

URINE PROTEIN ANALYSIS BY CAPILLARY ELECTROPHORESIS IN CANINE AND FELINE SAMPLES

OBJECTIVES

- To learn using the MINICAP equipment by capillary electrophoresis (CE).
- To develop a laboratory protocol on capillary electrophoresis with dog and cat urine samples.
- To analyze the electrophoretic profile of urine samples from the Veterinary Clinical Biochemistry Service and the Veterinary Teaching Hospital.
- To compare results with bibliographic information with other methods that measure urine proteins.

RESULTS AND DISCUSSION



PROTOCOL FOR URINE ANALYSIS OF DOGS AND CATS BY CAPILLARY ELECTROPHORESIS MINICAP URINE (Vivaspin 500 µL)

NOTES:

- Quantify total protein concentration in the urine sample to be analyzed.
- For longer storage periods, it is recommended to keep sample frozen at -20°C.
- The centrifuge must be of a fixed angle.
- Working dialysis buffer: dilution ½ the dialysis buffer in distilled water.

1) Centrifuge 500 µL of urine at 3000 x g for 10 minutes.

2) Prepare each sample in a new dialysis system according to its total protein (TP) concentration:

TP	Urine	Distilled water
≤100 mg/dL	500 µL	0 µL
100 and 200 mg/dL	250 µL	250 µL

If the TP is >200 mg/dL, dilute with distilled water to <200 mg/dL.

3) Centrifuge at 10000 x g for 5 minutes.

4) Add 400 µL of working dialysis buffer.

5) Centrifuge at 10000 x g for 5 minutes.

6) Add working dialysis buffer to the sample tubes until 100 µL total volume.

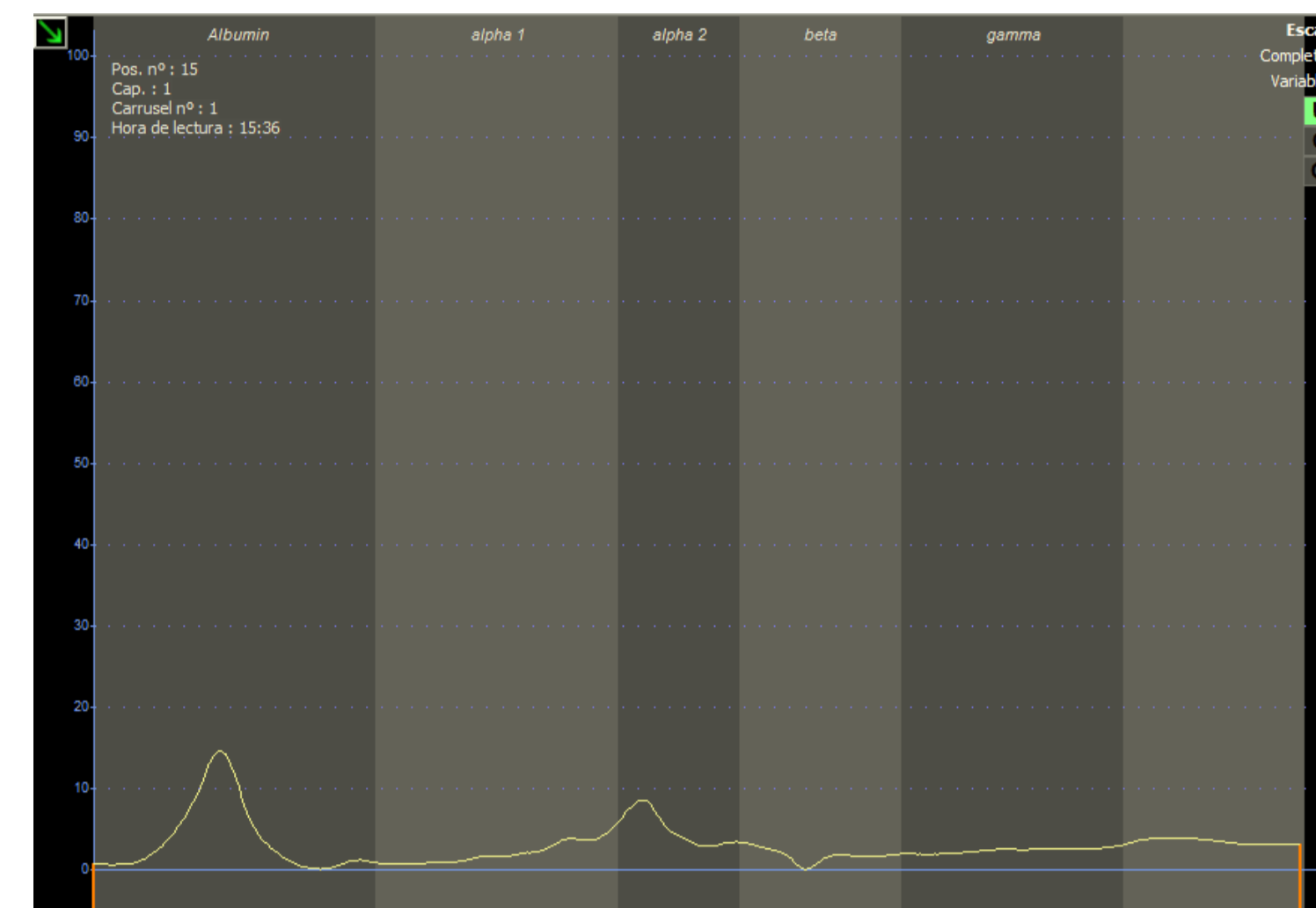
7) Dispense the urine in a microcentrifuge tube for further analysis in the MINICAP.

