Tests for Glucose

Benedict's Qualitative (Semi quantitative) Glucose test

In this method the cupric ion is reduced to Cu_2O (Cuprous oxide). If only 0.1% or less of glucose is present, the precipitate may not appear until cooling. To 5 ml of Benedict's qualitative reagent, add 8 drops of urine (0.5 ml). Heat to boiling and set in a boiling water bath for 5 minutes or else boil it over a flame for 2 minutes.

Read as follows:

Blue to cloudy Green color	= Negative, 0
Yellow – green	= + (< 0.5 % glucose)
Greenish yellow	= ++ (0.5 – 1 % glucose)
Yellow	= +++ (1 – 2 % glucose)
Orange to brick red	= ++++ (over 2 % glucose)

Sensitivity of this test is 50 mg% or more.

Glucose oxidase methods: Glucose oxidase reacts with glucose to yield gluconic acid and hydrogen peroxide. Hydrogen peroxide and orthotolidine yield a blue color. This is a specific test. The reagents may be impregnated on paper strips and dipping them in urine provide the result in lesser time as compared to Benedict's method (sensitivity =0.1%).

Benedict's Quantitative Glucose test

Place a small quantity of powdered pumice, 10 gm of anhydrous sodium carbonate and 25 ml of quantitative Benedict's reagent in a 250 ml container and heat. While the mixture is boiling, add urine rapidly from a buret until the blue color begins to fade, then add urine drop by

drop until all blue color is gone and only a grey color remains. The amount of urine used contains 0.05 gm of glucose. To calculate grams of glucose per 100 ml of urine, divide 5 by the number of ml of urine used.

Quantitative Method

Urinary sugar can also be detected by routine biochemical kits too.

Ketone bodies in urine

The three ketone bodies that can be detected in urine are:

1- Acetone (2%)
2- Acetoacetic acid (20%)
3- β-Hydroxybutyric acid (78%)

ketone bodies are products of incomplete fat metabolism and their presence is indicative of **acidosis**.

Tests for ketone bodies

(Never heat urine specimen before performing the tests).

1- Rothera's Test

Saturate 5 ml of urine with ammonium sulfate, add a few crystals of sodium nitroprusside, shake. Add liquor ammonia from the side of the test tube, formation of a purple ring at the junction indicates a positive test.

Sensitivity > 1-5 mg% acetoacetic acid, or > 10-25 mg% of acetone.

2- Legal's Test

Take 10 ml of urine in a test tube and add a few crystals of sodium nitroprusside. Acidify with glacial acetic acid, invert to mix. Overlay with strong liquor ammonia, let stand for 5 minutes. **A violet ring indicates a positive test**.

3- Paper Strip

These contain sodium nitroprusside, aminoacetic acid and disodium phosphate. A positive test is indicated by development of a purple color.

4- Diacetic Acid Test

Not a very sensitive test. Perform this test if the test for acetone was positive. Precipitate the phosphates in 5 ml of urine with 10% ferric chloride solution, drop – by – drop , filter and add more ferric chloride. If a purple – red color appears, it indicates presence of 0.05% or more of diacetic acid. False-positive test may appear with salicylates, sodium bicarbonate, etc.

Bile Salts

Bile salts when present decrease the surface tension of urine. When sulfur powder is added on the surface of urine, sulfur particles sink to the bottom of the test tube. In normal urine sample sulfur particles float on the surface of the urine.

Method

1- Take about 10 ml urine in a test tube.
2- Sprinkle a little dry sulfur powder on the surface of urine.
3- Observe the sulfur practices.

Interpretation

1- Sulfur particles sink to the bottom: bile salts present.
2- Sulfur particles remain floating: bile salts absent.

Bile Pigments

Bile pigments (always use fresh specimen). Normal level of bile pigments is urine in < 0.02 mg%.

1- Foam Test

Not very accurate as proteins can also form foam. Shake 5 ml of urine in a test tube, bile produces a yellowish foam which persists.

2- Iodine Ring Test

A sensitive cum reliable test. Layer a solution of 10% alcoholic iodine on urine in test tube. A green ring indicates presence of bile.

