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CAPI 3 PROTEIN(E) 6

Ref. 2503

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INTENDED USE

The CAPI 3 PROTEIN(E) 6 kit is designed for the separation of human serum proteins by capillary electrophoresis in alkaline buffer (pH 9.9) with the CAPILLARYS 3 instrument.
Normal serum proteins separate into six major fractions.
The CAPILLARYS 3 instrument performs all sequences automatically to obtain a protein profile for qualitative or quantitative analysis. The proteins, separated in silica capillaries, are directly detected at an absorbance of 200 nm. The electrophoregrams can be interpreted visually to screen for any pattern abnormalities. Direct detection provides accurate relative quantification of individual protein fractions.

For *In Vitro* Diagnostic Use.

NOTE : In this instruction sheet, the name "CAPILLARYS 3" is used for the SEBIA CAPILLARYS 3 OCTA, CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA automated instruments.

PRINCIPLE OF THE TEST

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening samples for protein abnormalities. Besides the electrophoresis techniques performed on different media, including agarose gel, the capillary electrophoresis has been developed to provide a 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.
The CAPILLARYS 3 OCTA instrument has 8 capillaries functioning in parallel allowing 8 simultaneous analyses, the CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA instruments have 12 capillaries functioning in parallel allowing 12 simultaneous analyses. A sample dilution with buffer is prepared and injected by aspiration at the anodic end of the capillary. 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.
By using alkaline pH buffer, proteins are detected in the following order : gamma globulins, beta-2 globulins, beta-1 globulins, alpha-2 globulins, alpha-1 globulins and albumin with each zone containing one or more proteins.

REAGENTS AND MATERIALS SUPPLIED IN THE CAPI 3 PROTEIN(E) 6 KIT

WARNING : See the safety data sheets.

ITEMS	PN 2503
Buffer (ready to use)	3 vials, 700 mL each
Filters	4 filters

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. BUFFER

Preparation

The buffer is ready to use. It contains : buffer solution pH 9.9 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

Buffer for protein analysis in capillary electrophoresis.

Storage, stability and signs of deterioration

Store the buffer at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C). It is stable until the expiration date indicated on the kit package or buffer vial labels. Avoid storage close to a window or to a heat source.

NOTE : When analysis buffer is stored between 2 and 8 °C, it is recommended to allow reagent to come to room temperature prior to use.

DO NOT FREEZE.
Once the buffer vial has been opened and positioned on the CAPILLARYS 3 instrument, it is stable for a maximum of 2 months (accumulated). If the buffer vial is planned to be used for more than 2 months, it must be removed from the instrument after each use and stored at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C), it is then stable until the expiration date indicated on the buffer vial label.
Discard buffer if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

2. FILTERS

Use

Disposable filters for filtration of analysis buffer and distilled or deionized water (used for capillaries rinsing).

IMPORTANT : When kit replacement, change systematically all the filters. Wear clean gloves for handling and installation of filters.

Screw one filter at the connector situated at the extremity of each tube plunging in vials of buffer and distilled or deionized water. When setting filters on the instrument, rinse the connectors and the tubes with distilled or deionized water.

Storage

Before use, store the filters in their sealed package in a dry place at room temperature (15 to 30 °C) or refrigerated (between 2 and 8 °C).

REAGENTS REQUIRED BUT NOT SUPPLIED

WARNING : See the safety data sheets.

1. 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.*

2. 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 (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.

3. 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.

4. 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, 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 PROTEIN(E) 6 (1), SEBIA, PN 1381, to launch automatically a technique change to PROTEIN(E) 6 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 8 samples with CAPILLARYS 3 OCTA and 12 samples with CAPILLARYS 3 TERA and CAPILLARYS 3 TERA TLA.

   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 2 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 integration 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 aspect before analysis (cases of hemolysis, cryoglobulins or turbidity).

Samples to avoid

- Avoid hemolysed serum samples. Hemolysis induces a double alpha-2 zone.
- Avoid aged, improperly stored serum samples, beta-2 fraction would be decreased.
- Avoid plasma samples. Fibrinogen migrates in beta-2 position (shoulder on beta-2 or superimposed with the beta-2 zone with possibly an increase of this fraction). When it is present in some samples (plasma, serum not totally defibrinated or patient with anticoagulant treatment), fibrinogen may interfere on the analysis and makes interpretation inaccurate (suspicion of monoclonal band or beta-2 fraction increase). When analyzing an aged plasma sample (not recommended), the C3 complement which is labile over the time is partially degraded, the beta-2 zone then corresponds essentially to fibrinogen.

