

**DEPARTMENT OF CHEMICAL PATHOLOGY
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN**

Undergraduate Practical Classes (Urinalysis)

For use during Block I and Block II Postings

Introduction: Examination of the urine specimen may be considered from two general standpoints. First, for the diagnosis and management of renal and/or urinary tract diseases and secondly, for the detection of metabolic or systemic diseases that may not be directly related and/or referable to the kidneys.

In Chemical Pathology, we use two groups of methods for urine examination viz:

- the physico-chemical methods
- the chemical methods.

We use the physico-chemical methods to assist us in detecting the cause or causes of abnormal colour, abnormal odour, abnormal composition, abnormal concentration and abnormal reaction of urine.

On the other hand, we use the chemical methods to assist us in detecting the absence, or presence in normal quantities or presence in abnormal quantities of certain substances in the urine.

Physico-Chemical Methods

(a) Colour:

Normal urine colour is pale to dark yellow.

If colour of urine deviates from this range the individual must be thoroughly investigated to find the cause or causes.

In clinical medicine, four ranges of abnormal colour of urine have been described.

These are:

- yellow-brown to deep green
- red
- orange red to orange brown
- dark-brown to black.

Inspect for these and report your findings. Suggest pathways whereby the colour that you report may arise.

Note: For this procedure and the subsequent ones, clear and fresh urine should be used. Avoid cloudy urine by filtration, centrifugation or by allowing it to

stand for some minutes and then decant the supernatant liquid. It is essential to remember that there is a difference between a cloudy urine and a turbid one.

Continue the physical examination by inspecting for particles, precipitates and turbidity and report your findings.

(b) **Odour of Urine**

Perceive the odour of the urine and record any characteristic odour. Amongst the characteristic odour of urine are:

- sharp, sweet smell
- obnoxious smell
- maple syrup smell
- mousy smell

Report your findings and suggest probable cause or causes for the perceived odour.

(c) **Reaction or Urine (pH)**

The pH of urine is a reflection of the ability of the normal kidney to maintain normal H⁺ in plasma and other extra-cellular fluids.

The average adult on a normal diet and normal fluid intake excretes urine of about pH 6.0. However, in health, urine pH varies from 4.6 – 8.0 depending on the type of diet.

For this exercise, you are provided with pH Test paper. Dip one end of a piece of the wide range pH indicator strip into the urine and shake to remove any excess urine on the paper. Compare, within the time limit stipulated, with the standard colours shown on the cover of the test paper booklet.

- Report your finding and comment on the result.

(d) **Concentration of Urine (S.G.)**

Normal adults, with normal diets and normal fluid intake will produce urine of specific gravity SG 1.010 – 1.025 during a 24 hr period.

Procedure:

Fill the graduated glass cylinder with distilled water to the 50 ml mark. Place the urinometer in the cylinder and allow it to float freely. Record the level of floatation. Decant the distilled water and repeat the same procedure using unfiltered urine instead of the distilled water.

If the specific gravity of the distilled water is not exactly 1.000 (which should normally be the situation) you will now go ahead to make necessary adjustments and corrections.

This is accomplished in the following manner:

Observed S.G. of distilled water	=	0.995
Observed S.G. of urine	=	1.016
Expected S.G. of distilled water	=	1.000
Correction factor: 1.000 - 0.995	=	0.005
∴ Corrected SG of urine: 1.016 + 0.005 =		1.021

Chemical Methods

- (a) Proteins: Normally, there is a scant amount of protein in urine up to 150mg/24 hr or 20mg/dl. However, this cannot be detected by proprietary reagents you will use in these exercises.
- In clinical medicine, proteinuria is viewed under three categories. viz: minimal, moderate and heavy.

(i) **Albustix test strip:**

Dip the test end of the strip in urine and remove immediately. Compare the colour of the dipped end with the colour scale provided. This varies from yellow through green to blue depending on the amount of protein present.

Note: High alkaline urine may give a false positive result.

(ii) **Sulphosalicylic Acid Test:** *

To about 5ml. urine in a test tube add 20% sulphosalicylic acid solution drop-wise. Watch each drop of the acid as it travels through the urine to the bottom of the tube. Cloudiness indicates the presence of protein. The degree of cloudiness is proportional to the amount of protein present. Report your findings.