8) Change the work program to URINE.

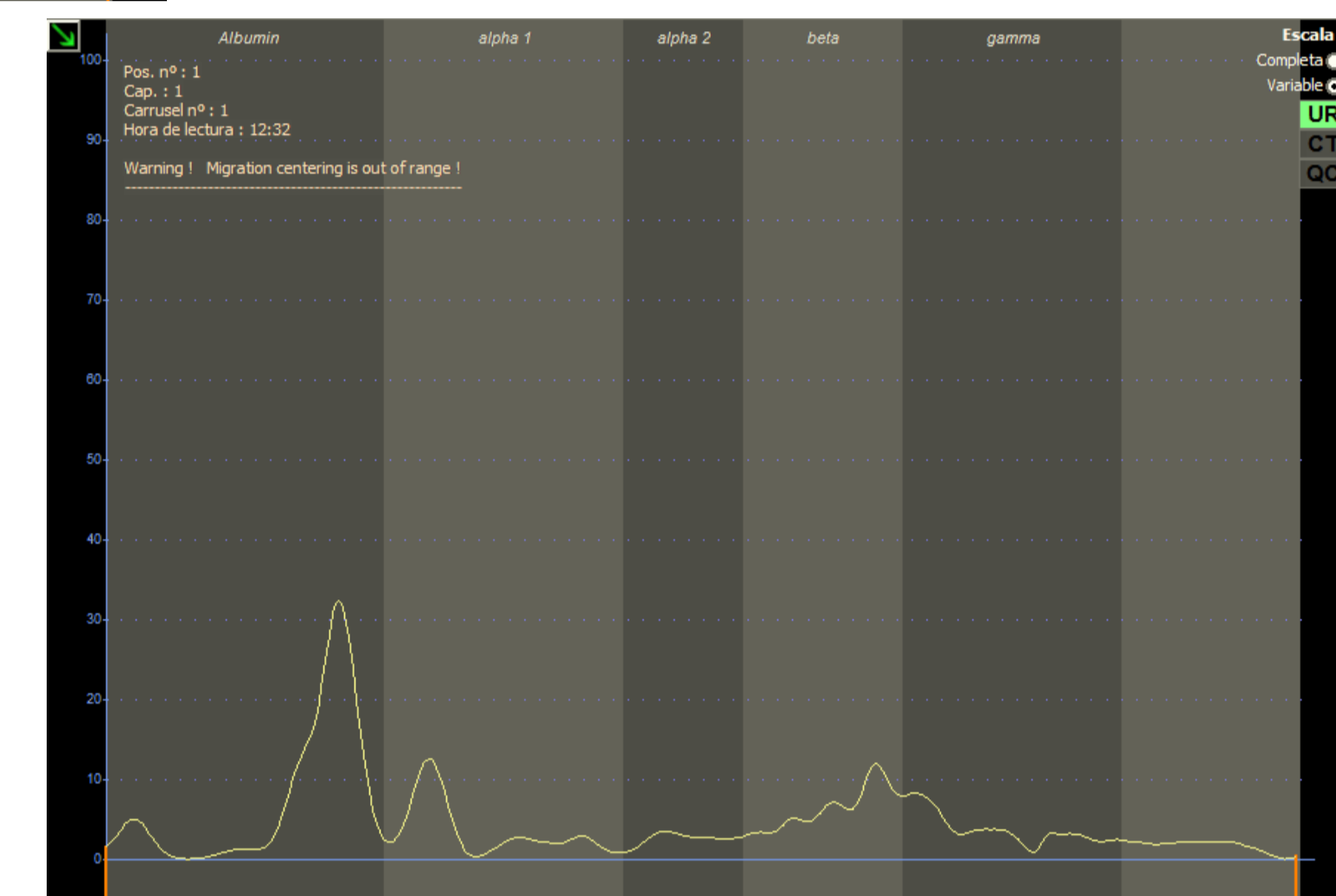
9) Fill the carousel and analyze the samples.

Technical problems:

- The final peak that comes out after the γ-globulin is an artifact caused by the dialysis buffer used in the protocol.
- The albumin peak may go out of scale. For quantification of protein fractions diluted further the urine.
- The displacement to the right of albumin in the feline species.