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 system is a multiparameter instrument for serum proteins analysis on 8 or 12 parallel capillaries 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,
- capillary washing,
- injection of diluted samples,
- protein analysis and direct detection on capillaries.

The manual steps include :
- placement of sample tubes in sample racks,
- 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.

PLEASE CAREFULLY READ THE CAPILLARYS 3 INSTRUCTION MANUAL.

I. PREPARATION OF ELECTROPHORETIC ANALYSIS

1. Switch on CAPILLARYS 3 instrument and computer.
2. Wait until the instrument is completely initialized.
3. Start the PHORESIS software installed on the computer for data processing.
4. The CAPI 3 PROTEIN(E) 6 kit is intended to run with "PROTEIN(E) 6" analysis program from the CAPILLARYS 3 instrument.
   To select "PROTEIN(E) 6" 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.
5. The sample rack contains 8 positions for sample tubes. Position up to 8 sample tubes on each sample rack ; the bar code of each tube must be visible in the openings of the sample rack.
6. 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).
7. Place a new bin for used reagent cups into the CAPILLARYS 3 instrument at the location intended for this purpose.
8. 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.
   NOTE : When analyzing a control serum, it is advised to use the sample rack No. 0 intended for control serum.
9. Remove analyzed sample racks from the plate on the left side of the instrument.
10. 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. Serum samples are diluted in analysis buffer and the sample probe is rinsed after each sample.
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 pattern are transmitted from the instrument to the computer equipped with the software for data processing.

NOTE : Please read the CAPILLARYS 3 TERA TLA instruction manual for the analysis of tubes delivered by a laboratory automation system.

II. RESULT ANALYSIS

At the end of each analysis, the corresponding data are transmitted by the instrument to the software for data processing and an electrophoretic profile appears on the screen of the computer. Relative quantification of individual zones is made automatically and profiles can be analyzed.
With the total protein concentration, the software will calculate each fraction concentration.
The electrophoregrams are interpreted visually for pattern abnormalities.
Electrophoretic profiles are visualized by default using the re-drawn mode : then, the alpha-1 fraction is closer to albumin.
Optionally, the standard mode allows to visualize the initial pattern obtained with raw data.

PLEASE CAREFULLY READ THE PHORESIS INSTRUCTION MANUAL.

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.

QUALITY CONTROL

It is advised to include a control serum with each sequence of analysis.

RESULTS

Values

Direct detection at 200 nm in capillaries yields relative concentrations (percentages) of individual protein zones.
Reference values (mean ± 2 SD) for individual major electrophoretic serum protein zones have been established from a healthy population of 246 adults with normal triglycerides levels (men and women) :

	CAPI 3 PROTEIN(E) 6
Albumin	55.8 – 66.1 %
Alpha-1 globulins	2.9 – 4.9 %
Alpha-2 globulins	7.1 – 11.8 %
Beta globulins	8.4 – 13.1 %
Beta-1 globulins	4.7 – 7.2 %
Beta-2 globulins	3.2 – 6.5 %
Gamma globulins	11.1 – 18.8 %

It is recommended each laboratory establish its own reference values.

NOTE : Reference values have been established using the standard parameters of the PHORESIS software (smoothing 2 and automatic drift).

Interpretation

A distortion of the electrophoretic pattern compared to a normal one indicates an abnormality, especially the appearance of an additional thin peak in the gammaglobulins zone.
The C4 complement migrates between beta-1 and beta-2 zones ; CRP migrates in beta-2 position, see ELECTROPHORETIC PATTERNS.
A relative increase of the beta-2 zone compared to the beta-1 zone, without any clinical context of inflammatory disease, must be a warning signal for necessary complementary analyses.
In case of doubt concerning the interpretation of the pattern and / or the positioning of minima (particularly during the analysis of an external control), it is necessary to overlay the obtained pattern with that of the Normal Control Serum (SEBIA, PN 4785).
A monoclonal component may be suspected in the serum sample when a single protein electrophoretic pattern is delayed or distorted or in the case of impossibility for the software to redraw the albumin / alpha-1 zone. The following warning message is then displayed on the electrophoretic pattern "Warning: Migration time out of range" with a red warning signal. This red warning signal is also displayed on the curves mosaic and in the result table for the sample concerned. To confirm the presence of a monoclonal component in such sample, it is necessary to treat the sample with beta-mercaptoethanol and to repeat the analysis on the sample after reducing treatment. 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 instrument.

