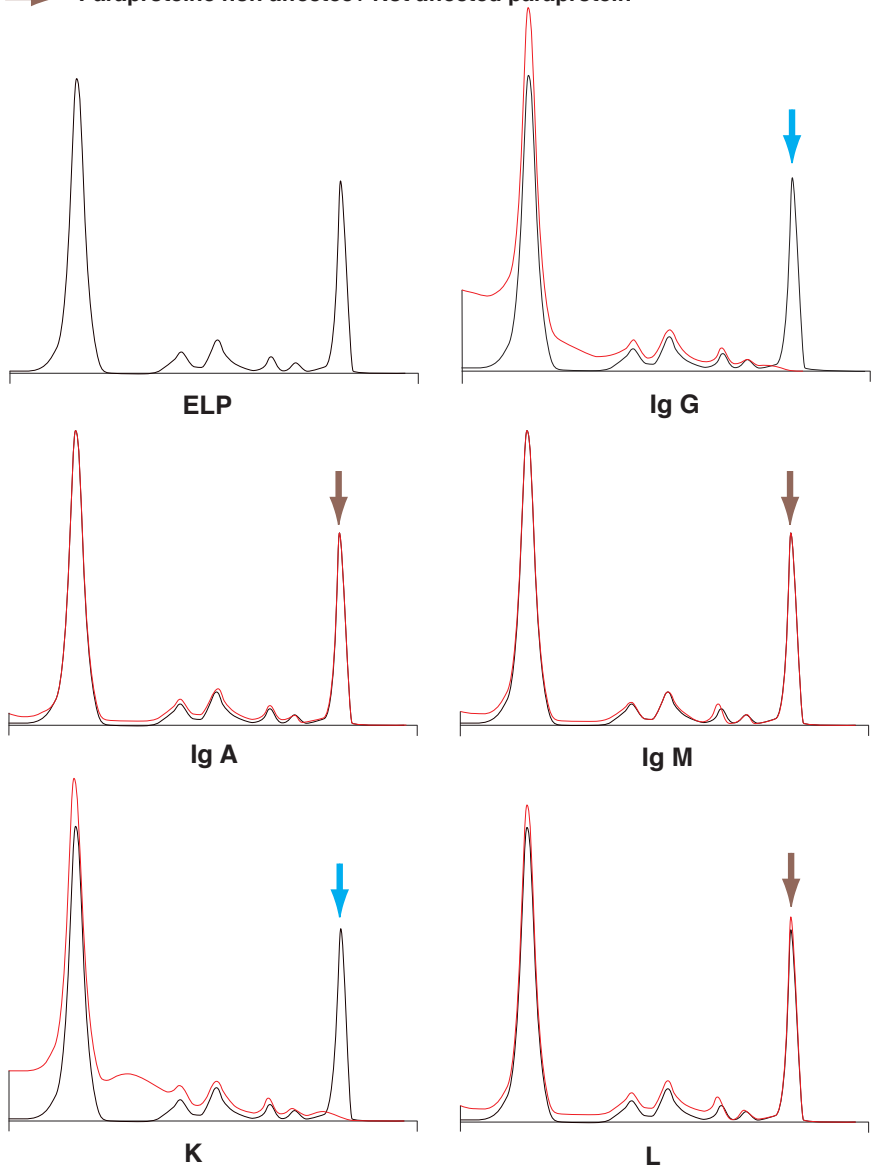
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

