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# Introduction

Urine is a unique body fluid formed by a process of glomerular filtration and selective tubular secretion and reabsorption of constituents by the kidneys. It contains all the constituents found in the blood, but at different concentrations (Table 1). Urine is continuously formed and secreted from the body. It is the main route of water excretion and removal of waste products of metabolism from the body. Indeed, urine is responsible for the excretion of more solids than any other routes in the body.

The study of physical and biochemical properties (urinalysis) which has a long historical heritage is now a major component of laboratory medicine in almost all parts of the world.

Urine as a great source of immense clinical data provides important information on the metabolic state of the body. Valuable and relevant information obtainable from urinalysis relates to specific organs, tissues, physiological and metabolic processes in the body (Table 2).

## **ADVANTAGES OF URINE TESTING (URINALYSIS)**

- (i) Urine specimen is readily available.
- (ii) Convenience quick tests (dip-sticks or tablets) are now commercially available.
- (iii) Tests are highly reliable as sensitive indicators of disease or normal health.

- (iv) Urine can be tested anywhere (e.g. laboratory, ward, office, house etc).
- (v) May not require the expertise of a trained staff all the time.
- (vi) Relatively inexpensive tests are now available.

**Table 1****Comparison of Typical Serum and Urine Normal Constituent Concentrations**

	Serum or Plasma (normal fasting levels)	Urine (24 hour)
Sodium	140 mmol/l	70mmol
Chloride	1000 mmol/l	200 mmol
Creatinine	10 mg/l	150 mg
Glucose	900 mg/l	10 mg
Lead	150 mg/l	100 ug
Phosphorus	40 mmg/l	100 mg
Potassium	4 mmol/l	80 mmol
Protein	70 g/l	<150 mg
Urea	4 mmol/l	415 mmol
Uric acid	50 mg/l	400 mg

Levels are essentially mean concentrations.

The study of urine has a long historical heritage and is a major component of clinical laboratory medicine in almost all parts of the world. The ready availability of urine, availability of convenience quick tests, (dip-stick or test tablets) relative

**Table 2**  
**Information obtainable from Urine Study**

Important and very relevant information obtainable from urinalysis relates to specific organs, tissues, physiological and metabolic processes (as in A-D).

A.	Kidneys Liver Pancreas Blood Bone Muscle Endocrine Nutrition
B.	Acid Base equilibrium Water balance Carbohydrate, and protein metabolism Pregnancy
C	Inborn errors of metabolism Drug abuse Intoxications or Poisoning Urinary tract Gastrointestinal tract Hormones
D.	Cardiovascular System Infection Respiratory System Central Nervous System

inexpensiveness and high reliability of tests as sensitive indicators of the presence of disease or normal health are great advantages in almost all situations in medical diagnosis. Also urine can be tested anywhere, laboratory, ward, office, house etc.

### **CLINICAL APPLICATION OF URINALYSIS**

Urinalysis is frequently carried out at the time of the patient's hospital admission, as a follow up of treatment while the patient is hospitalised and medical screening test in apparently normal subjects. There are two major purposes:

- (a) to detect intrinsic conditions that may adversely affect the kidneys or urinary tract. The kidneys function normally in regulating the volume and composition of body fluids and in maintaining body homeostasis. In conditions where the kidneys are diseased, the normal functions are affected and thus substances normally retained by the kidney or excreted in small amounts may appear in the urine in large quantities, while substances normally excreted may be retained. Also structural elements, such as red blood cells, leukocytes, cells from the urinary tract and casts from the diseased kidneys may appear in the urine in such situations.
- (b) In conditions where kidney functions remain normal, simple urinalysis can serve as a useful aid in detecting or assessing metabolic abnormalities or breakdown of normal body homeostasis where the kidneys excrete abnormal amounts of metabolic end-products produced by the particular disease (e.g. ketone bodies in ketoacidosis).

**Table 3**  
**A typical normal profile of urine constituents in a random urine specimen in humans**

Volumes: 1000-1.500ml/24hrs, 50-500ml/Spot specimen

Appearance: Sparkling clear yellow/amber colour

Odour: Aromatic (fresh) – ammoniacal (stale)

A.	Specific gravity:	1.002 – 1.030
	Total solids	50 – 70g/24hr
	PH:	5–9
<b>B</b>		
Qualitative test results:		
Glucose – negative		
Reducing substances – Negative to Trace		
Protein – negative to trace		
Ketones – negative		
Urobilinogen – negative		
Nitrate (indicator of bacteriuria) – negative		
<b>C</b>		
Sediments		
Epithelial cells, crystals and casts-occasional/negative		
Leukocyte esterase – negative		

### **COMMON PROCEDURES**

The major procedures in routine urinalysis can be divided into three subheadings.

- (a) Urine sample collection and preservation
- (b) Assessment of physical characteristics (Appearance, Odour, pH, specific gravity and volume)

- (c) Measurement of chemical characteristics (presence or absence of protein, reducing substances, particularly sugars, bile pigments, ketones, blood and urobilinogen, etc).

However, other specialised procedures (both qualitative and quantitative) may be carried out on request for further management of patients (e.g. Pregnancy tests, hormones, electrolytes, drugs, phenylketonuria, enzymes, trace elements, catecholamines, amino acids, etc.).

#### COLLECTION OF URINE SPECIMENS

The most general factors which provide for obtaining good results are related to proper collection and care of the urine specimen. Therefore, for accurate results of urinalysis it is important that a properly collected urine specimen must be used for the tests. The different types of urine specimens commonly used for routine urinalysis include:

- (A) **Freshly voided early morning or random urine:** This involves a single voided specimen without regard to the time of or the interval of time over which the urine is secreted by the ureters into the bladder. A freshly voided urine specimen is adequate for most urinalysis in a Clinical Chemistry laboratory. The patient should be instructed to void directly into a clean dry container or into a clean dry bedpan from where the specimen can be transferred directly into an appropriate container. Specimens from infants and young children can be collected in a disposable collection apparatus, consisting of a plastic bag with an adhesive backing around the opening to fasten it to the child so that he voids directly into the bag. If a urine specimen is likely to be contaminated with

vaginal discharge or menstrual blood (in the case of females) then a clean-voided specimen must be obtained. The procedure most commonly used for obtaining a suitable sample in such conditions is the collection of a clean-voided midstream specimen. Bladder catheterisation and percutaneous suprapubic aspiration of the bladder may be used, but only in very rare and unusual circumstances. All urine specimens should be examined within one hour of collection to avoid changes in physical and/or chemical properties. However, urine samples not in use should be preserved and stored refrigerated or frozen until analysed.

#### Advantages of Random Urine

- (i) Easy to collect
- (ii) Provides a great amount of clinical information

#### Limitations

The sample is subject to considerable variations in the concentrations of constituents.

- (B) **Timed Urine collections:** This involves collection of the total volume of urine excreted over a defined period of time (e.g. 2 hours, 12 hours, or 24 hours). The concentration of urine varies throughout a 24-hour period depending on the patient's fluid intake and physical activities. Diurnal variations in concentrations of chemical components of urine may occur in health and in disease, but in general, a more concentrated urine is preferred for urine examination than a dilute specimen. Consequently, the first routine analysis must be carried out on early morning urine

specimen. In cases where the first morning specimen cannot be collected, a randomly voided urine specimen can be used. Thus, the effect of the concentration of a sample as measured by the specific gravity should be considered in the interpretation of the results.

Random urine samples can be used for qualitative tests, but only urine collected over a precisely measured period of time should be used for quantitative tests. For example a 24-hour urine specimen may provide a more representative quantity of a solute excreted in urine. This is the preferred procedure in clearance tests.

#### **Advantages of Timed Urine Collection**

- (i) Useful in certain metabolic studies
- (ii) More concentrated than random urine specimen.

#### **Limitation**

- (i) Quite cumbersome to collect and may lead to inaccuracies in the collection of sample.
- (ii) Errors may occur in the interpretation of results.
- (iii) Deterioration of certain constituents commonly occurs during long period of urine collection.

(C) **Post-prandial urine:** This involves collection of urine sample after taking a meal and this is very useful in screening for hyperglycaemia.

#### **Advantages**

- (i) Useful in detection of early diabetes mellitus.
- (ii) More sensitive than random sample in assessing moderate disturbances in carbohydrate metabolism.

#### **Limitations**

- (i) Errors may occur in the interpretation of results.
- (ii) Less concentrated than early morning urine specimen.