When many electrophoretic patterns show the same warning signal, call SEBIA Technical Service.
An identification is recommended to characterize monoclonal or oligoclonal components :

- by immunotyping with SEBIA CAPI 3 IMMUNOTYPING kit or,
- by immunofixation with SEBIA HYDRAGEL IF kits.

As an aid in interpretation of serum protein electrophoregrams, see BIBLIOGRAPHY.

Alpha-2 zone :

- In some samples and according to the haptoglobin phenotype, alpha-2 zone can be split, see ELECTROPHORETIC PATTERNS.

Interference and Limitations

See SAMPLES FOR ANALYSIS.

Lipoproteins / triglycerides or biliary pigments (with a characteristic yellow – green color of the serum) at high concentration in the sample may lead to the visual impression of a bisalbuminemia on the electrophoretic pattern.

In the case of suspected contamination between two samples (very rare), due to the presence of some monoclonal components (in high concentration for example), it is recommended to repeat the test on those investigated samples (contaminating and potentially contaminated) by reversing their analysis order.

Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some monoclonal components may not be detected with this method.

A monoclonal component may be not detected (i.e., polymerized immunoglobulin spread or hidden in the polyclonal background). Conversely, a slight distortion of the electrophoretic pattern may indicate the presence of a monoclonal immunoglobulin. In all cases, the clinical context must be analyzed and if a gammopathy is suspected, it is then recommended to perform an immunotyping analysis on the sample. If an uncertainty persists, confirm the result by an immunofixation technique on agarose gel.

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

Precision

The precision of the CAPI 3 PROTEIN(E) 6 procedure was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP5-A2 guideline "Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition".

The means and coefficients of variation (CV %) of percentages (%) for each protein fraction from each sample were calculated using statistical tools recommended by CLSI.

Reproducibility between capillaries from the same instrument

8 different serum samples were run using the CAPI 3 PROTEIN(E) 6 procedure on the CAPILLARYS 3 instrument. The analyzed serum samples included 2 normal samples, 5 samples with abnormalities especially in beta and gamma zones and 1 sample with abnormalities including increased values in alpha zone.

In this study, each serum sample was analyzed on all capillaries from the same instrument and with 1 lot of CAPI 3 PROTEIN(E) 6 kit, including 6 runs over 3 working days (at 2 different times of the day). Within each run, samples were analyzed in duplicate.

CV ranges were obtained for each fraction by conducting this study with 3 lots of kit on 3 different instruments.

The following table summarizes the repeatability and the total reproducibility CV (%) ranges for percentages (%) of each protein fraction from all samples.

Fraction	Mean ranges		Repeatability						Total reproducibility					
			Instrument No. 1		Instrument No. 2		Instrument No. 3		Instrument No. 1		Instrument No. 2		Instrument No. 3	
	Min %	Max %	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)
Albumin	42.7	62.7	0.4	1.0	0.3	1.1	0.3	1.0	0.6	1.1	0.6	1.2	0.4	1.4
Alpha-1	3.1	8.5	1.0	5.2	0.8	5.4	0.6	6.2	1.0	5.8	0.8	5.7	1.5	7.2
Alpha-2	5.8	17.4	0.7	4.1	0.8	3.2	0.7	4.9	0.7	4.6	1.0	3.3	0.9	5.1
Beta-1	4.7	6.8	0.6	2.8	0.7	3.1	0.8	3.5	1.2	3.7	1.1	3.6	1.2	3.8
Beta-2	3.5	30.2	0.4	5.1	0.5	4.1	0.4	4.6	0.9	5.3	0.7	5.8	0.8	4.8
Gamma	6.6	31.0	0.5	1.9	0.5	2.7	0.5	2.0	0.9	2.2	0.8	2.8	0.7	2.4

Reproducibility between lots and between instruments

8 different serum samples were run using the CAPI 3 PROTEIN(E) 6 procedure on the CAPILLARYS 3 instrument. The analyzed serum samples included 2 normal samples, 5 samples with abnormalities especially in beta and gamma zones and 1 sample with abnormalities including increased values in alpha zone.

In this study, each serum sample was analyzed at 2 different times of the day on all capillaries from 3 different instruments and with 3 lots of CAPI 3 PROTEIN(E) 6 kit. Within each run, samples were analyzed in duplicate.

