

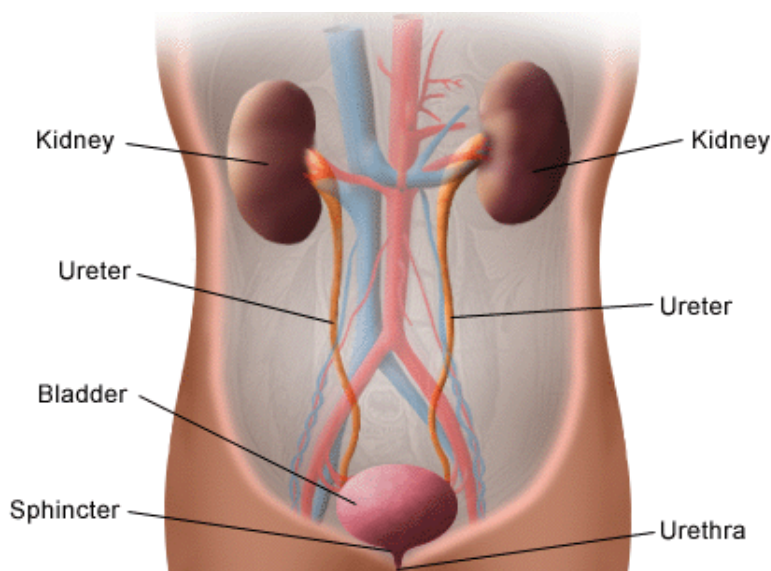
BCM 101

BIOCHEMISTRY

Week 9 Practical

“Urine analysis”

“**Urine**” is a liquid produced by animals and humans through the kidneys, and is collected in the bladder and excreted through the urethra. Kidneys make urine by filtering out unwanted water, waste products, chemicals, sodium and potassium ions from the blood. Through a complex process, kidneys return an exact amount of sodium and potassium ions and some water to the blood stream so as to maintain a constant water and salt balance in the body.



Changes in the composition of urine occur very early in many “**diseases**”, often before the patient is aware of any symptoms. For this reason and because urine can be obtained relatively easily, “**urine analysis**” was one of the first laboratory tests performed and related to diseases.

The **aim** of this practical session is to:

1. Obtain a simplified knowledge about routine urine analysis.
2. Perform simple chemical analyses on the provided urine sample.
3. Interpret results and comment on the case.

Collection of urine samples

The best sample for a routine urine analysis is a “**clean-catch**” or “**midstream**” sample collected after the external genitalia have been cleansed with an antiseptic solution. In this technique, the first portion of voided urine is discarded and the next portion is collected in a “**clean**” container (N.B. a sterile container is necessary if bacterial culture is requested).



The container should be “**clearly labeled**” and urine must be analyzed soon after collection because most urine elements deteriorate at room temperature within one hour. A refrigerated specimen will retain its integrity only up to four hours.

Routine urine analysis

Urine analysis is a group of “**qualitative**” and “**semiquantitative**” analyses performed on urine samples. A routine urine analysis usually includes the following:

- I. **Physical examination**
- II. **Chemical analysis**
- III. **Microscopic examination**

I. Physical examination:

Physical examination of urine includes observation of the **color**, **odor**, **turbidity**, and determination of **pH** and **specific gravity**.

Color

The normal colors of urine range from straw yellow to amber and are the result of normal metabolic end products such as **urochrome** and **urobilin**. Abnormal colors include red (due to presence of red blood cells or red pigments of beets), yellow-brown (due to presence of bilirubin), and orange (due to presence of some drugs); vitamin B complex can turn urine bright yellow.

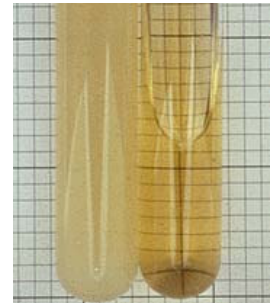


Odor

The odor of urine is described as “**urinoid**”. Diabetic ketoacidosis (in uncontrolled diabetes mellitus), starvation or a very low carbohydrate diet may cause the urine to have a sweet “**fruity**” odor due to the presence of acetone. Patients with urinary tract infections often have urine with a “**pungent**” odor.

