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AN EPITOME OF BIOMEDICINE

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AN EPITOME OF BIOMEDICINE

1.URINALYSIS

INTRODUCTION

Urinalysis is a laboratory examination of a person's urine used to detect and manage a wide range of disorders related to kidney diseases, urinary tract infection, diabetes and to carry out pregnancy test.

It involves checking the appearance, concentration and content of urine which are eventually used in analyzing the urine's contents like sugar and protein-and the types and number of cells it contains.

Diagnosis depends on specific urinalysis tests which include;

- ✓ **Urine pH level test**
- ✓ **Ketones urine test**
- ✓ **Urine specific gravity test**
- ✓ **Bilirubin urine test**
- ✓ **Protein urine test**
- ✓ **Glucose urine test**
- ✓ **RBC urine test**
- ✓ **Urobilinogen**
- ✓ **Nitrites**
- ✓ **Leukocyte esterase(White Blood Cells count)**

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Urinalysis Principle

Routine urinalysis consists of color, clarity specific gravity and a chemical examination including pH, albumin, ketones, glucose, bile, leukocyte esterase, nitrites and blood. Microscopic examination, if indicated, includes cell identification, casts, crystals, bacteria, and miscellaneous.

The Principle of Dipstick Urinalysis

Multistix urinalysis test kits (Choiceline 10) Takes three (3) minutes.

A totally negative dipstick test which is associated with negative microscopy in 90-95 % of cases. Faulty negative results ranging from 5-10%.

Bayer Multistix 10-SG Reagent Strips contain reagent areas for testing glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrites and leukocytes. Test results may provide information regarding the status of carbohydrates metabolism, kidney and liver function, acid-base balance and UTI.

The test relies upon reaction of glucose with glucose oxidase to form hydrogen peroxide which eventually causes color change.

N/B: Dipsticks employing the glucose-oxidase/peroxidase reaction for screening are specific to glucose(dextrose) and not any other sugar i.e galactose and fructose(levulose) and are not suitable for testing newborn and infant urine.

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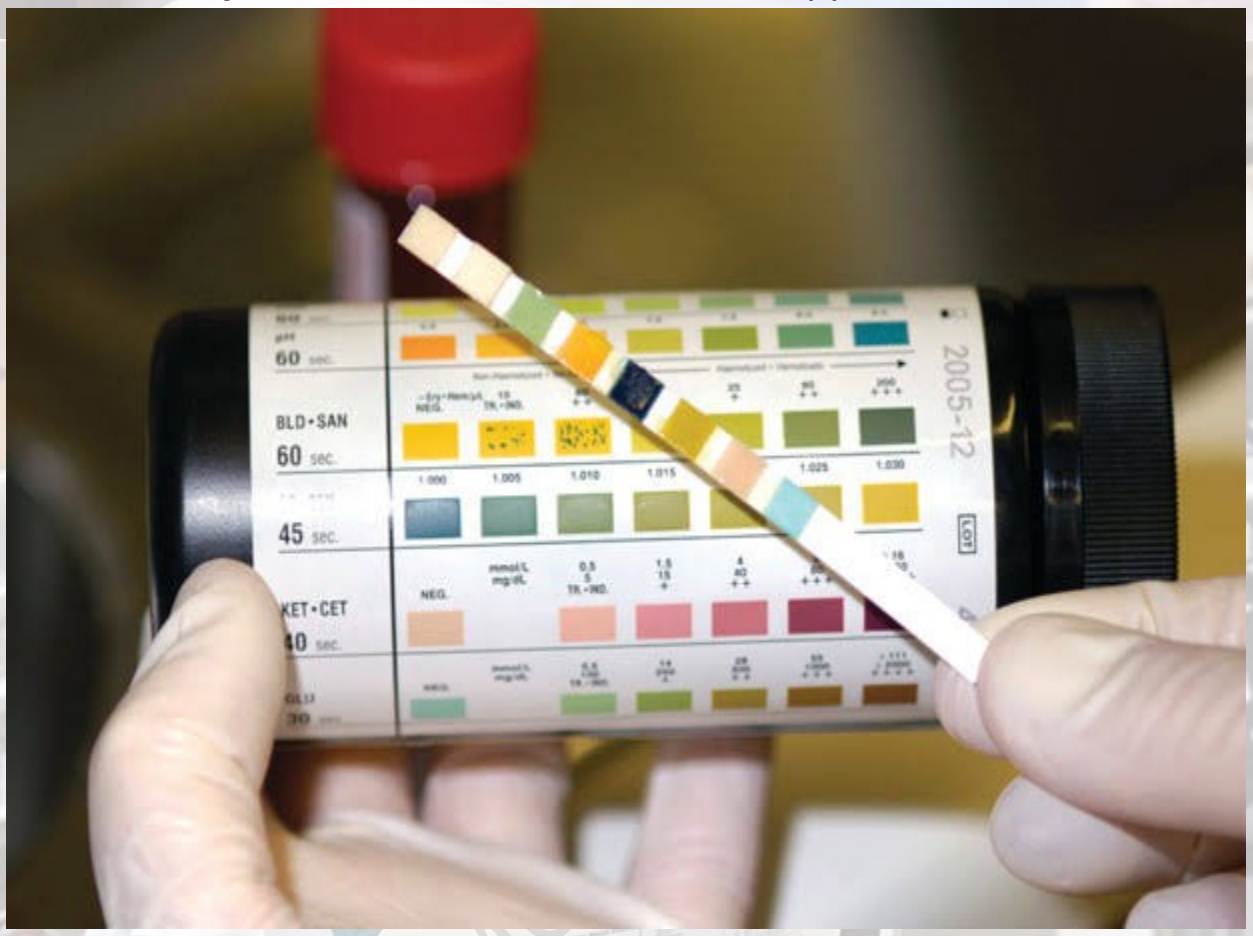
Alternatively, use a modified Benedict’s copper reduction test.