Tables 4 and 5 summarise some of the characteristics of the various types of urine samples:

**Table 4**  
**Types of Urine Specimen (SPOT)**

#### **FIRST MORNING SPECIMEN**

- Most concentrated
- Bladder incubated
- Best for analysis of Nitrate, Protein, and Sediments

#### **RANDOM SPECIMEN**

- Most convenient for patient and analyst, especially for chemical qualitative screening tests

#### **SECOND VOIDED SPECIMEN**

To obtain the second voided specimen, the first morning specimen is discarded and the second specimen collected and tested for components of interest. The specimen offers a good reflection of:

- blood glucose level
- formed elements in urine (which are kept intact).

**Table 5****Types of Timed-Urine Specimens****(a) POST PRANDIAL**

Urine specimen collected after a meal, and this is adequate in tests designed to assess moderate disturbances in carbohydrate metabolism.

**(b) 2-HOUR VOLUME**

The urine specimen is good for urobilinogen and glucose tests.

**(c) 24-HOUR VOLUME**

- This is necessary for a true quantitative estimation of urinary components to overcome any diurnal variations in constituent concentrations. This is the urine sample of choice in clearance and quantitative tests.

**SUITABLE CONTAINERS**

A urine specimen must be collected in a clean dry container (plastic or glass), which may vary in size depending on the volume of urine required for specific tests to be performed on the sample. Commercial disposable specimen containers are now available. Also special pliable polyethylene bags are available for collection of urine from infants and children who are not toilet-trained.

**PRESERVATION and STORAGE of URINE**

When analysis is delayed for periods over one hour, special precautions are necessary to prevent deterioration of chemical and cellular elements of the urine specimen. Bacterial

multiplication regularly occurs in urine that are left at room temperature for several hours. Bacteria may utilise glucose in urine and urea - splitting organisms may convert urea to ammonia, and thus producing an alkaline urine. Alkaline urine may give false positive or false negative results for certain components of the sample. In addition, casts decompose in alkaline urine and blood cells may be haemolysed. Routinely, refrigeration at 5°C is often the only precaution needed to preserve the urine for routine analysis. Chemical preservation may however be required when a specimen cannot be refrigerated - for example, during transportation from home to office or laboratory; when the specimen is sent by postal system or during long storage. The common preservatives include formaldehyde producing tablets (e.g. Urokeep Cargille tablets, King Bury-Clank tablets); toluene, benzoic acid, thymol crystals and 0.8% boric acid.

The choice of a specific preservative must be determined by the procedures/tests to be performed since some preservatives may interfere with certain tests. For the different preservatives, excess amount may interfere with common routine tests in urinalysis. For example, thymol at concentrations of 0.1 gm/dl or more may give false positive reactions for albumin in some test procedures. A urine sample for glucose determination is best preserved with benzoic acid. For determination of urine pH, any preservative that affects acidity should be avoided. Storage at very low temperatures (in the freezer) is encouraged for quantitative tests (e.g. urinary electrolytes, etc.)

**MAJOR ERRORS IN URINE TESTING**

- Failure to test fresh urine or adequately preserved and stored urine specimen.
- Failure to mix urine (especially stored) sample well before tests are carried out (blood, casts, etc).

- (3) Inappropriate interpretation or inability to understand implications of a result.
- (4) Improper recording of results.
- (5) Poor methodology.
- (6) Disregard for a result, believing some other answer is more important.
- (7) Failure to recognise that any result is only part of the picture of the biochemical profile of a subject.
- (8) Inadequate care of reagents.
- (9) Use of unclean collection containers.
- (10) Inadequate understanding or recognition of interfering substances.

#### **What to Do to Ensure Good Result**

- (1) Collect appropriate urine specimen in a properly labelled clean container.
- (2) Select good methods using properly stored and high quality reagents.
- (3) Test well mixed fresh or adequately preserved urine specimen containing no interferences, following carefully the directions for the analytical procedures in a clean and well-lit area in the laboratory.
- (4) Record results carefully and dispatch results to the wards/ clinic promptly.
- (5) Use urine controls to ensure reliability of results (Quality Control).
- (6) Understand the interpretation of results, paying attention to details of clinical history and presentation of the subject.
- (7) Consult the laboratory whenever in doubt.

## **2 | Physical Properties of Urine**

### **APPEARANCE OF URINE**

This is the first observation commonly made on urine. Usually the urine is a sparkling clear fluid which is yellow or amber in colour. The yellow colour is due to the pigments urochrome, urobilin and porphyrin present in urine. Attention to details of clinical history and presentation of the subject can provide useful clues to the presence of many substances in the urine. For example, an intense yellow coloured urine may indicate a concentrated urine, while a pale colour may indicate a dilute urine. On the other hand, a reddish-brown colour is indicative of the presence of blood or food pigments in the urine. Turbidity of urine specimens in healthy persons is due to precipitation of phosphate salts or uric acid in the bladder. Such precipitation may occur due to changes in the acidity or alkalinity of the urine in the bladder. A trained observer is therefore able to gain some important clues about the urine by simple visual inspection of the urine specimen.

### **COLOUR**

This is affected by many components, e.g. concentration of food pigments, dyes, blood, metabolites of in-born errors of metabolism, etc. when they are present in significant amounts in the urine sample (Table 6).

**Table 6**  
**Common Colours that can be Exhibited by Urine**

Colour of Specimen:	Possible basis for Observed Colour
Colourless	Highly dilute
Pale yellow	Normal
Yellow	Normal
Amber	Normal
Deep Yellow	Normal
Orange	Concentrated, Riboflavin
Pink	Pyridium, Santonin, Chrysophanic acid Porphyrin, myoglobin, haemoglobin, beet root pigment
Red	Porphyrin, myoglobin, haemoglobin, beet root pigment, uroerythrin
Green	Oxidized bilirubin (biliverdin)
Blue	Dianox, methylene blue, indican
Brown	Bilirubin, haematin, methemoglobin
Gray	Drugs, e.g. Furazolidone, nitrofurantoin, nitrofurazone
Black	Melanin, homogenetic acid
Turbidity or Milkiness	Precipitated urates or phosphates, bacteria, fat globules, pus
Smoky	Red blood cells.

The yellow or amber colour of a normal urine is due to the presence of a yellow pigment called **urochrome** which is a degradation product of heme. The colour of urine changes in many disease states because of the presence of pigments that do not normally appear in urine from healthy individuals. The urine may assume many different colours in different conditions and following the ingestion of various dyes, foods,

and drugs. Substances taken into the body as foods or drugs which have unique colours may be excreted by the kidney and this gives rise to recognisable changes in the appearance or odour of the urine. However, the great majority of foods and drugs ingested by the body lose their colours or odours through either the process of digestion or metabolism, and thus do not have any recognisable effect on the urine. Common exceptions are beets, asparagus, pyridium, methylene blue, among others. In some cases, the perceived colour may manifest instantly or when the urine is left standing at room temperature. These examples clearly illustrate such situations which may be important in the overall clinical diagnosis:

- (a) Bile pigments – Yellow-Yellowish – Green-Green.
- (b) Porphyrins – Dark brown or greenish colour which turns to red colour on standing at room temperature.
- (c) Haemoglobin – Reddish brown colour.
- (d) Melanins – urine turns to a brownish – black colour on standing.
- (e) Alkaptonuria – urine turns dark brown or black colour on standing.
- (f) Ingestion of dyes – different colours depending on substances and drugs ingested.
- (g) Dietary pigments: Numerous dietary pigments may be directly excreted or may be altered to give products which contribute colour to the urine (e.g. riboflavin, carotene, the pigments of beets, etc.).

#### ODOUR

Normal freshly voided urine has a characteristic aromatic odour which is believed to be due to the presence of volatile acids. Urine that has stood on the bench for sometime develops ammoniacal odour arising from the decomposition of urea in

the specimen. Different odours may be perceived in different conditions either due to putrefaction or the presence of definite chemical substances in urine. For example, the urine of patients with diabetes mellitus may have a fruity odour owing to the presence of acetone. The urine of patients with urinary tract infections may be foul-smelling especially when the infecting organism is a coliform bacillus. Certain foods such as asparagus may impart a characteristic odour. The urine may have several characteristic odours, as a rule, the odour of the urine is not considered to be of diagnostic significance except in some rare cases of inherited metabolic diseases like PKU, which gives a mousy smell; maple syrup urine disease (MSUD); the smell of maple syrup and trimethylaminuria, the smell of rotten fish or in cases where it may help to recognise urine samples that have been left standing for a long time and therefore unsuitable for urinalysis, due to bacteria contamination.