Turbidity

Normal urine is clear. Turbidity can be caused by “**crystals**” or “**cells**” which separate after centrifugation of the urine sample. Crystals include amorphous urate, triple phosphate or calcium oxalate, and cells include pus cells or epithelial cells. Crystals and cells are detected by microscopic examination of the sediment.



pH

Normally, freshly voided urine is acidic. Some foods (e.g. citrus fruits) and drugs (e.g. antacids) can affect urine pH. Alkaline pH may be noted in urinary tract infection due to alkaline fermentation by bacteria. In some cases, urinary pH is made acidic or alkaline by certain treatments to prevent the formation of certain types of kidney stones.

Specific Gravity:

Specific gravity measures urine density and is directly proportional to the amount of solutes present in urine. The normal urinary specific gravity ranges from 1.002 to 1.030. Decreased specific gravity occurs in case of diabetes insipidus and excessive fluid intake while increased specific gravity occurs in diabetes mellitus, severe vomiting or diarrhea. The specific gravity of urine is measured by a device called “**refractometer**” in which the extent of refraction of a beam of light depends on the concentration of solutes present in urine.



Refractometer

II. Chemical analysis:

Different chemical tests are performed to detect **glucose, ketone bodies, protein, bilirubin** and **nitrite**.

Glucose: Glucose is normally absent or present in undetectable amounts in urine. When glucose level in blood exceeds the renal sugar threshold (160-180 mg/dl) glucose starts to appear in urine. Presence of glucose in urine (glucosuria) is usually an indication of diabetes mellitus.

Ketone bodies: When there is no adequate amount of carbohydrates in the diet, the body begins to utilize fatty acids to produce energy. When this increased metabolic pathway reaches a certain point, fatty acid utilization becomes incomplete and intermediary products occur in blood and urine. These products are the 3 ketone bodies: acetone, acetoacetate, and beta-hydroxybutyrate. Presence of ketone bodies in urine (ketonuria) is usually an indication of uncontrolled diabetes mellitus, starvation or a very low carbohydrate diet.

Proteins: Proteins are normally undetectable in urine. Presence of proteins in urine usually indicates kidney damage or glomerulonephritis.

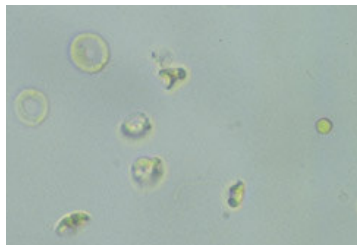
Bilirubin: Bilirubin is a yellow-brown breakdown product found in the bile. It results from normal heme catabolism. It is normally absent or present in undetectable amounts in urine. Most of the bilirubin produced is conjugated in the liver and excreted in the bile but a very small amount is reabsorbed from the intestine to the blood and is then excreted in urine. Elevated bilirubin in blood (and subsequent appearance in urine) indicates excessive bilirubin production due to increased destruction of RBCs (as in case of hemolytic anemia) or inability of the liver to adequately remove bilirubin due to obstruction of bile ducts or a liver disease (e.g. liver cirrhosis or acute hepatitis).

Nitrite: In urinary tract infection (UTI), bacteria produce an enzyme that converts urinary nitrates to nitrites. Thus, the presence of nitrites in urine indicates UTI.

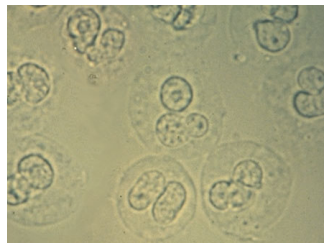
III. Microscopic examination:

Microscopic examination of urinary sediment reveals very few or no red or white blood cells or casts. No bacteria or parasites are present and few crystals are usually normal.

“**Red blood cells**” in urine may be caused by kidney stones or bladder tumor. “**White blood cells**” (pus) indicate a urinary tract infection. “**Schistosoma eggs**” can indicate Bilharziasis. Excessive amounts of “**crystals**” or the presence of certain types of crystals can indicate kidney stones. Depending on the type, “**casts**” can indicate inflammation in the kidneys.