The analysis of obtained results allows to demonstrate the reproducibility :

- between lots : from data obtained with 3 lots of CAPI 3 PROTEIN(E) 6 kit on the same instrument, including 18 runs over 9 working days. CV ranges were obtained for each fraction by conducting this study on 3 different instruments.
- between instruments : from data obtained with 3 instruments and 1 lot of CAPI 3 PROTEIN(E) 6 kit, including 18 runs over 3 working days. CV ranges were obtained for each fraction by conducting this study on 3 different lots.
- between lots and between instruments : from combined data obtained with the 3 instruments and the 3 lots of CAPI 3 PROTEIN(E) 6 kit, including 54 runs over 9 working days.

The following table summarizes the repeatability and the total reproducibility CV (%) ranges for percentages (%) of each protein fraction from all samples.

Fraction	Mean ranges		Reproducibility between lots				Reproducibility between instruments				Reproducibility between lots and between instruments			
			Repeatability		Total reproducibility		Repeatability		Total reproducibility		Repeatability		Total reproducibility	
	Min %	Max %	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)	CV min (%)	CV max (%)
Albumin	42.7	62.7	0.4	0.9	0.5	1.0	0.4	1.0	0.7	1.4	0.6	0.9	0.8	1.2
Alpha-1	3.1	8.5	1.1	5.5	1.1	5.9	0.9	5.1	1.2	5.3	1.2	4.5	1.4	4.8
Alpha-2	5.8	17.4	0.9	4.2	0.9	4.6	0.8	3.9	1.0	4.0	0.9	3.6	1.1	3.6
Beta-1	4.7	6.8	1.0	2.9	1.4	3.0	0.7	2.7	1.8	3.1	1.4	2.3	2.1	2.9
Beta-2	3.5	30.2	0.6	4.2	0.9	4.5	0.5	4.1	0.8	4.3	0.6	3.5	1.0	3.9
Gamma	6.6	31.0	0.6	2.3	0.8	2.3	0.6	1.9	0.9	2.3	0.9	1.7	1.0	2.0

Sensitivity

Serial dilutions of one serum sample with an abnormal protein in gamma zone at 1.198 g/dL was electrophoresed using the CAPI 3 PROTEIN(E) 6 procedure.
The highest dilution with a discernible abnormality corresponded to a concentration of 19 mg/dL of the abnormal protein.

NOTE : According to the position of the abnormal protein and polyclonal background in the gamma zone, the detection limit may vary.

Linearity

The linearity study of the CAPI 3 PROTEIN(E) 6 procedure was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP6-A guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A statistical Approach; Approved Guideline".
The results for albumin and gammaglobulins percentages (%) were analyzed using statistical tools recommended by CLSI.

A solution (A) with high albumin concentration and a solution (B) with high gammaglobulins concentration were mixed within different proportions from 10 to 10 (100 % solution A + 0 % solution B, 90 % + 10 %, etc..., 0 % solution A + 100 % solution B) and the mixtures were electrophoresed with the CAPI 3 PROTEIN(E) 6 procedure. For each mixture, samples were analyzed in triplicate.
The results demonstrated that the obtained percentage of each fraction is perfectly correlated with the theoretical percentage of each fraction within the mixture and that any variation may be detected with linearity using the CAPI 3 PROTEIN(E) 6 procedure.
The CAPI 3 PROTEIN(E) 6 procedure was determined to be linear for albumin and gammaglobulins fractions :
- between 0.2 and 91.6 % for albumin fraction (studied concentration range : up to 4.99 g/dL),
- between 0.0 and 96.4 % for gammaglobulins (studied concentration range : up to 3.01 g/dL).

Accuracy – Internal correlation

The internal concordance study of the CAPI 3 PROTEIN(E) 6 procedure performed with the CAPILLARYS 3 instrument was evaluated in a study based on the Clinical Laboratory Standards Institute (CLSI - USA) EP09-A2 guideline "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (Interim Revision)".
The results for percentages (%) of each protein fraction were analyzed using statistical tools recommended by CLSI.

NOTE : The results presented below have been obtained from 1 internal accuracy study. The analyzed serum samples were provided by 2 laboratories in France.

All the samples were exactly treated the same way with both techniques and followed the same guidelines in regards to sample integrity.