#### TURBIDITY

Normal freshly voided urine is usually clear or transparent, but an alkaline urine may be cloudy or turbid due to the presence of phosphates and carbonates or during urinary tract infection. This turbidity usually disappears on acidification. A pinkish turbid urine may indicate the presence of urates. Urine may be milky or turbid due to the presence of fistula from a large lymph duct within the bladder or other portions of the urinary tract. In such situations fat or cholesterol may appear in urine. Pathologic states may include excess dietary intake of phosphates and hyper-parathyroidism.

#### URINE VOLUME, SPECIFIC GRAVITY (SG) AND OSMOLALITY

The specific gravity (SG) of urine indicates the relative proportions of dissolved solid components to the total volume

of the urine specimen. It reflects the relative degree of concentration or dilution of the urine specimen. Accurate knowledge of the SG may be required in interpreting results of urinalysis.

Under appropriate and standardised conditions of fluid restriction or increased intake, the specific gravity of urine is a measure of the concentrating or diluting ability of the kidney respectively.

The specific gravity of urine may vary from 1.002 to 1.030, but usually remains between 1.010 and 1.025. The SG depends on the state of hydration of the person and the time of the day. It is highest at the beginning of the day. For the first morning (early morning) urine specimen an SG between 1.025-1.030 indicates a normal concentrating ability of the kidneys.

#### CLINICAL SIGNIFICANCE

The specific gravity of urine may be low, high or fixed.

##### (a) Low Specific Gravity

Diabetes insipidus, a disease caused by the absence of, or impairment of the normal functioning of the antidiuretic hormone (ADH), is the most outstanding and severe example of loss of effective concentrating ability of the kidney. This disorder is characterised by excretion of large urine volume of low specific gravity. Specific gravity in such cases usually ranges between 1.001 and 1.003.

Low specific gravity may also occur in patients with glomerulonephritis, pyelonephritis, and various renal disorders, where the concentrating ability of the kidney has been affected. In such cases, the kidney has lost its ability to concentrate the urine because of tubular damage.

##### (b) High Specific Gravity

This occurs in patients with

- (i) diabetes mellitus,
- (ii) adrenal insufficiency,
- (iii) hepatic disease and
- (iv) congestive cardiac failure.

The specific gravity of urine is also elevated in conditions where there has been

- (a) excessive loss of water (dehydration) as in excessive sweating, high fevers, vomiting and diarrhoea,
- (b) in conditions where abnormally high amounts of some urinary constituents in particular glucose and protein are encountered. For example an increase in the SG leading to measurement up to 1.050 or more in the urine may occur in some patients with diabetes mellitus or nephrosis. The SG increases by 0.004 for every 1% increase in glucose and 0.003 for every 1% increase in protein in the urine specimen.

#### **(c) Fixed Specific Gravity**

Urine with a fixed low specific gravity (approximately 1.010) which varies little from specimen to specimen is known as isothenuric. This condition is indicative of severe renal damage with disturbances in both the concentrating and diluting abilities of the kidneys.

#### **Measurement of Specific Gravity:**

The specific gravity is a measure of the density of urine, derived from the ratio of the weight of a given volume of urine to that of the same volume of water, under standardised conditions.

i.e.,

$$\text{S.G.} = \frac{\text{Weight of Urine}}{\text{Weight of equal vol. of Water}}$$

This can be referred to as the relative mass density of urine. Water has a specific gravity of 1.000 and since urine is a solution of minerals, salts and organic compounds in water, the SG is greater than 1.000. The relative difference reflects the degree of concentration of the urine specimen. In practice, the S.G. of urine can be measured using different methods which include:

#### **(A) MEASUREMENT USING A URINOMETER (HYDROMETER)**

##### **Principle**

The urinometer (hydrometer) is a weighted bulb shaped instrument that has a cylindrical stem which contains a scale calibrated in specific gravity readings. This instrument is floated in a cylinder containing urine and the depth to which it sinks in the urine indicates the specific gravity of the urine. This is read on the urinometer scale at the junction of the urine with the air.

##### **Notes**

The urinometer is calibrated to read 1.000 in distilled water at a specific temperature, indicated on each instrument. There is a change in the SG of 0.001 for a change of 1°C in temperature. Determination of SG of water under laboratory conditions may not always give the expected result of 1.000 as the observed measured value is greatly influenced by temperature, pressure and overall purity of water. If the urinometer does not read 1.000, appropriate corrections as specified under procedure must be made on measured values for urine.

#### **Procedure for Specific Gravity Measurement**

Fill the graduated specific gravity cylinder with distilled water to the 50ml mark. Place the urinometer in it, and allow it float freely. Then record the level of floatation, starting from the top graduation. Discard the distilled water and repeat the same

procedure with unfiltered urine. If the specific gravity of the distilled water is not 1.000 make the necessary correction to account for changes in temperature and pressure in the laboratory where the test is carried out. The procedure for a typical observation is as follows:

Observed S.G. of distilled water	=	0.996
Observed S.G. of urine	=	1.016
Correction factor	=	1.000-0.996
	=	0.004
Therefore the corrected S.G. of urine	=	0.004 + 1.016 1.020

Appropriate corrections are also recommended when glucose and protein are present in large amounts in the urine sample. It is recommended that 0.003 be subtracted for each 1000mg/dl glucose or protein. The major disadvantage of this method is the requirement of a large volume of urine for the procedure.

#### (B) The Direct-Falling Drop Method

In this method, a drop of urine is allowed to fall into a series of columns each filled with solvent mixtures of increasing steps of known specific gravity. If the drop of urine comes to rest after its initial momentum is dissipated and then neither rises nor falls, the S.G. of the urine is the same as the solvent mixture of the particular column. Some commonly employed solvent mixture-systems include xylene and bromobenzene; chloroform and benzene; and bromobenzene and kerosene. The advantage of this method is that only few drops of urine sample are required. This method is however not popular because of the obvious length of time required to set up the system.

#### (C) The Indirect-Refractive Index (TS Meter) Method

This indirect method of measuring S.G. is based on the principle

of refractometry. The total solid Meter (e.g. American Optical) measures the refractive index of the solution. The refractive index is the ratio of the velocity of light in air to the velocity of light in solution. The refractive index varies with, but is not identical to the SG of urine. Although the instrument measures the refractive index of a solution, the scale readings have been calibrated in terms of SG refractive index and total solids. The instrument requires daily calibration and it is temperature compensated between 60°C and 100°C. The scale reads in increments of 0.001 from 1.000-1.035. Urines of SG less than 1.017 are read more accurately using the indirect - Refractive Index method. Digital urometers based on the principle of refractive index measurement using low - (Low-cal) and high-mode (Hi-cal) calibrators are now commercially available for easy application in urinalysis.

#### (D) The Ionization of Polyelectrolyte Method

This is the newest method for SG measurement. The SG reagent area has three primary ingredients impregnated into the reagent papers:

- a polyelectrolyte-polymethylvinyl ether/maleic acid, partially neutralised.
- an indicator, bromothymol blue; and
- specific buffers.

In the specific gravity reagent area of the multiple strips (Multistix), the polyelectrolyte, Polymethyl/Vinylether/maleic acid, is sensitive to the number of ions in the urine specimen. When the concentration of electrolyte increases an indication of high specific gravity of the urine, Pka of the polyelectrolyte in the reagent strip decreases thus causing a decrease in the pH of the medium. As a result of the change in PH caused by increasing ionic strength of the solution, the bromothymol blue

indicator changes colours from blue to green to yellow-green. The principle of the specific gravity reagent area is based on the  $\text{pKa}$  change of certain pretreated electrolytes in relation to the ionised concentration of solids in urines. This change in colour is empirically related to SG values and the calibration is in SG units.

**READING:** The SG colour scale ranges from 1.000 – 1.030.

### OSMOLALITY

Osmolality is an index of osmotic concentration and this is related to the number of particles a solute contributes to a solution, whereas the specific gravity depends on both the quantity and the precise nature of particles in solution. Large dense particles like proteins, glucose and intravenously injected dyes will increase SG more than they will do to the osmolality of urine.

In routine urinalysis, SG measurement, which serves quite adequately for the majority of clinical conditions is generally performed, but where more precise information is required, measurement of osmolality is the preferred choice. Osmolality of urine gives the same information as SG, but it is unaffected by the spurious results that may be obtained in urine samples containing large amounts of glucose or protein. It is more accurate than measurement of SG in conditions where the urine contains large quantities of solutes like glucose or protein.

The unit of measurement is in milliosmols/kg water. Healthy kidneys are capable of diluting and concentrating urine from a minimal range of 40-80 mosmol/kg water during water diuresis to a maximal range of 800-1400 mosmols kg water during fluid deprivation in man.

The normal range of values for urine osmolality in subjects on normal fluid and food intake is between 500-800 mosmol/kg water.