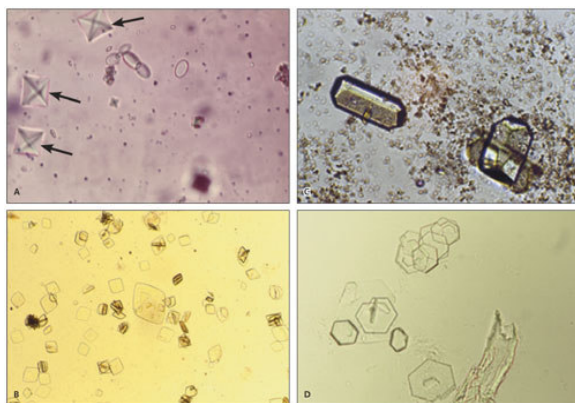
Red blood cells



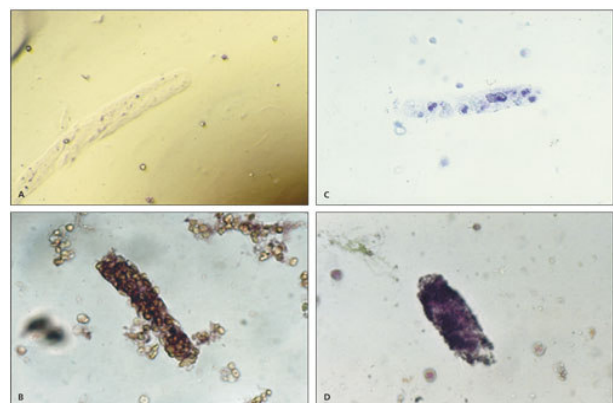
White blood cells



Schistosoma hematobium egg



Urine crystals



Urine casts

Dipstick analysis

Dipstick analysis is an easy and convenient method for the detection of leukocytes, nitrite, protein, blood, ketone bodies, glucose and bilirubin in urine and for the determination of urine pH and specific gravity.

A “**dipstick**” is a paper strip with patches impregnated with chemicals that undergo a color change when certain constituents of urine are present in a certain concentration. The strip is dipped into the urine sample, and after the appropriate number of seconds, the color change is compared to a standard chart to determine urine constituents.



Leukocyte esterase	120		Tr	+	++	+++		
Nitrite	60		Pos	Pos				
pH	60	5.0	5.5	6.0	6.5	7.0	7.5	8.0
Protein	60		Tr	+	++	+++	++	
Blood	60		Tr	+	Tr	+	++	+++
Specific gravity	45	1.005	1.010	1.015	1.020	1.025	1.030	
Ketones	40		Tr	+	++	+++	++	
Glucose	30		Tr	+	++	+++	++	
Bilirubin	30		+	++	+++			

Practical

Using the provided urine sample, perform the following tests to detect **glucose, proteins, ketone bodies** and **bilirubin**:

Tests for glucose:

1. **Fehling's test:** in a test tube, add 1 ml Fehling A solution and 1 ml Fehling B solution to 2 ml urine sample. Mix well and put in a boiling water bath. A change in color indicates the presence of glucose.
2. **Benedict's test:** in a test tube, add 3 ml Benedict's reagent to 1 ml urine sample. Mix well and put in a boiling water bath. A change in color indicates the presence of glucose.

Test for ketone bodies:

Rothera's test: in a test tube, add 0.5 ml of saturated ammonium sulfate to 3 ml urine sample. Add 2-3 drops of ammonia solution and 2-3 drops 5% sodium nitroprusside and shake well. A permanganate-like color indicates the presence of ketone bodies.

Tests for proteins:

1. **Heat coagulation test:** in a test tube, put 2 ml urine sample. Incline the test tube and heat the surface. The formation of a coagulum indicates the presence of albumin.
2. **Heller's test:** in a test tube, add 2 ml of urine slowly to 2 ml of conc. HNO_3 . A white coagulum is formed at the junction between the 2 layers indicating the presence of proteins.

Test for bilirubin:

Hay's test: The test depends on the surface activity of bilirubin as it lowers the surface tension of urine. Sprinkle a little of precipitated sulfur powder on the surface of 2 ml urine. If bilirubin is present, sulfur powder will sink to the bottom of urine. If bile is absent, sulfur will remain on the surface of urine.

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Student Name: **Student number:**

Laboratory exercise:

Using the provided urine sample N° (.....), perform the tests in the table below illustrating your observations and results.

	Test	Observation	Result
Glucose	Fehling’s test		
	Benedict’s test		
Ketone bodies	Rothera’s test		
Proteins	Heat coagulation test		
	Heller’s test		
Bilirubin	Hay’s test		

Comment on the case:

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