Total Protein Methods

Kjeldahl – Determination of protein by nitrogen.

Assumption of 16% Nitrogen mass of protein is made, no protein loss into supernatant during precipitation.

Proteins are precipitated using TCA or Tungstic acid, the supernatant is discarded.

Protein digested with H_2SO_4 and 350°C heat. Cupric sulfate catalyzes the reaction. Potassium sulfate introduced to increase boiling point and improve efficiency.

Nitrogen becomes NH_4HSO_4, which is measured by adding alkali and distilling ammonia into a standard boric acid solution.

The resultant ammonium borate $NH_4H_2BO_3$ is titrated with HCL to determine amount of nitrogen in solution.

Biuret- Determination of peptide bonds using Copper

Cu^{2+} ions will bind with tripeptide bonds forming a violet complex. Absorbance measured at 540 nm

Sodium potassium tartrate stops the complex from falling out of solution

Reacts more strongly with larger peptide chains

Dye Binding- Proteins will bind dyes, shifting abs. peaks

Bromophenol blue, Ponceau S, amido black 10B, lissamine green, and Coomassie brilliant blue all used to stain proteins for electrophoresis

Coomassie brilliant blue 250 shifts absorbance from 465-595 nm, increase is proportional to protein concentration

Not all proteins bind with similar affinity or avidity.

UV Method- Amino Acid R-groups produce absorbance peak at 280 nm

Albumin Methods

Dye Binding- Anionic dyes attracted to cationic albumin (acid pH buffer).

Absorbance peaks change, proportional to the amount of albumin present

HABA-Low sensitivity, specific for albumin, interfered with by salicylates, penicillin, bilirubin, sulfonamides.

Methyl Orange-Non-specific towards albumin, alpha globulins and lipoproteins also bind

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BCG- No interfering substances, but hemoglobin will also bind, overestimates in patients with low albumin

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BCP-Not subject to most interferences, precise, excellent correlation with reference method, Underestimates albumin in renal patients.

Protein in Fluids Methods

Urine Turbidimetric- Anionic organic chemicals lead to turbidity when mixed SSA, TCA, benzethonium chloride all used. Do not react equally well with all proteins, but better than dipstick. Does not react with normal urine proteins

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Urine Biuret- Precipitate proteins, then dissolve, measure with biuret

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Folin-Ciocalteu: Phosphotungstomolybdic acid, changes from yellow to blue when reacting with tyrosine, tryptophan and histidine residues

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Folin-Lowry- Biuret before Folin-Ciocalteu increased sensitivity 100x, also may use pyrogallol red-molybdate.

Immunoassay- Used for microalbumin assays

Dipstick- Protein error of indicator. Acid buffer keeps pad at low pH. Proteins are able to change the absorbance peak of pH indicator without changing the pH. Most sensitive for albumin, will not detect microalbumin amounts.