Newer concepts in assessment of the concentrating/diluting ability of the kidneys have evolved over the years. Measurement of plasma to urine osmolality ratio, osmolar clearance and free water clearance are now effective methods for comprehensive evaluation of the functional status of the kidneys in health and disease.

### PLASMA/URINE OSMOLALITY RATIO

It is calculated from measured values for plasma osmolality and urine osmolality in a subject. This ratio measures the concentrating ability of the kidneys and the normal range of values is 3.0 to 4.7.

### OSMOLAL CLEARANCE

This reflects the ability of the kidneys to conserve or excrete water.

$$\text{Osmolal clearance} = \frac{\text{Urine Osmolality} \times \text{Plasma Osmolality} \times R}{\text{Where } R = \text{Urine flow in ml/min.}}$$

### FREE WATER CLEARANCE

This is a better measure of kidney function as compared to the other measurements listed above.

$$\text{Free water clearance} = \frac{\text{Urine flow Rate} - \text{Urine Osmolality} \times \text{Urine flow}}{\text{Plasma Osmolality}}$$

Free water clearance is negative during water concentration tests.

Free water clearance can be altered in disease states as illustrated in Table 7.

**Table 7**  
**Changes in free water clearance in Disease States**

Decreased	Increased
Excess anti-diuretic hormone secretion	During water dilution tests
Heart failure	Diabetes insipidus
Liver disease	Adrenal insufficiency Certain head injuries

#### Method for determination of Urine/Plasma Osmolality

Osmolality of urine or plasma can be determined indirectly by measuring freezing point depression with a Freezing Point Osmometer. A one molal solution (1000 mosmol/kg water) depresses the freezing point to  $1.86^{\circ}\text{C}$  below  $0^{\circ}\text{C}$  freezing point.

The Osmometer is calibrated for both temperature and osmolality readings. The volume of urine/plasma required may be as low as 3ml. Recently, new developments have led to the introduction of vapour pressure osmometer for which only few drops of urine are required for measurement of urine/plasma osmolality. The advantage of this new method is that urine/plasma osmolality can easily be measured at any selected temperature in the laboratory.

#### URINE VOLUME

About 1 percent of the glomerular filtrate is excreted as urine. The volume of urine formed is directly the result of a balance between glomerular filtration and tubular reabsorption. Urine volume, although not measured except in timed-urine collections, is roughly identified each time a person voids. A typical urine output in an adult is between 750ml and 2,000ml per day, but may be less under conditions of water deprivation.

The urine output over a given period is directly related to

- (a) total fluid intake,
- (b) temperature and climatic conditions,
- (c) overall level of perspiration,
- (d) age,
- (e) degree of cellular hydration or dehydration,
- (f) efficiency of blood circulation,
- (g) overall concentration of certain electrolytes and solutes, and
- (h) Hormones of the posterior pituitary and adrenal cortex, etc.

Urine output in children is smaller than in adults, but the level increases with age in proportion to the body size. Normal values of urine volume in different age groups are as follows: Newborn/infants – 30 to 60 ml per 24 hour; Children 8–14 years 800 to 1,400 per 24 hours and Adults – 1000 to 2000 ml with an average of about 1,500 mls per 24 hour.

#### ABNORMALITIES IN URINE OUTPUT

##### Polyuria

Polyuria which is defined as excretion of an increased volume of urine over a given time frame may occur as a physiological response in different conditions. Such conditions include:

- (a) Increased intake of water and fluids (polydipsia).
- (b) Ingestion of diuretic medications.
- (c) Ingestion of certain diuretic drinks (e.g. coffee, tea, alcohol, etc.).
- (d) Exposure to excessive cold.
- (e) Nervousness and anxiety.
- (f) Increased ingestion of protein-rich foods.
- (g) Intravenous infusion of fluids.

- (h) Increased intake of salts (because excretion of excess salt or sodium is accompanied by excretion of water).

Polyuria may be a characteristic feature in the following pathological states in man:

- (a) Diabetes insipidus – a group of conditions including: Idiopathic and secondary absence of ADH (neurogenic) or primary and secondary (nephrogenic) type in which the kidney does not respond to ADH. Acquired nephrogenic diabetes insipidus can occur as a result of several clinical conditions like (i) Hypokalaemia, Hypercalcaemia, Sickle cell disease (SCD), anaemia, Amyloidosis, (ii) intake of drugs like: lithium, demeclocycline.
- (b) Osmotic diuresis in conditions like
  - (i) **Diabetes mellitus** – when there is glucosuria which leads to osmotic diuresis.
  - (ii) Hypercatabolic states – where excess urea leads to osmotic diuresis.
  - (iii) Chronic renal disease – due to excess urea which leads to osmotic diuresis and also to loss of concentrating ability of the kidneys (e.g. compensated renal insufficiency in chronic nephritis).
- (c) Certain tumours of the spinal cord.
- (d) **Acromegaly** – due to hormonal imbalance – over-production of growth hormone, somatostatin.
- (e) **Myxoedema** – due to water imbalance.
- (f) Addison's disease or adrenalectomy.
- (g) During healing phase of acute tubular damage.

### Oliguria and Anuria

Oliguria which is defined as urine output of less than 500ml/

24 hours may occur as a physiologic response to

- (a) Decreased intake of water and fluids.
- (b) Increased injection of salt and.
- (c) Excessive perspiration (common in tropical environment).

Oliguria may occur in pathological states in man:

Pre-renal (fluid losses)

- (a) vomiting and diarrhea and high fevers – due to excessive loss of body fluids.
- (b) oedema (nephritic).
- (c) oedema associated with heart failure.
- (d) ascites and oedema due to hepatic cirrhosis.

### Others

- (a) Mechanical obstruction to urinary flow.
- (b) Hypotension (e.g. Addison's disease).
- (c) Cardiac insufficiency.

Complete suppression of urine formation or excretion termed, anuria occurs in the following conditions:

- (a) Severe impairment of renal blood flow, total suppression of urine formation and the urinary bladder remains empty.
- (b) Obstruction to the outflow of urine. Most common cause is stones in both ureters, bladder-neck obstruction (prostate enlargement in old age and valve in paediatric cases).
- (c) Lesions causing acute renal insufficiency because of pathological changes within the nephrons.
- (d) Effect of toxic materials.

Further biochemical tests recommended in patients presenting with any abnormalities in urine output include:

- (i) Plasma Electrolytes and Urea.
- (ii) Comprehensive renal function tests (Plasma creatinine, urea and possibly clearance tests).
- (iii) Fasting/Random Blood glucose and possibly Glucose tolerance test.
- (iv) Urine concentration tests (SG, Osmolality).
- (v) Growth hormone assay.
- (vi) Markers of brain tumours.
- (vii) Thyroid function tests ( $T_4$ ,  $T_3$ , TSH).
- (viii) Total cholesterol, and
- (ix) Analysis of drugs in urine.

## 3 | Biochemical Properties of Urine

The major constituents of urine are electrolytes, trace elements, non-protein nitrogenous components (urea, uric acid and creatinine), indoles (metabolite of tryptophan), porphyrins, amino acids, organic acids, hormones, vitamins and enzymes. Total nitrogen excretion is closely related to dietary intake of nitrogen containing food components (e.g. protein).

### PH AND ACID-BASE BALANCE

The kidneys and the lungs are the two major organs responsible for the regulation of acid-base balance in the human body. The lungs excrete carbon dioxide, while the kidneys regulate the excretion of the non-volatile acids produced by the normal metabolic processes of the body tissues. The pH of urine is influenced to a significant degree by the acidic or basic salts which are in the specimen. These include primarily sodium and potassium, mono- and dihydrogen phosphates, sodium citrate, ammonium salts, sodium bicarbonate and carbonic acid, and to a little extent organic acids like pyruvic acid, lactic and citric acids (intermediate metabolites of Kreb's cycle). By the mechanism of regulated excretion of acidic or alkaline urine, the body can eliminate large quantities of either acids and/ or bases and maintain a constant homeostatic state. The adjustment of urinary pH by the kidney is accomplished primarily through reabsorption of a variable amount of sodium

ion ( $\text{Na}^+$ ) by the renal tubules accompanied by concomitant secretion of hydrogen ( $\text{H}^+$ ) and ammonium ( $\text{NH}_4^+$ ) ions in exchange.

The pH of urine is a measure of the  $\text{H}^+$  ion concentration in urine and a pH below 7 is acidic, while that above 7 is basic.

The pH of fresh urine from healthy individuals can vary considerably from approximately pH 4.8 to pH 8.5. Freshly voided urine from subjects on normal diets has an approximate pH value of 6.0.

