Suggested CAP Validation Procedures

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Protein Recovery by Concentration Factor Test *(See Note Below)*

Determine the initial total protein concentration (TP1) using a clinical chemistry analyzer.

Fill the concentrator with the sample volume (V1) and perform the concentration.

Measure the final concentrate volume (V2) accurately and then measure final TP (TP2) using a clinical analyzer.

Calculate the Concentration Factor (CF) according to the equation: **CF = V1 / V2**.

Calculate the Recovery (R) according to the equation: **R = TP2 / (CF x 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%

Protein Recovery by Sample Dilution Test *(See Note Below)*

Determine the initial total protein concentration (TP1) using a clinical chemistry analyzer.

Fill the concentrator with the sample and perform the concentration.

Add water to dilute the volume back to the starting volume, mix well and then immediately withdraw all of the volume.

Determine the TP of the re-constituted solution from the concentrator (TP2) using a clinical chemistry analyzer.

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%

Electrophoresis Test

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

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

If the observed results do not perform as expected, repeat the steps with another urine sample.

NOTE: *Using Total Protein (TP) is not a completely accurate method to measure recovery of Bence Jones proteins. Labs have observed loss of small urine proteins through the filter membranes that are measured by clinical analyzers. Some decreased recovery can be attributed to the loss of these proteins, which are not clinically significant. Samples from patients with known disease are generally better in this regard for CAP validation. To minimize this effect,* ***it is recommended to use samples with an initial TP of 50 mg/dL or higher****.*