3- Harrison Test

A sensitive test. To 5 ml of urine, add 5 ml of 10% barium chloride in a test tube. Shake. Filter it off. Let the filter paper dry. When dry, add 1-2 drops of Fouchet's reagent to the dried precipitate. A green (disregard all other colors) color indicates bilirubinuria.

4- Diazo Test

p-nitrobenzene diazonium p-toluene sulfonate is the active reagent. Place 5 drops of the urine on the mat provided in the kit. Bilirubin, if present shall be absorbed onto the mat surface. Place a reagent tablet on it. Let 2 drops of water flow over the tablet. A positive test is indicated by the appearance of a blue to purple color within 30 seconds. Pink/red color is negative. Sensitivity > 0.1 to 0.05 mg% of bilirubin in urine.

5- Paper strip method

After dipping the strip in urine, match with the color chart provided by the manufactures.

Urobilinogen and Urobilin

Urobilinogen is colorless, and on standing it gets oxidized to urobilin which has brown color. It is best to perform tests for urobilinogen on fresh specimens. If delay is inevitable, collect the sample

in a dark bottle, provide a surface layer of petroleum ether and add sodium carbonate (5 gm for 24 hours volume) and refrigerate the sample.

1- Urobilinogen

To 10 ml of fresh sample at room temperature add 1 ml of Ehrlich's reagent, invert several times and let stand for 5 minutes. A pink color is normal, cherry or darker red color indicate abnormal amounts of urobilinogen. Normal values: 0.1 – 1 Ehrlich/dl.

2- Urobilin (Schlesinger's Test)

Convert urobilinogen to urobilin by adding a few drops of Lugol's solution. Mix 10 ml of urine with an equal quantity of saturated alcoholic solution of zinc acetate filter into a dry test tube.

Abnormal amounts of urobilin give the filtrate a green fluorescence which is best seen against a dark background with a light source from the side, or in sunlight against a black background.

Porphyrine

Perform Ehrlich's test for urobilinogen by mixing equal parts of urine and Ehrlich's reagent. Add 2 parts of saturated sodium acetate solution and mix. If turbid, filter. Shake with a small quantity of chloroform. Urobilinogen is soluble in chloroform, prophobilinogen is not. If after several extractions with chloroform the aqueous phase is still pink, the test is positive for porphobilinogen.

Normal Values

Prophobilinogens : 2mg/ 24 hr or negative

Porphyrins : 50-300 mg/ 24 hr

DAL or ALA : 1-710 mg/24 hr (delta-aminolevulinic acid)

Fluorescent : Negative

Blood in Urine(Haematuria)

Haematuria can be gross, urine appears reddish due to blood, it can also be microscopic, when it is not visible to the naked eye, here various tests are performed for confirmation.

1-Guaic Test

In one test tube, mix 2 ml of 10% acetic acid, 5 ml of urine and 5 ml ether. In a second test place 5 ml of 95% alcohol, 2 ml fresh hydrogen peroxide and a pinch of powdered guaic. Now pour the guaic solution slowly down the side of the first tube. Blood in the urine causes blue color to appear at the zone of contact between the guaic and ether.

2-Benzidine Test

Saturate 2 ml of glacial acetic acid with benzidine and pour off the clear supernatant fluid. Add 1 ml of fresh hydrogen peroxide and 2 ml of urine. Development of blue color indicates a positive test (if the blue color develops before the addition of urine, the glassware is contaminated).

3- paper Strips

Blood reacts with the peroxide – orthotolidine reagent to produce a blue color.

Nitrite/Bacteria

Normal values: Negative for bacteria.

Explanation of test: There are two methods that are used to detect bacteria in the urine during routine – microscopic examination and clinical testing. The sediment when examined microscopically can reveal bacteria when present. Chemical dipstick testing is also commonly done. The nitrite area in the multiple reagent strip is calibrated so that any shade of pink color that develops within 30 seconds indicates an amount of nitrite produced by 10^5 or more organisms per ml in the urine specimen.