Understanding the utility of urinary pH measurement is somewhat more difficult than in understanding the utility of many other tests that are applied to urine. This is because the range of pH values encountered in urine specimens from normal individuals is not different from that observed in persons with abnormalities where urinary pH measurement makes an important contribution to either diagnosing or monitoring the disorder.

Meaningful use of pH measurements can be achieved only when used in conjunction with other clinical information and laboratory measurements in the subject. The types of application of urine pH are listed in Table 8.

**Table 8**  
**Urine pH in Screening, Diagnosis and Monitoring**

Renal tubular acidosis
Urinary tract infections
Drug therapy monitoring
Prophylaxis of renal calculi
Renal tubular alkalosis
Respiratory acidosis
Respiratory alkalosis
Metabolic acidosis
Metabolic alkalosis

### ACIDIC URINE

In acidic urine pH is less or equal to 6.0 ( $\text{pH} \leq 6.0$ ). Common conditions where urine is acidic include:

- (1) Consumption of high protein diets.
- (2) Ingestion of drugs (e.g. Ammonium chloride, Mandelic acid, etc).
- (3) Acidosis, and
- (4) Uncontrolled diabetes mellitus (ketosis).

### ALKALINE URINE

In an alkaline urine pH is greater than 7.0 ( $\text{pH} > 7.0$ ). Common conditions where urine is alkaline include:

- (i) Consumption of diets high in vegetables, milk and other dairy products.
- (ii) Ingestion of drugs (e.g. sodium bicarbonate, potassium citrate, acetacetamide, etc.).
- \* (iii) Renal tubular acidosis (RTA).
- (iv) Fanconi syndrome.
- (v) Urinary tract infection.
- (vi) Bacteria contamination of urine specimen with urea splitting organisms.

In the treatment of certain diseases, it is important that the urine be kept at an acid pH. Such needs are particularly pertinent in the treatment of urinary tract infection and persistent bacteriuria, especially where urea splitting organisms are involved. It is also quite important to keep the urine acid in the management of urinary calculi such as those due to calcium phosphate, calcium carbonate and magnesium phosphate which develop in alkaline urine. Excretion of some drugs/metabolites shows some relationship with the urinary pH.

\* The tubules show loss of ability to maximally excrete an acid load.

Salicylates, porphyrins and urobilinogens are excreted from the body much more rapidly if the urine is alkaline, whereas amphetamine and pethidine are excreted in much larger quantities in an acid urine. Also streptomycin is effective in genitourinary tract infection treatment only if the urine is alkaline. In such situations as listed above, it is important to monitor frequently urine specimens with a pH test.

Renal stone formation partially depends on urine pH. Phosphate and calcium calculi develop in alkaline urine, while uric acid, cysteine and calcium oxalate stones are common in acid urine.

### METHODOLOGY

For routine analysis, urinary pH may be measured with indicator paper strips and a colour chart. The pH test paper contains the indicators methyl red and bromothymol blue. This combination gives a series of distinct colour changes from orange via green to blue over a pH range of 5-9.

When more exact determinations are required, a pH meter is employed.

## 4 | Urinary Protein

**Normal:** Protein between 40 and 80mg is excreted per 24 hours, but a value between 100 to 150mg/24h may also be considered normal. The approximate urinary protein concentration varies from 2-8mg/dl, with albumin constituting about 33.3 per cent of the total protein and the remaining being beta- and gamma-globulins. The wide range in the reference value for urinary protein concentration is the result of biological variations and differences in methods for total protein determination.

### CLINICAL APPLICATION

#### PROTEINURIA

##### Definition

Proteinuria refers to an increased amount of protein in the urine, usually detectable by common methods used in routine urinalysis. The results of epidemiological studies indicate a limit of 30mg/100ml for physiological proteinuria, in first morning urine specimens. The use of first morning urine is unanimously recommended for tests to determine proteinuria. It is probably one of the single most important indicators of renal disease. Detection of protein in urine combined with the microscopic examination of urinary sediments (usually carried out in Medical Microbiology laboratory) forms the basis of the differential laboratory diagnosis of renal disorders. Other causes include non-nephrogenous pathologic conditions: e.g.

Proteinuria due to cardiac insufficiency; excretion of abnormal types of proteins (e.g. Bence-Jones Protein. Tamm-Horsfall Protein) and benign conditions in healthy individuals (e.g. orthostatic). Proteinuria can be classified as listed under classification of Proteinuria using anatomical description or amount of protein excreted.

### **Classification of Proteinuria**

(a) Benign – Like in orthostatic (postural) and lordotic proteinuria, physical exertion (sports), hypothermia, pregnancy, and the administration of vaso-constricting drugs.

#### Characteristics

- healthy kidneys
- **intermittent**
- negative in the first morning urine
- positive-(minimal-moderate) in the course of the day (urinary protein can be up to 500mg/100ml in some cases).

Benign, on the part of subjects with healthy kidneys – observed primarily in the age group below 30 years – and this constitutes up to 90% of all proteinurias affecting this age group in some populations.

(b) Pathological Renal proteinuria

#### Characteristics

- damaged kidneys
- **persistent**
- values of urine protein usually > 25mg/100ml (most pronounced in nephrosis)
- values between 200-300mg/100ml in glomerulonephropathy; micro haematuria usually present in marked proteinuria.

### **Tubular Proteinuria**

#### Characteristics

- lesions of renal tubules and/or impaired protein reabsorption from the glomerular filtrate by the tubules
- also in pyelonephritis, cystic kidneys, phenacetin induced nephropathies; granular atrophy of the kidney in gout.

### *Excretion of abnormal types of proteins*

#### Characteristics

- excretion of low molecular weight proteins as in multiple myeloma, (Bence-Jones Protein); heavy chain disease, (Franklin's disease).

### **Multiple Myeloma**

Multiple myeloma is a malignant proliferation of plasma cells in the bone marrow which results in an abnormally high concentration of serum immunoglobulins usually IgG or IgA less commonly IgM. Very rarely IgD and IgE may be involved.

During the synthesis of immunoglobulins in the lymphocytic cells, the heavy-chain polypeptides are usually assembled on a different ribosome from the one for light chain assembly. This process operates somewhat synchronously as the light chains are attached to two heavy chains to form a complete immunoglobulin.

In the disease multiple myeloma, light chains are frequently produced in excess over the available heavy chains; since they are of relatively low molecular weight, they pass through the glomerular membrane and are detectable in urine. They possess peculiar solubility properties which was first reported by Dr. Henry Bence-Jones. These proteins precipitate when urine is heated from 45°C to 60°C and redissolve when heated above 80°C. The disease is also called light chain disease. The chain

may be composed of either kappa or lambda. The protein can be demonstrated by serum protein electrophoresis characterised by a discrete (sharp band) at the gamma-globulin fraction or myeloma 'M' spike. Immunodiffusion in agar gel is used for quantitative studies while immunofixation procedure is used for definitive identification. There are also typical serum biochemical and haematological patterns such as markedly raised plasma total protein, calcium, uric acid, creatinine, urea and decreased albumin concentrations. The erythrocyte sedimentation rate (ESR) is usually rapid and high, haemoglobin level is low and marrow smear (aspirate) shows excessive abnormal plasma cells. Other haematological abnormalities are normocytic anaemia, leucopenia and thrombocytopenia. The clinical features of bone pain, fractures, infections and renal failure are largely due to the abnormal protein. X-rays may show typical osteolytic areas.

#### *Non-nephrogenous Proteinuria*

- kidneys not involved.

#### Characteristics

- disappear after elimination of the cause due to colic infarction, cardiac insufficiency or febrile states.

The type of protein excreted in urine in disease states is generally related to the serum proteins; and in severe cases, direct leakage of serum proteins into urine occurs. Smaller proteins, such as albumin and alpha-1 globulin are excreted more readily than larger ones and in fact albumin constitutes between 60 and 90% of protein excreted in most disease states. Certain diseases are characterised by excretion of specific globulins rather than by diffuse or generalised proteinuria common in others. The urine of patients with multiple myeloma for instance contains increased amounts of a low molecular

weight globulin known as Bence-Jones protein. This type of protein with an abnormal molecular weight may also be found in some patients with Waldenstrom macroglobulinaemia and primary systemic amyloidosis.

An increased excretion of a specific globulin that is similar to Bence-Jones protein occurs in Franklin's disease (heavy chain disease) and patients with renal tubular disorders such as Fanconi syndrome, show a predominant increase in the quantities of globulins excreted in the urine.

The degree of proteinuria depends upon the precise nature of the clinical and pathological disorders as well as the severity of the specific disease. Proteinuria may be intermittent, transient or persistent and commonly continuous. Transient or intermittent proteinuria is caused by physiological changes or conditions of renal vasoconstriction rather than by renal diseases.