Individual Test Principles and Clinical Significances.

The urinalysis is a composite examination that is usually carried out with a single specimen of urine performed and recorded in a systematic sequence. This examination offers many invaluable clues in the detection, differential diagnosis and evaluation of urinary tract disorders, especially in the areas of metabolic and renal disorders.

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Glucose

This test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. On the other hand, enzyme peroxidase catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.

Glycosuria occurs when the blood glucose levels exceed the reabsorption capacity of the renal tubules. The condition may be a benign condition, renal glycosuria, occurring after ingestion of a carbohydrate ladened meal or in conjunction with emotional stress, or a pathological condition, diabetes mellitus (hyperglycaemia), occurring from a marked elevation of the blood glucose and insulin resistance or by impaired insulin production.

Glycosuria is also associated with certain drugs such as cephalosporins, penicillin, nitrofurantoin, methyldopa, tetracycline, lithium, carbamazepine, phenothiazines, steroids and thiazides.

False positive- Hydrogen peroxide or bleach.
False negative- Ascorbic acid or fruit juices. However, some dipsticks are affected by increased specific gravity or ketonuria.

❖ Bilirubin

This test is based jo the coupling of bilirubin with diazotized dichloroaniline in a strongly acidic medium. The color ranges through various shades of tan.

Bilirubin is not present in the urine of anormal healthy person. The presence of bilirubin may be an early indicator of liver disease and occur before the clinical signs of jaundice development.

Bilirubin is formed as a by-product of red blood cell degradation in the liver, and then conjugated with the solubilizing sugar, glucuronic acid and excreted in the bile. Within the intestine, the bilirubin is converted into stercobilin (excreted in the faeces) and urobilinogen (excreted by the kidneys).

Failure of conjugated bilirubin to reach the intestines (e.g biliary obstruction) will result in bilirubinuria. Only conjugated bilirubin can be excreted as bilirubinuria. A positive test for urine bilirubin confirms the presence of conjugated hyperbilirubinaemia.