(b) **Reducing Substances**

(i) **Benedict's Test:**

To 5ml of Benedict's reagent add eight drops (0.5ml) of urine. Shake and place in boiling water for precisely 5 min. Remove and cool. If a reducing sugar is present, a precipitate will form varying in colour from green to yellowish-brown to reddish-brown to brick-red depending on the amount of sugar present.

(ii) **Clinitest Tablet Test:**

Place 5 drops of urine and 10 drops of water in a test tube. Drop into the mixture, one tablet of clinitest. Wait for the reaction to stop and 15 seconds after compare the resulting colour with the colours reflected on the manufacturer's scale.

louh
benet
Droste
green
brown

- (iii) **Clinist Test Strip:**
Dip the end of strip in urine and remove immediately. Compare resulting colour with the colour chart provided
Report your findings in the three tests and comment on them.

- (c) **Ketone Bodies:**
Ketone bodies are the products of incomplete fat metabolism. Their presence in urine is indicative of acidosis. Ketonuria is commonly found in uncontrolled Diabetes Mellitus.
In Ketonuria the three ketone bodies present are acetoacetic acid, (beta)-hydroxybutyric acid, and acetone

- (i) **Rothera's test:**
Saturate 5 ml of urine with Rothera's reagent and add 1 ml of concentrated ammonia solution. Shake well and allow to stand for a few minutes. A characteristic permanganate colour indicates the presence of acetone or acetoacetic acid or both. Report your findings.

- (ii) **Acetone tablet test:**
Place an acetone tablet on the clean mat provided or on a filter paper. Put a drop of urine on the tablet. After 30 seconds, compare the resulting colour of the tablet with the colour scale provided.

Report your findings.
Comment on the results obtained for the two tests.

- (d) **Bilirubin:**
- (i) **Foam Test:** Shake vigorously about 10ml of urine. A yellow froth indicates the presence of bile pigments in the urine.
- (ii) **Ictotest Tablet Test:**
Place two drops of urine on the centre of the special "Ictotest mat" provided. Put an Ictotest tablet on the urine. Place two drops of water around the tablet. A bluish-purple colour on the mat around the tablet indicates the presence of bilirubin. The time frame for the observation is about thirty seconds.
- (iii) **Fouchet's Test:**

(iii) **Fouchet's Test:**

Acidify about 10ml of urine with the dilute sulphuric acid provided and add 5ml. of 10% Barium chloride solution, mix well, then filter to remove the precipitate. Unfold the filter paper and add a drop of Fouchet's reagent on the precipitate. A green to blue colour indicates the presence of bilirubin.

Report your findings on the three tests and comment on the results.

(e)

Bile Salts:

Gently sprinkle fine flowers of sulphur on the surface of the urine in a test tube. Allow to stand undisturbed for two minutes. Presence of a great excess of bile salts is indicated by the sulphur particles sinking to the bottom of the tube. Report your findings and comment on the result.

(f)

Urobilinogen:

To 1ml of fresh urine add 1ml of Ehrlich's aldehyde reagent, mix, and then add 4ml. of supersaturated aqueous solution of sodium acetate. Add 5ml of chloroform and shake vigorously. Urobilinogen forms a purple red aldehyde soluble in the chloroform layer. The intensity of the colour is directly related to the amount of urobilinogen present in the urine.

Occultest Tablet Test:

Place one drop of mixed uncentrifuged urine on a special test paper which is provided. Place an occultest tablet in centre of the moist area of the paper. Allow one drop of water to flow on the tablet, wait for 5-10 seconds, and flow a second drop of water on the tablet so that it runs down the sides on to the test paper. A positive result of presence of blood in the urine is shown by the reaction site turning blue to bluish-green. The time frame for the observation is two minutes.

Report your findings and comment on the results.

Conclusion: Summarise all your findings and comments on all results obtained during the course of the various investigations.

Provide a provisional diagnosis and suggest further tests that you can carry out to establish a definite diagnosis.

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