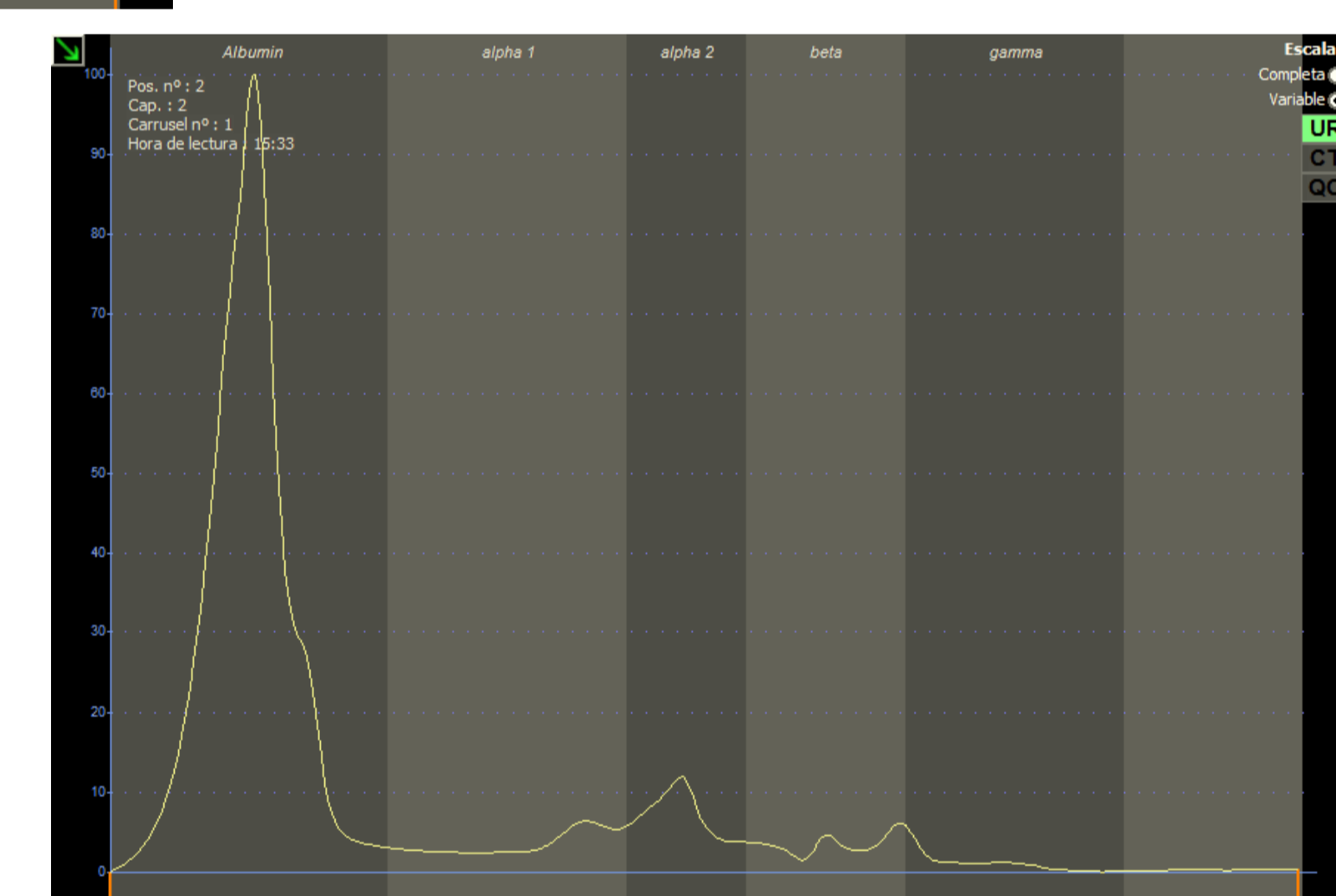
Dog urine protein CE
UPC → 0.3



Dog urine protein CE
UPC → 0.5



Dog urine protein CE
UPC → 1.5
Dialysis buffer peak



Dog urine protein CE
UPC → 3.2



Cat urine protein CE
UPC → 1.8

Urine Protein to Creatinine Ratio (UPC)

Proteinuria

Urine protein CE

0.1 a 0.3

Non proteinuria

Poorly visible and flat peak.

0.3 a 0.5

Proteinuria limit

More visible and defined albumin peak.

0.5 a 1.5

Proteinuria

Elevated and defined albumin peak. In some instances elevated globulin peaks.

>1.5

Serious proteinuria

Larger and noticeable albumin peak. Larger α1 and α2 globulin peaks and in some instances elevated β-globulins.

CONCLUSIONS

The objectives established at the beginning of the study have been achieved:

- It has been learned to use the MINICAP equipment and fluency has been achieved in the laboratory work.
- The technique and the protocol are valid to perform urine protein CE based on the obtained results.
- Urine protein CE is a valid methodology for the potential diagnosis and monitoring of some diseases, for example Leishmaniasis and kidney failure.
- As a test sample, urine has certain advantages in relation to other biological fluids.