Raised conjugated bilirubinaemia (with bilirubinuria) is associated with hepatocellular disease, cirrhosis, viral and drug induced hepatitis, biliary tract obstruction (e.g choledocholithiasis), pancreatic causes of obstructive jaundice (e.g carcinoma of the head of pancreas) and recurrent idiopathic jaundice of pregnancy.

False positive- Phenothiazines

False negative- Ascorbic acid, aged samples (conjugated bilirubin hydrolizes to unconjugated bilirubin under normal room temperature), rifampicin and exposure to UV light(converts bilirubin to biliverdin).

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❖ Ketone

This test is based on the development of colors ranging from buff-pink, for a negative reading, to purple when acetoacetic acid reacts with nitroprusside.
Ketones (acetone, acetoacetic acid, beta-hydroxybutyric acid) are the end-point of incomplete fat metabolism. They accumulate in the plasma and are excreted in the urine.
Ketonuria is associated with low carbohydrate (high fat/protein) diets, starvation, diabetes, alcoholism, eclampsia and hyperthyroidism.
Ketonuria is also associated with overdose of insulin, isoniazid and isopropyl alcohol.
Most urinalysis reagent tests utilize the nitroprusside test which is most sensitive to acetoacetic acid, less sensitive to acetone, and not sensitive to beta-hydroxybutyric acid.
False negative- Heavily pigmented urine. Drugs such as captopril, L-dopa, salicylates, phenothiazines.
False positive- Negative nitroprusside tests for ketonuria underestimate the presence of ketonemia due to increased beta-hydroxybutyric acid concentrations.

❖ Specific Gravity

This test is based on the apparent pKa (acid) change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, color changes from deep blue-green in the urine of low ionic concentration through green and yellow-green in urine of increasing ionic concentration.

Specific gravity of urine is a measure of the number of solutes dissolved in urine as compared to water (1.000).

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S.G measures the ability of the kidney to concentrate or dilute the urine and is directly proportional to urine osmolality (solute concentration).

Specific gravity between 1.002 and 1.035 on a random sample is normal if kidney function is normal.

Decreased S.G :<1.005

-Inability to concentrate urine or excessive hydration (volume resuscitation with iv fluids).

-Nephrogenic diabetes insipidus, acute glomerulonephritis, pyelonephritis, acute tubular necrosis.

-**Note**-The renal concentration ability may be impaired if S.G is not >1.022 after a 12 hour period without food or water. The patient will therefore have either a generalized renal impairment or nephrogenic diabetes insipidus.

-Falsely low specific gravity can be associated with alkaline urine.

Fixed: 1.010

-The glomerular filtrate in Bowman's space ranges from 1.007 to 1.010, any measurement below this range indicates dehydration and any measurement above this indicates relative dehydration.

-In end stage renal disease, specific gravity tends towards 1.010.

-Chronic Renal Failure (CRF), Chronic glomerulonephritis (GN).

Increased: >1.035

-Increased specific gravity indicates concentrated urine with a large volume of dissolved solutes.

-Dehydration (fever, vomiting, diarrhoea), SIADH, adrenal insufficiency, pre-renal failure, hyponatraemia with oedema, liver failure, CCF, nephrotic syndrome.
-Elevation in specific gravity also occurs with glycosuria (e.g diabetes mellitus or IV glucose administration), proteinuria, IV contast, urine contamination, LMW dextran solution (colloid).

❖ **Blood**

This test is based on the peroxidase-like activity of haemoglobin, which catalyzes the reaction to diisopropylbenzene and 3,3' , 5,5'-tetramethylbenzidine. The resulting color ranges from orange through green, very high levels of blood may cause the color development to continue to blue.
Occult blood occurs in the urine as intact RBCs and haemoglobin which can occur during urological, nephrological and bleeding disorders. Blood is often found in the urine of menstruating females.
-Dipstick is able to detect haemolysed and and non-haemolysed blood in the urine.
-The pseudoperoxidase reaction of erythrocytes, free haemoglobin or myoglobin catalyses chromogen oxidation on the dipstick to produce a color change.
-A positive result may be indicative of haematuria from trauma, infectiin, inflammation, infarction, calculi, neoplasia, clotting disorders or chronic infection.
-Haemoglobinuria may be associated with intravascular haemolysis, burns, sudden cold, eclampsia, sickle cell crisis, multiple myeloma, alkaloids (mushroom) and transfusion reactions.

