

Understanding nutrition

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??? ???? ?????? ?????? Al-Quds University Body Fluid Lab. Report Chemical Examination of Urine Prepared By : Lucia Principles Benedict's Test for Reducing Sugars Urinary sugars when boiled in Benedict's reagent reduce copper sulphate to a reddish cuprous oxide precipitate in hot alkaline medium, the intensity of which is proportional to the amount of sugar present in the urine. The results are reported as 1+, 2+, etc. depending upon the colour and intensity of the cuprous oxide precipitate. turbidimetric method protein in urine Quantitative Tests for 24-Hour Specimens.

Trichloroacetic acid (TCA) test.

The addition of TCA to a urine specimen precipitates the protein in a fine suspension that is quantified spectrophotometrically at 420 nm (nanometers) by comparison with a similarly treated standard. Heat and acetic acid method Heat test: Based on the principle of heat coagulation and precipitation of proteins. If any turbidity appears, add 2 drops of 33% acetic acid. (Acidification is necessary because in alkaline medium heating may precipitate phosphates). If the precipitate is due to proteins, it will increase on acidification and if it is due to phosphates, it will dissolve again.

Sulphosalicylic acid method Urine Protein Sulfosalicylic Acid Precipitation Test (SSA) Principle: Three percent (3%) Sulfosalicylic Acid (SSA reagent) is added to a small and equal volume of clear urine. The acidification causes precipitation of protein in the sample (seen as increasing turbidity), which is subjectively graded as trace, 1+, 2+, 3+ or 4+. Bence Jones protein Bence Jones reaction involves heating urine to 140°F (60°C). At this temperature, the Bence Jones proteins will clump. The clumping disappears if the urine is further heated to boiling and reappears when the urine is cooled.

Other clumping procedures using salts, acids, and other chemicals are also used to detect these proteins. These types of test will reveal whether or not Bence Jones proteins are present, but not how much is present. Hemosiderin stain is used to indicate the presence of iron storage granules called hemosiderin by microscopic examination of urine sediment. Granules of hemosiderin stain blue when potassium ferrocyanide is added to the sample. The Prussian blue stain may also be used to identify siderocytes (iron-containing red blood cells [RBCs]) in peripheral blood.

The presence of siderocytes in circulating RBCs is abnormal. Urobilinogen This test is based on a modified Ehrlich reaction in which p-diethylaminobenzaldehyde reacts with urobilinogen in a strongly acid medium. Colors range from light pink to bright magenta. Results * Benedict's Test result for cup # G3 : 4+ Brown color appear. * Turbidimetric method result for cup of 24-hrs urine: Tube| Absorbance| Test+test-blank| 0. 058| Standard+water blank| 0. 010| Calculation : Total protein (mg/dl)= $A_t/A_s \times \text{conc. St}$ $0.058/0.010 \times 100 = 580$ mg/dl Total protien(mg/24 hrs) = urine protein (mg/dl)? urine volume(ml)/100 = $580 \times 2000/100 = 11600$ mg /dl Normal values : 0-150 mg/24 hrs * Heat and acetic acid method and Sulphosalicylic acid method for cup # P4: 4+ precipitation appeared. * Bence Jones protein for cup # P4 : Clear after 15 min of boiling so negative for Bence Jones protein. * Hemosiderin results : Few Hemosiderin granules was seen under Microscope * Urobilinogen result for cup # G3: Negative result (no appearance of red color). Interpretation Benedict's Test for Reducing Sugars Normal urine does not contain any reducing sugar. If protein is

present in large amounts, it may interfere with the precipitation of the cuprous oxide.

To overcome this problem, precipitate the proteins using 3% SSA filter using a Whatman filter paper and use the filtrate to test the amount of sugar present. As a quality control measure, standards containing known amounts of glucose are prepared in saturated benzoic acid and one of the standards is used every day to check the reliability of the patient's results. The standard results may be transformed in the following semi-quantitative way.

turbidimetric method protein in urine For turbidimetric methods, there were no apparent problems of comparative bias between human albumin and serum-based materials and urines used in this study.

Perhaps this was because all materials were diluted in 9 g/L saline: turbidimetric methods generally suffer from failure of standards and samples to form precipitates identically, and precipitation may not occur at low protein concentrations in urines of high ionic strength. Heat and acetic acid method This test is sensitive enough to detect protein down to a concentration of 2-3 mg%. If an alkaline urine is boiled, the protein may be converted into the so-called "alkaline metaprotein", which is not coagulated by heat. Therefore it is always better to acidify the urine before doing this test.

If too much acetic acid is added, the protein may be converted to the so-called "acid metaprotein", which is also not coagulated by heat. Therefore the urine should be only mildly acidic. Sulphosalicylic acid method The sulphosalicylic acid method will not detect protein in a normal urine, but will be sensitive enough to detect protein present down to 20mg%. As a

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quality control measure, a 22g/dl albumin solution can be diluted appropriately with 0.9 g/dl sodium chloride to get standards containing 20, 50, 200, 500 and 2500 mg/dl proteins.

These standards are stable for one month when stored at 2-8°C. Bence Jones protein Monoclonal light chain proteinuria (Bence Jones proteinuria) is seen in patients with light chain myeloma, in approximately 50% of those with IgG and IgA myeloma, and in some patients with other lymphoproliferative disorders (eg, macroglobulinaemia) and plasma cell dyscrasias (amyloid). Entire paraprotein molecules may also be detected in serum. Urine protein dipsticks do not detect Bence-Jones protein. Hemosiderin Hemosiderin is present in diseases involving a true siderosis of kidney parenchyma (hemochromatosis).

It is also present 2-3 days after an acute hemolytic episode that produces hemoglobinemia and hemoglobinuria. Hemosiderin granules are found in intact renal tubular epithelial cells or occasionally in casts and may also be seen extracellularly. Urobilinogen Interpretation of results will depend upon several factors: the variability of color perception; the presence or absence of inhibitory factors; the presence or absence of inhibitory factors typically found in urine, the specific gravity or the pH; and the lighting conditions under which the product is used.