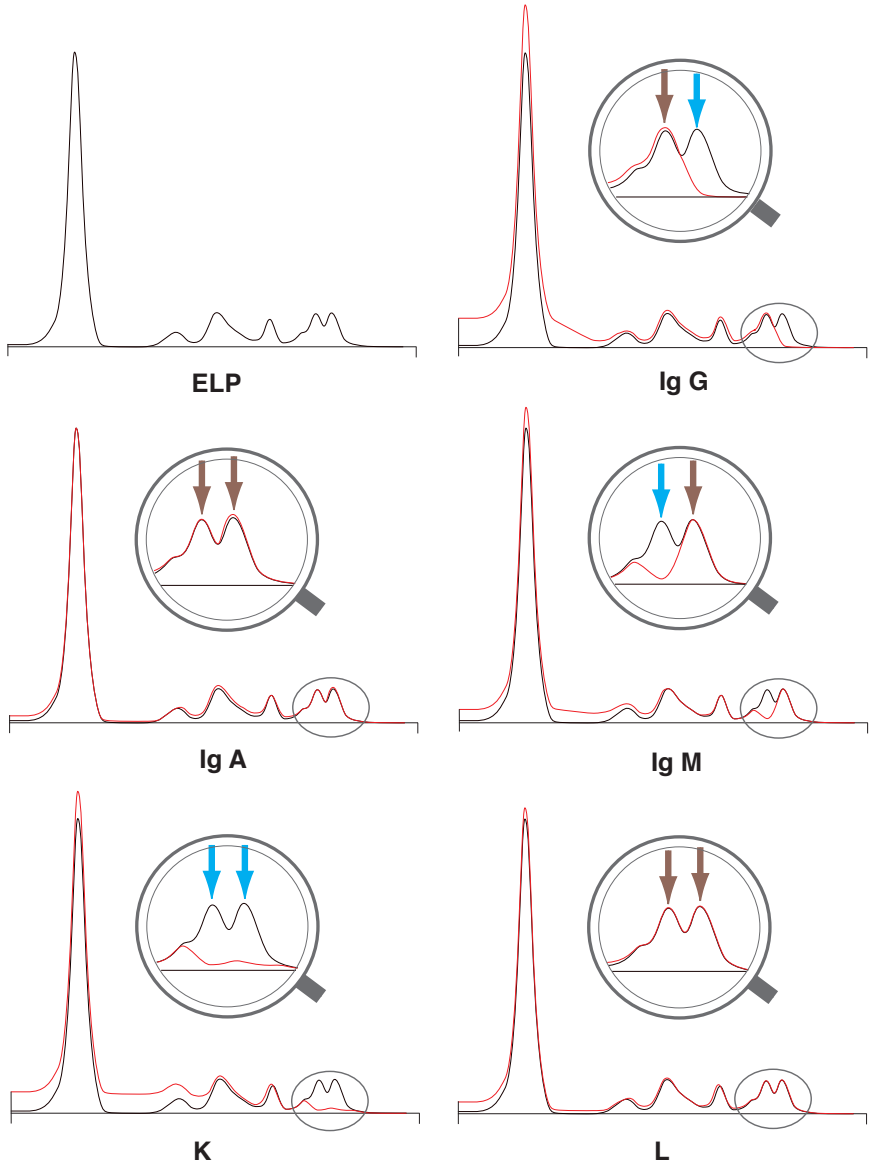
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

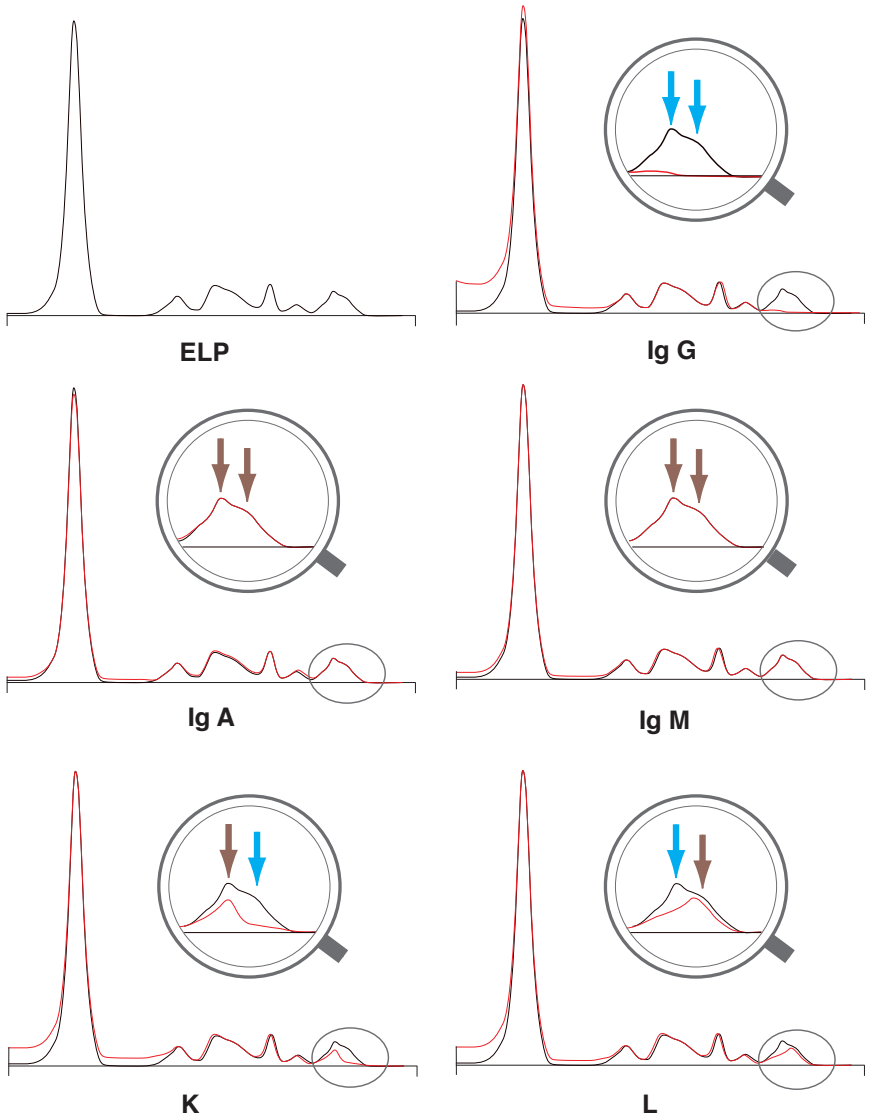
PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