❖ pH

This test is based on a double indicator principle that gives a broad range of colors covering the entire urinary pH range. Colors range from orange through yellow and green to blue. Urine pH is a general indicator of the acid-base balance of the body controlled in part of the kidney. Certain dietary conditions can produce acid or alkaline urines, which can be useful in the treatment of some calculi.

-The kidneys play an important role in acid-base regulation within the body to maintain a normal urinary pH range between 5.5-6.5 but may vary from as low as 4.5 to as high as 8.0.

-The glomerular filtrate of blood plasma is usually acidified by renal tubules and collecting ducts from a pH of 7.4 to about 6 in the final urine. Control of pH is important in the managerial of several diseases, including bacteriuria, renal calculi, and drug therapy.

High Urinary pH (Alkali Urine)

-Vegetarian diet, low carbohydrate diet or ingestion of citrus fruit (although citrus fruits are acidic – the digestion process leaves an alkali ash)

-Systemic alkalosis (metabolic or respiratory)

-Renal tubular acidosis (RTA I (distal)), Fanconi syndrome.

-UTI (bacteriuria with urea splitting organisms)

-Drugs: Amphotercin B, carbonic anhydrase inhibitors (acetazolamide), $NaHCO_3$, salicylate OD

-Stale ammoniacal sample

Low Urinary pH (Acidic urine)

-High protein diet or fruits such as cranberries.

-Systemic acidosis (metabolic or respiratory)

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-Diabetes mellitus, starvation, diarrhoea, malabsorption.
-Phenylketonuria, alkaptonuria, renal tuberculosis.

❖ **Protein**

This test is based on the protein-error-of-indicators principle. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow, for "negative" through yellow-green to green to green-blue for "positive" reactions.
Protein in the urine can be a result of urological and nephrological disorders, glomerular or tubular abnormalities and temporarily elevated in the absence of renal abnormality by strenuous exercise, orthostatic proteinuria, dehydration, urinary tract infections and acute illness with fever.
-Normal daily protein excretion should not exceed 150mg/24 hours or 10mg/100ml. Proteinuria is defined by the production of> 150mg/day with nephrotic syndrome producing >3.5g/day.
-Dipstick urinalysis detects protein with Bromphenol blue indicator dye and is most sensitive to albumin and less sensitive to Bence-Jones protein and globulins. Trace positive results are equivalent to 10mg/100 ml or about 150 mg/24 hours (the upper limit of normal)

True Protein Elevation:

- **Renal:** Increased renal tubular secretion, increased glomerular filtration (glomerular disease), nephrotic syndrome, pyelonephritis, glomerularnephritis, malignant hypertension.
- **CVS:** Benign HT, CCF, SBE

- **Functional proteinuria (albuminuria):**

  Feel, cold exposure, stress, pregnancy, eclampsia, CHF, shock, severe exercise.

- **Other:**

  Orthostatic proteinuria, electric current injury, hypokalaemia, cushing syndrome.

- **Drugs:**

  Aminoglycosides, gold, amphotericin, NSAID, sulphonamides, penicilins.

- **False Positive:**

  Concentrated urine (UO<2.L/day), alkaline urine (pH >7.5), trace residue of bleach, aceazolomide, cephalosporins, NaHCO3.

- **False Negative:**

  Dilute urine (UO >5.0 litres/day) or acidic urine (pH <5)

Dipstick Protein Reading	**Protein Excretion gm/24 hours**	**Protein excretion mg/dL**
Negative	<0.1	<10
Trace	0.1-0.2	15
1+	0.2-0.5	30
2+	0.5-1.5	100
3+	2.0-5.0	300
4+	>5.0	>1000

- **Note:**

Bence Jones globulin associated with multiple myeloma, lymphoma and macroglobulinaemia is not detected by dipstick urinalysis.

