



Suggested CAP Validation Procedures

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(1) Protein Recovery by Concentration Factor Test (See Notes Below)

- 1) Determine the initial total protein concentration (TP1) using a clinical chemistry analyzer.
- 2) Fill the concentrator with the sample volume (V1) and perform the concentration.
- 3) Measure the final concentrate volume (V2) accurately and then measure final TP (TP2) using a clinical analyzer.
- 4) Calculate the Concentration Factor (CF) according to the equation: $CF = V1 / V2$.
- 5) Calculate the Recovery (R) with the equation: $R = TP2 / (CF \times TP1)$. Multiply by 100 to convert to a percentage.

NOTE: A spreadsheet for this method can be downloaded using the “CAP EXCEL SHEET” link on this site.

Example: Initial TP = 80 mg/dL and sample volume = 5 mL

Concentrate volume = 100 µL (0.1 mL) and final TP = 3.4 g/dL (3400 mg/dL)

Concentration Factor = 5 / 0.1 = 50x. Recovery = 3400 / (50 x 80) = 3400 / 4000 = 0.85 = 85%

(2) Protein Recovery by Sample Dilution Test (See Notes Below)

- 1) Determine the initial total protein concentration (TP1) using a clinical chemistry analyzer.
- 2) Fill the concentrator with the sample and perform the concentration.
- 3) Add water to dilute the volume to the starting volume, mix well and then immediately withdraw all of the volume.
- 4) Determine the TP of the re-constituted solution from the concentrator (TP2) using a clinical chemistry analyzer.
- 5) Calculate the Recovery (R) according to the equation: $R = TP2 / TP1$. Multiply by 100 to convert to a percentage.

Example: Initial TP = 80 mg/dL

TP of re-constituted solution from test concentrator = 70 mg/dL

Recovery = 70 / 80 = 0.875 = 87.5%

(3) Electrophoresis Test

Use a urine sample with at least 30 ml of total volume and perform serial concentration tests as described below.

One series of concentrations could be: (1) Neat (Unconcentrated), (2) 10x, (3) 25x, (4) 50x and (5) 100x. For this series, fill four sample wells with urine and concentrate to the desired target factor. Other concentrations may be used.

After concentrating to the desired level, perform electrophoresis on the samples to observe enhancement of the protein bands. As the concentration factor increases, the bands should become darker in appearance. Also test the neat sample in the same manner but some bands may not be visible, depending on the protein levels and the gel being used.

NOTE FOR CAPILLARY ELECTROPHORESIS USE

For urine samples analyzed by capillary electrophoresis, the overall procedure is validated against the previous or current method or to send out results. Procedurally, this proves that the concentrators perform as indicated with the new instrument and assay as compared to the previous method.

To satisfy CAP requirements for just the concentrators, it would be best to test the concentrators without involvement of the capillary electrophoresis urine procedure. Above method (1) or (2) may be used for such validations since they do not involve electrophoresis. Labs may also contact CAP for information on verifying protein recovery.

NOTE ON TOTAL PROTEIN LEVELS FOR TEST SAMPLES

Using Total Protein (TP) is not a completely accurate method to measure recovery of M proteins. Labs have observed the loss of small urine proteins through the membranes. Some decreased recovery can be attributed to the loss of these proteins, which are not clinically significant. To minimize this effect, it is recommended to use samples with an initial TP of 50 mg/dL or higher for CAP validation.