#### **CLASSIFICATION OF DEGREE OF PROTEINURIA**

Proteinuria may be classified into (i) marked, (ii) moderate and (iii) minimal proteinuria.

##### **(i) Marked Proteinuria**

This is characterised by the excretion of more than 4g of protein per day. It is typical of the nephritic syndrome, but also occurs in severe cases of:

- (a) glomerulonephritis
- (b) nephrosclerosis
- (c) amyloid disease
- (d) systemic lupus erythematosus (SLE)
- (e) and severe venous congestion of the kidney produced by renal vein thrombosis, congestive heart failure or obstructive pericarditis.

##### **(ii) Moderate Proteinuria**

This is a condition where daily excretion of protein is between

0.5 and 4g per day. This is the more commonly encountered type of proteinuria, found in the vast majority of renal diseases including all the disorders listed above for marked proteinuria, as well as chronic glomerulonephritis, diabetic nephropathy, multiple myeloma, toxic nephropathy, pre-eclampsia and inflammatory diseases. Other conditions include malignancy, degenerative and irritative conditions of the lower urinary tract, including nephrolithiasis.

### (iii) Minimal Proteinuria

In this type of proteinuria, the excretion of protein is less than 0.5g per day. It is associated with chronic glomerulonephritis, polycystic disease of the kidneys, renal tubular disorders, the healing phase of acute glomerulonephritis, latent or inactive stages of glomerulonephritis and various disorders of the lower urinary tract.

There is another type of proteinuria called Postural Proteinuria. This describes the excretion of excess protein by subjects who are erect or in lordotic position. The proteinuria is intermittent and disappears when the individual lies down. The daily protein output in this condition is less than 1g/day. Postural proteinuria occurs in three to five percent (3-5%) of healthy young adults.

### **Orthostatic Proteinuria (OP)**

This has been attributed to inferior venacaval compression between the liver and the vertebral column in a lordotic posture. There are no other symptoms of renal disease. Serum urea, creatinine concentrations as well as blood pressure are usually normal. Haematuria is absent, but hyaline and granular casts are often present. The degree of proteinuria may vary from day to day but characteristically vanishes at night. It therefore fits into the classification of intermittent proteinuria.

It may be differentiated from other forms of proteinuria by testing for protein in urine specimens collected before and after the individual has been erect. The subject voids and discards this urine at bedtime. He collects a urine specimen immediately after awakening and before he is upright for more than a moment. He collects another specimen after he has remained erect or walking for a period of at least two hours. The first specimen should be negative for protein, while the second specimen will be positive for protein if the subject has postural proteinuria.

There is yet another type called functional proteinuria. This defines the conditions in which excess protein excretion is associated with fever, exposure to heat or cold, excessive exercise and emotional stress. The underlying physiologic mechanism inducing proteinuria in these conditions is renal vasoconstriction.

### **BENCE-JONES PROTEIN**

This deserves special mention because of the important clinical significance of its detection in patients. Bence-Jones Protein is a specific low molecular weight protein (fragments of light chains of immuno-globulins) excreted in the urine of more than 50% of patients suffering from multiple myeloma. It is also found in macroglobulinaemia. Bence-Jones Protein represents a portion (fragment) of the high molecular weight plasma myeloma globulin, which has heat stability properties different from all other urinary proteins. In particular it coagulates on heating to temperatures between 45°C and 60°C and then redissolves on further heating to the boiling point.

### **CLINICAL APPLICATION**

A test for the presence or absence of protein is one of the most frequently performed procedures in routine urinalysis. It is well established that urinalysis is cost effective and easy to perform

**Table 9**  
**Types of Protein in Urine in Different Conditions**

Protein	Condition(s)
Albumin	Strenuous physical exercise, emotional stress, infections, glomerulonephritis Pregnancy Newborns (first week)
Globulins	Glomerulonephritis, tubular dysfunction
Haemoglobin	Haematuria, haemoglobinuria
Fibrinogen	Severe renal disease
Nucleoproteins	WBC's in urine, epithelial cells in urine
Bence-Jones	Multiple myeloma, leukaemia

and it should be the first line of clinical examination for all subjects undergoing routine medical checkup and patients in all health institutions including primary and secondary health centres. The summary for routine screening test for urinary protein is as shown in Table 10.

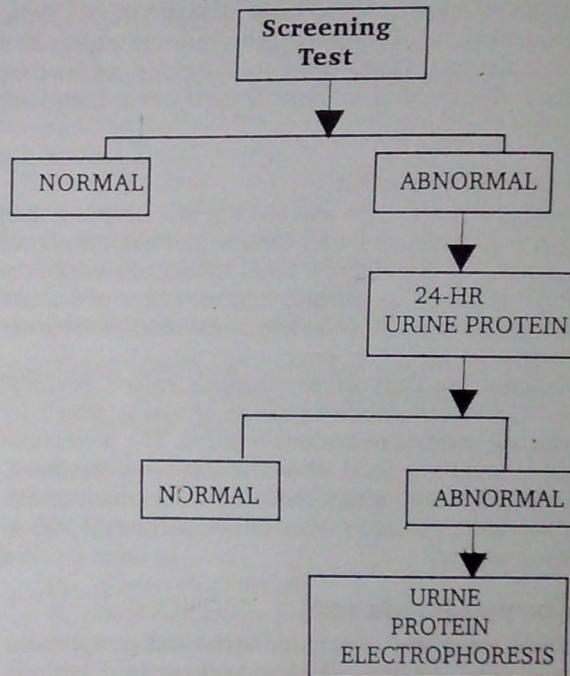
#### METHODS

For more than a century, clinical tests for protein in urine were based on precipitation phenomena involving the coagulation of proteins by heat, and by various chemical agents, including concentrated nitric acid, trichloroacetic acid and sulfosalicylic acid (SSA). Presently, a number of simple, semi quantitative and more complex quantitative tests are available for determination of different proteins (e.g. albumin, globulin, Bence-Jones Protein, etc.) in urine.

#### COLOURIMETRIC REAGENT STRIP TEST

The colourimetric dip-and-read protein test has achieved widespread acceptance in practically all parts of the world.

**Table 10**  
**Summary of Approach to Urine Screening Test**



Single dip-stick as well as the protein testing position of all multiple dip-stick strips is based on the so called "protein error of indicators". In simple terms, the colorimetric reagent strip test is based upon the ability of proteins to alter the colour of some acid-base indicators without altering the pH. When an

indicator such as tetrabromophenol blue is buffered at pH 3, it is yellow in solution without protein, but in the presence of protein, the colour will change to green and then to blue with increasing protein concentration. The commonly commercially available single dip-stick strips include Albustix (Ames Co) and Albym - Test (Boehringer Mannhem Co.), while multiple strips include Uristix, Combistix, Hema-combistix, Labstix, Bililabstix, Multistix and Multistix-SG reagent strips.

The colour change is compared with the Maker's colour chart where semi-quantitative values for urinary protein concentration can be obtained (Range from Trace to + + + +).

#### SEMI-QUANTITATIVE PRECIPITATION TEST

The heat, acetic acid, sulfosalicylic acid (SSA) and the concentrated nitric acid protein precipitation methods are simple methods for semi-quantitation of protein concentration in urine. The results are interpreted as follows:

- (1) Negative means no turbidity.
- (2) Trace is a faint precipitate visible against a black background, equivalent to about 5mg/dL protein.
- (3) One plus (+) is a small degree of turbidity, equivalent to 10 to 30mg/dL.
- (4) Two pluses (++) is a moderate turbidity equivalent to 40 to 100mg/dL.
- (5) Three pluses (+++) is heavy turbidity equivalent to 200 to 500mg/dL.
- (6) Four pluses (++++) is heavy flocculation equivalent to 500mg/dL or more.

**Sulfosalicylic acid Test:** To about 5ml of urine in a test tube, add 20% sulphosalicylic acid solution dropwise. Watch each drop of the acid snake its way through the urine to the bottom of the tube. Cloudiness observed against a dark

background in its wake or at the bottom of the tube where it settles indicates the presence of protein.

**Albustix:** Dip test end of strip in urine and remove immediately and compare colour of the strip with the Maker's colour scale which varies from yellow (when no protein is present), through green to blue depending on the amount of protein present. High alkaline urine may give a false positive result.

#### QUANTITATIVE 24-HOUR PROTEIN DETERMINATION

Simple estimates of the protein content of urine are performed by quantitating the amount of precipitate formed following the addition of some protein precipitants, measured either by comparison with known standards or by recording the height of the column of precipitate in a specially devised tube. More complex quantitative tests involve the measurement of protein precipitation with a nephelometer or photometer (spectrophotometer). These tests are general adaptations of one of the after precipitation tests such as the sulphosalicylic acid (SSA) turbidity test.