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

Figure 6

Paraprotéine éliminée / Decreased paraprotein

Paraprotéine non affectée / Not affected paraprotein



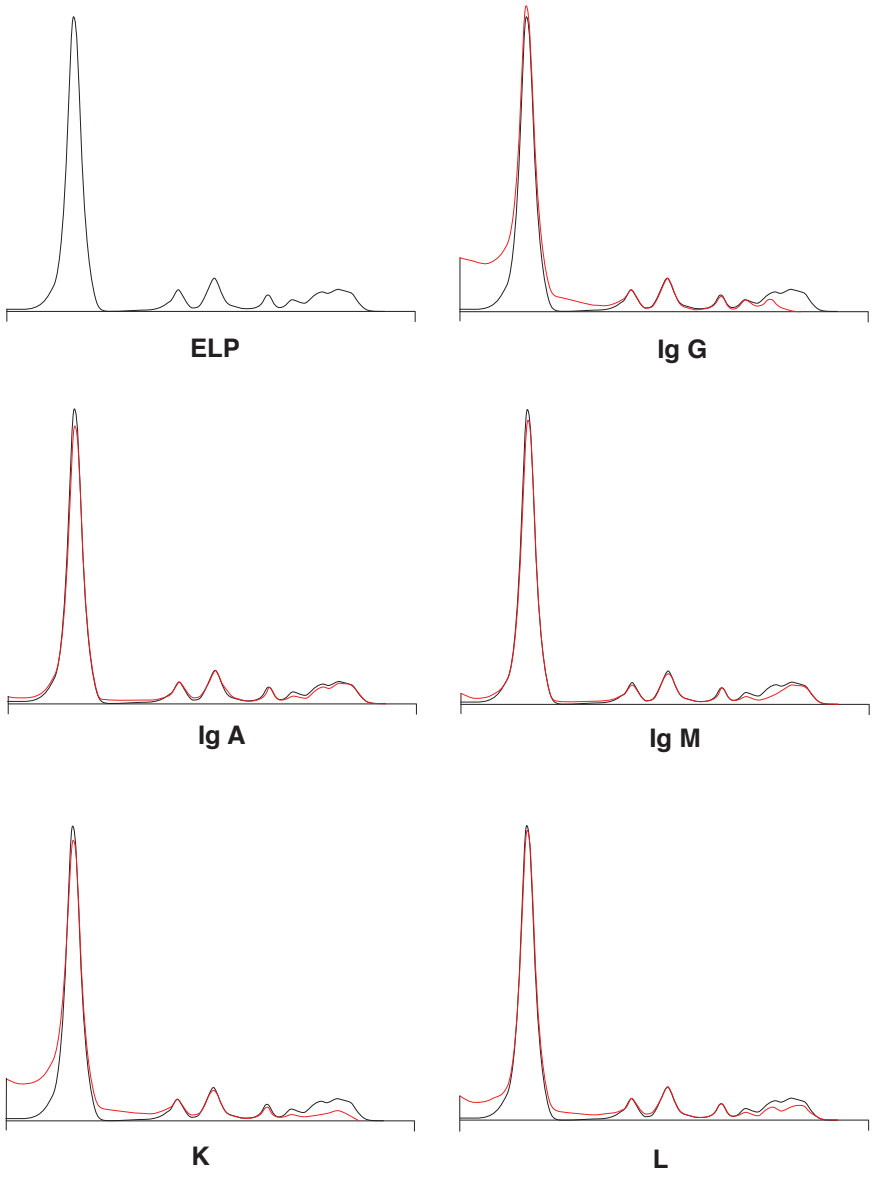
Interpretation : Ig G, Kappa + Ig G, Lambda

SCHÉMAS / FIGURES

PROFILS ÉLECTROPHORÉTIQUES DE SÉRUMS - ELECTROPHORETIC PATTERNS OF SERUM SAMPLES

Profil oligoclonal / Oligoclonal pattern

Figure 7



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