The levels of each protein fraction were measured in 118 serum samples, including 18 normal samples and 100 pathological samples, both by electrophoretic separations obtained with the CAPI 3 PROTEIN(E) 6 procedure on the CAPILLARYS 3 instrument and another commercially available capillary electrophoresis technique for proteins analysis.
The measured values of percentages of the 6 protein fractions from both procedures were analyzed by a linear regression statistical procedure. The obtained results have demonstrated a perfect correlation between both procedures for proteins quantification.
The results of linear regression analysis are tabulated below (y = CAPI 3 PROTEIN(E) 6), the sensibility and specificity of the CAPI 3 PROTEIN(E) 6 procedure compared to the reference procedure have been calculated using the recommended method (Wendling, 1986).

Fraction	Correlation coefficient	y-Intercept	Slope	Range of % values CAPI 3 PROTEIN(E) 6	Sensibility (%)	Specificity (%)
Albumin	0.999	- 0.230	1.013	24.3 – 74.7	93.7	100.0
Alpha-1	0.998	- 0.056	0.981	2.0 – 16.1	90.7	98.4
Alpha-2	0.998	- 0.383	1.016	4.2 – 23.1	97.6	98.7
Beta-1	0.998	- 0.131	1.004	2.3 – 41.0	78.1	98.8
Beta-2	1.000	0.028	1.013	1.3 – 45.7	96.9	98.8
Gamma	1.000	- 0.329	1.014	1.7 – 64.2	97.1	100.0

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SCHÉMAS / FIGURES

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PROFILS ÉLECTROPHORÉTIQUES - ELECTROPHORETIC PATTERNS

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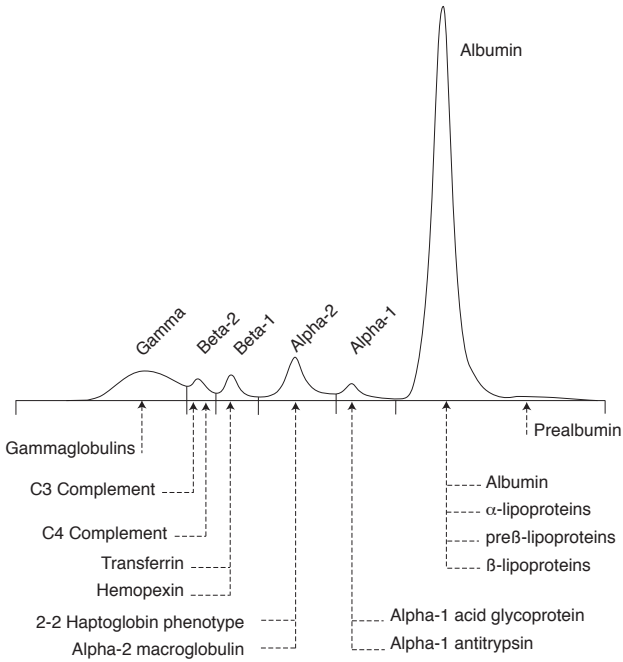

SCHÉMAS / FIGURES

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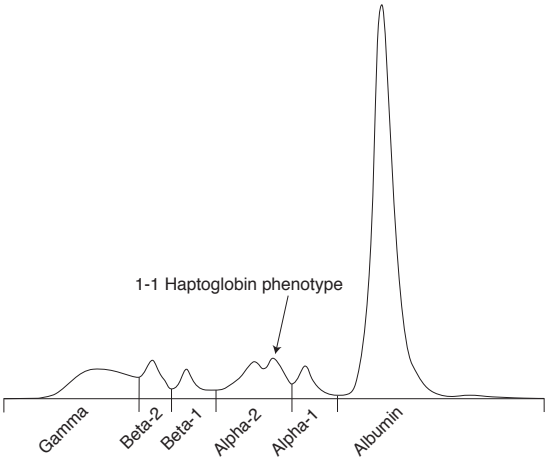

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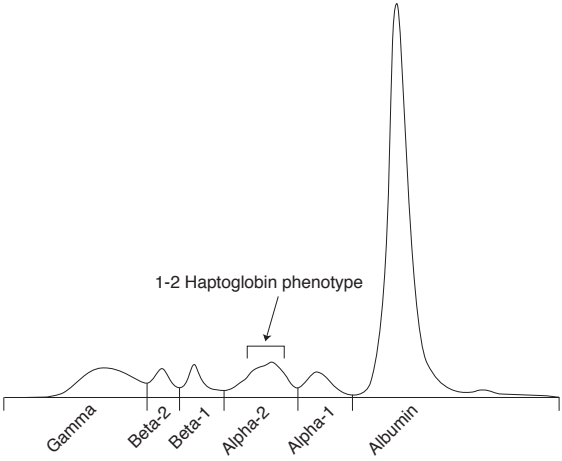

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