A short description of the procedure is as follows:

- (1) Pipette 2.5ml of centrifuged or filtered urine into a test tube.
- (2) Add 7.5ml of 3% SSA (3g in 100ml of distilled water).
- (3) Invert to mix several times.
- (4) Allow to stand for 10 minutes at room temperature, and
- (5) Compare the resulting turbidity with those of known protein standards prepared from solutions containing 10, 20, 30, 40, 50, 75 and 100mg/dL, to estimate the concentration of the unknown. If the subject's urine sample contains more than 1000mg/dL protein, the urine should be diluted and the test repeated on the diluted specimen.

### BENCE-JONES PROTEIN DETERMINATION

Bence-Jones protein is soluble at room and body temperatures. It however, precipitates upon heating between 45° and 60° and redissolves when the urine is heated further up to the boiling point. The Bradshaw's test where a small amount of concentrated HCl is carefully added to the urine. A precipitate at the interface indicates presence of Bence-Jones Protein (BJP).

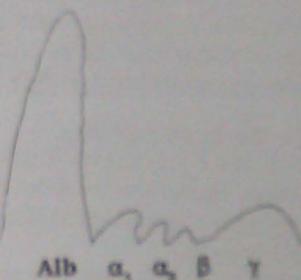
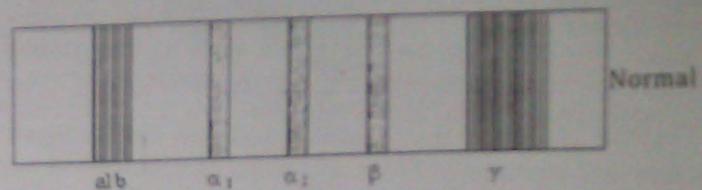
### SCREENING METHOD

Gradual heating of a urine sample to the boiling point is the simplest screening method for the detection of Bence-Jones protein.

When present, a precipitate will first appear and then redissolves as the urine is further heated. The presence of larger amounts of other proteins or phosphates may decrease the accuracy of this test. These interfering proteins can usually be removed by cooling the heated urine to room temperature, filtering and repeating the heating process on the filtrate.

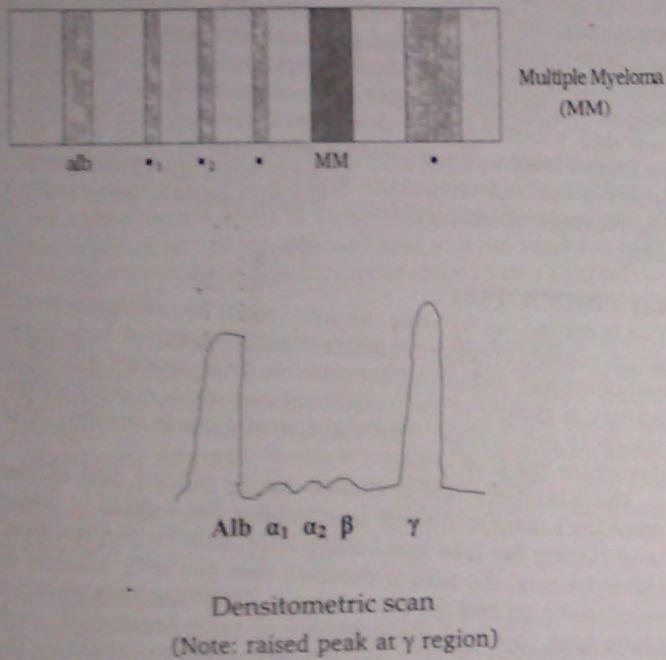
A more definitive test on urine is protein electrophoresis of concentrated urine. This shows a typical intense band between beta and gamma fraction as follows (Figures 1 and 2).

**Figure 1**  
Urine Protein Electrophoresis



Densitometric impression of serum protein

**Figure 2**  
Urine Protein Electrophoresis



## 5

# Glucose and Other Reducing Substances

Glucose is the reducing sugar most commonly found in urine; although other reducing sugars such as lactose, fructose, galactose and pentose may also be found under certain conditions.

The presence of a detectable amount of glucose in urine is known as glycosuria. During fasting, the concentration of glucose in the blood is ordinarily between 65 and 80mg/100ml. When blood glucose concentration exceeds 180mg/100ml, (200mg/plasma) there will be a corresponding increase in the concentration of glucose in the glomerular filtrate. Glycosuria occurs in such instance when the blood glucose level exceeds the reabsorption capacity of the renal tubules, i.e. when the glomerular filtrate contains more glucose than the tubules are able to reabsorb (renal threshold). Glycosuria may be either benign or pathological, and the physician must distinguish between the two types.

**Clinical Application:** The clinical utility of urine glucose tests is of great importance

- (1) in screening healthy persons for the identification of asymptomatic disease, as part of the diagnostic work up in the recognition of diabetes mellitus,
- (2) as part of the differential diagnosis in resolving the procedures of the crises of diabetes mellitus, and

- (3) in providing an important monitoring mechanism for diabetic patients to assess the effectiveness of their control by medication or diet (compliance).

### THE TYPES OF GLYCOSURIA

- (i) *Renal glycosuria.* This occurs in subjects with normal blood glucose levels where tubular reabsorption of glucose is below normal, lowered threshold thus leading to glucose 'spill over' into the urine. This is a benign condition, e.g. occurrence of glycosuria after eating a heavy meal or during emotional stress.
- (ii) *Pathological glycosuria.* The clinical condition known as diabetes mellitus is a major cause of glycosuria. This pathological state presents with marked elevation of blood glucose levels and usually associated with an increase in urine output. The urine is usually light in colour with increased specific gravity due to the extra load of dissolved solid. Glycosuria is not conclusive evidence for the presence of diabetes mellitus in an individual as it can also occur in other conditions like renal damage, pregnancy, thyrotoxicosis, phaeochromocytoma, Cushing's syndrome, growth Hormone excess, etc.

### METHODS

There are a variety of tests for glucose determination that can be applied for the quantitation of urinary glucose. The chemical basis of practically all the commonly used tests for qualitative and quantitative detection of glucose in urine involves either the reduction of glucose by copper in a hot alkaline medium or specific enzymatic catalysis of glucose oxidation by oxygen in the air.

### TECHNICAL NOTES

#### (1) Reducing Tests for Substances

The reduction of metallic ions such as  $\text{Cu}^{2+}$  is not specific for glucose since a positive reaction may be given by any other reducing substance present in urine. Such common reducing substances include creatinine, uric acid, ascorbic acid, glutathione, some drugs or other non-glucose reducing sugars. Non-carbohydrate components seldomly interfere, but occasionally in very concentrated urines, some interference from such substances may be observed. The non-specificity of the copper reducing tests has an advantage of being able to detect other reducing sugars other than glucose, but a disadvantage of high incidence of false positives for glucose tests.

#### BENEDICT'S TEST

This is carried out by using the well known Benedict's reagent, which consists of copper sulphate ( $\text{CuSO}_4$ ) dissolved in sodium carbonate and sodium citrate solution. The powerful reducing activity of glucose in hot alkaline solution yields cuprous oxide which is red-orange. The amount of glucose present will determine the final colour.

The Benedict's test is carried out by placing 5ml of the Benedict's reagent in a test tube, adding 5 to 8 drops of urine, and placing the tube in a boiling water bath for five minutes. After heating, the tube is removed from the bath, allowed to cool in the air and the colour noted. The colour change varies from blue (no reducing substance or sugar present) through green to yellow, to orange to a brick-red colour indicating the presence of varying concentrations of reducing substances in the urine specimen. The colour change can be used to estimate the amount of a reducing substance present in the urine by comparing the test urine with glucose solutions of known

concentrations. A glucose concentration as little as 0.1% (containing 100mg/dl glucose) is capable of giving a positive reaction with Benedict's reagent.

**Clinitest - Reagent Tablet:** The test which is based on the reduction of copper uses cupric sulphate as the source of cupric ions. The copper reduction test has been greatly simplified by the availability of the CLINITEST tablet which consists of copper sulphate compounded into an effervescent tablet containing sodium carbonate, citric acid and sodium hydroxide.

**Procedure:** The routine procedure is as outlined below: Place one Clinitest tablet in a dry test tube, add 10 drops of water and then 5 drops of urine. The tablet dissolves with the evolution of carbon dioxide and heat and the resultant colour is compared with the Maker's Colour chart 15 secs after reaction has stopped.