❖ **Urobilinogen**

-Urobilinogen is normally present in the urine in low concentrations (0.2-1.0 mg/dL or <17 micromol/L). Bilirubin is converted to urobilinogen by intestinal bacteria in the duodenum. Most urobilinogen is excreted in the faeces or transported back to the liver and converted into bile. The remaining urobilinogen (<1%) is excreted in the urine.
-Urobilinogen is present in increased concentrations in the urine in patients with cirrhosis, infective hepatitis, extravascular haemolysis, haemolytic anaemia, pernicious anaemia, malaria and hepatitis secondary to infectious mononucleosis.
-Very sensitive but non-specific test to determine liver damage, haemolytic disease and severe infections. Urobilinogen levels are decreased or absent in obstructive jaundice and elevated levels of bilirubinuria.

Dipstick Urinalysis	**Normal**	**Biliary obstruction**	**Hepatic disease**	**Haemolytic disease**
Bilirubin	Negative	Positive	Positive	Negative
Urobilinogen	Positive	Negative/ decreased	Increased	Increased

❖ Nitrite

This test depends upon the conversion of nitrate (derived from the diet) to nitrite by the action of gram-negative bacteria in the urine. At the acid pH of the reagent area, nitrite in the urine reacts with p-arsanilic acid to form a diazonium compound. This diazonium compound turn couples with 1,2,3,4-tetrahydrobenzo(h)quinoline-3-ol to produce pink color.

Many enteric gram-negative microorganisms possess the characteristics of conversion of dietary nitrates to nitrite and yield a positive nitrite when given adequate bladder incubation. Significant bacteriuria is probable with a positive nitrite result, but not ruled out with a negative nitrate result.

-Nitrates in the presence of gram Gram-negative bacteria such as E. coli and Klebsiella are converted to nitrites in the urine.

-A positive nitrite test is a surrogate marker of bacteriuria.

-Positive test strongly suggests infection but negative test does not exclude it (PPV 95% and NPV 25-70%)

False negative: Drugs or foods that color the urine red. Certain bacteria such S. saprophyticus, Acinetobacter and most enterococci.

	Sensitivity	**Specificity**	**PPV**	**NPV**
Dipstick				
Leucocyte esterase	75-90	95	50	92
Nitrite	35-85	95	96	27-70
Nitrite and	75-90	70	75-93	41-90

leucocyte esterase				
Microscopy				
WWC>8 x 10^6	91	50	67	83
Culture				
>10^5 bacteria/L	95	85	88	94
>10^8 bacteria/L	51	59	98	94

-Sensitivity Proportions of positive cases are correctly identified as positive.
-Specificity Proportions of negative cases that are correctly identified as negative.
- +ve predictor % of cases with +ve results that are diseased.
- -ve predictor % of cases with negative test results that are non-diseased.

❖ **Leukocyte esterase (White Blood Cells Count)**

Granulocytic leukocytes contain esterase that catalyze the hydrolysis of the derivatized pyrrole amino acid ester to liberate 3-hydrozy-5-phenyl pyrrole. This pyrrole then reacts with a diazonium salt to produce a purple product.
An increase in leukocytes is an indication of pyuria found in nearly all diseases/infections of the kidney and urinary tract.

- Leukocyte esterase activity is detected by determining the presence of whole or lysed white cells in the urine (pyuria).
- A positive leukocyte esterase test correlates well with pyuria. However, the diagnosis may be missed in up to 20% of cases if negative urinalysis dipstick is used to exclude UTI.

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- **False positive:** Contaminated specimen, trichomonas vaginalis, drugs or foods that color the urine red.
- **False negative:** Intercurrent or recent antibiotic therapy (especially gentamicin, tetracycline and cephalosporins), glycosuria, proteinuria, high specific gravity. Low bacteria count UTI (especially in women).

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