**Result:** If a reducing substance such as glucose is present, the colour changes from blue to orange, depending upon the amount of sugar present. Just as for Benedict's test, by comparing the colour with a reference colour chart, the amount of reducing substance in the urine can be semi-quantitated.

A typical result is indicated as negative (blue); ¼% (dark green); ½% (yellow-brown), 1% (brown) and 2% glucose (orange).

#### NYLANDER'S TEST

This is a test frequently used as a test for glucose. Nylander's test is based upon the reduction of alkaline bismuth subnitrate by glucose. When heated, a mixture of Nylander's reagent and urine containing glucose will produce a black precipitate of free bismuth. Galatest, which employs bismuth oxide in an alkaline powder, is now used as a test for total reducing sugar in urine. The procedure involves moistening of 'small amount'

of the powder with urine and recording the colour produced. The powder will remain white or yellow if there is no reducing sugar, but will turn brown or black if a reducing sugar is present.

#### ENZYMATIC METHODS

These methods are specific for detection of glucose, but some urine components such as ascorbic acid (Vitamin C) may inhibit or interfere with the tests. These tests are mainly based on the glucose oxidase principle.

#### GLUCOSE OXIDASE TEST

The enzymatic glucose oxidase methods for glucose as applied to urine (they are also used extensively for blood glucose determination) are specific for glucose. In these tests, glucose oxidase catalyses the oxidation of glucose to gluconolactone and a peroxide. The peroxide in the presence of a peroxidase is used to oxidize an indicator which in turn produces a colour change.

Other sugars such as lactose, fructose and pentose are not substrates for glucose oxidase and therefore do not give positive reactions with this test.

Many reagent strips such as Clinistix, Diastix, Uristix, N-Uristix, Combistix, Labstix, Bililabstix, Multistix, and N-Multistix-SG used for detection of glucose are based on this principle.

In practice, the test strips are dipped into the urine sample, the excess removed by touching the side of the container and the resulting glucose colour reaction compared to a colour chart. The manufacturer's instruction on time should be strictly adhered to.

**Non-Glucose Reducing Sugars:** Galactose, fructose, xylose,

sucrose, mannose, and other sugars are not actively reabsorbed by the kidney in the same way as glucose. When these sugars are present in the blood, they will appear in the glomerular filtrate and will not be as rapidly reabsorbed as the water or glucose of the filtrate. Accordingly, there will be a sizeable quantity of such reducing sugars in the final urine which is very much greater than in the blood. None of these other reducing sugars, unlike glucose, appear to have a renal threshold.

The need to detect these other reducing sugars in urine is important because of the important position they occupy in screening procedures for in-born errors of carbohydrate metabolism, particularly in paediatric medical practice.

When urine samples containing any of the common non-glucose reducing sugars such as lactose, galactose, fructose or pentose are tested with either the Benedict's reagent or Clinitest tablet a positive reaction will be observed, but a negative reaction will be observed with Clinistix. Urine chromatography for sugars is helpful for characterisation of these other sugars. The clinical conditions where these sugars are present and the implications are briefly discussed below:

### (i) Lactose

This is the sugar found in milk. It may appear in the urine of lactating women. This is usually a temporary condition which corrects itself upon cessation of lactation. Lactose may also be found in trace amounts in the urine of three- to five-day old infants before their digestive systems have become fully developed, and in other children and adults who are deficient in intestinal lactase.

The presence of lactose in urine can be detected by the Clinitest tablet method. Identifying the sugar as lactose can be achieved by the mucic test, the osazone test, paper chromatography, and hydrolysing the sugar and testing for galactose

by the galactose oxidase method. These are however, not routine procedures. The presence of lactose in urine is usually considered physiological rather than pathological. It may however be responsible for a form of osmotic diarrhea in children.

### (ii) Galactose

Galactose is found in the urine of infants suffering from galactosaemia, a metabolic disease caused by an inherited deficiency of the enzyme galactose - 1-phosphate-1-uridylyl transferase (Gal-1-PUT). This enzyme is necessary for converting galactose to glucose. It is a severe condition which can be treated by eliminating galactose from the diet. If not corrected (or treated), the infants will rapidly deteriorate physically and mentally which may lead to an early death. Occasionally, adults, who ingest large quantities of milk or other lactose-containing foods will show trace amounts of galactose in the urine. This has no clinical significance and it disappears from the urine upon the reduction of galactose consumption. Galactose can be detected initially with clinitest and identified by osazone test or paper chromatography, and by the galactose oxidase test. Since paediatricians commonly screen urine of infants with clinitest followed by Clinistix to detect the presence of non-glucose reducing substances (i.e. positive clinitest, negative clinistix) in urine, identification of non-glucose reducing sugars in such conditions is not problematic in routine urinalysis.

### FRUCTOSE

Fructose is occasionally seen in the urine of patients with hepatic disease and those who have consumed excess amounts of fruit. The presence of fructose can be detected with clinitest. It can be identified by Seliwanoff's test and by paper chromatography. These latter tests are not routine procedures.

**PENTOSE**

Pentosuria may be associated with certain types of drug therapy and with some hereditary conditions. In both cases, its presence is considered benign. Pentose is positive with clinitest and it reduces Benedict's solution (reagent) at room temperature.

Identification can be performed by using the osazone test, and paper chromatography.

**6****Ketone Bodies in Urine**

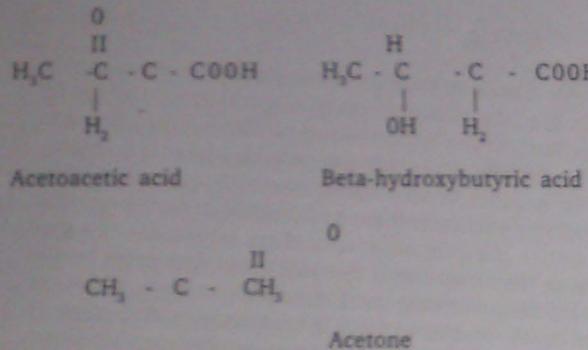
The body normally metabolizes fats completely to carbon dioxide and water. The intermediary metabolites of fatty acid oxidation are ketone bodies. The ketone bodies circulate in the blood and are used by the majority of body's peripheral tissues as an energy source. In normal health the acetoacetic acid and beta-hydroxybutyric acid formed are metabolized at such a rate that there is only a minimal amount of these substances in either the blood or the urine - such that this is not detectable by any of the methods commonly used in routine analysis of ketone bodies in urine. Whenever there is inadequate carbohydrate in the diet or a defect in carbohydrate metabolism or absorption, the body metabolizes increasing amounts of fatty acids to meet body energy requirements. Starvation with low carbohydrate diets or in diabetic patients as in keto acidosis, the amount of ketone bodies formed in the body exceeds the rate of peripheral tissue utilisation and, accordingly, there is an increase in serum ketone bodies which are excreted in the urine. These intermediates are the three ketone bodies; aceto acetic acid, acetone and beta-hydroxybutyric acid.

All the three ketone bodies are present in the urine of patients with ketonuria in relative proportions of 30% acetoacetic acid, 2% acetone and 68% beta-hydroxybutyric acid.

**CLINICAL UTILITY**

The most important disorder in which ketonuria occurs is diabetes mellitus. In Type 1 diabetes mellitus, glucose

Figure 3



metabolism is sufficiently impaired that fatty acids are utilised to meet the body's energy requirements. When this type of diabetes is untreated, or not well controlled excessive amounts of fatty acids are metabolized with the result that ketone bodies accumulate in blood (ketosis) and are excreted in urine (ketonuria). The presence of excess ketone bodies may lead to a decrease in carbon dioxide combining power leading to systematic acidosis (metabolic acidosis) in such patients. Progressive diabetic keto acidosis in diabetics can lead to coma and eventual death. Urine ketone tests have gradually assumed a role of increasing importance in the clinical monitoring of diabetes mellitus. Thus, detection of ketonuria in a patient with diabetes mellitus is of great significance as a change in insulin dosage or other management procedures is often indicated.

During periods of acute infections, surgery, G.I.T. disturbances, or other stress and anytime the management

routine does not adequately control the disease, the urine of all diabetic patients should be checked for the presence of ketone bodies. The tests can also be used in the differential diagnosis of coma. Ketonuria also accompanies the restricted carbohydrate intake that occurs in association with fevers, anorexia, G.I.T. disturbances, fasting, starvation, coeliac disease, vomiting of pregnancy, cachexia, following anesthesia, and as a result of certain neurologic disorders. During persistent intake of some popular low carbohydrate diets, it is advisable that the adequacy of such diets should be monitored over time by testing for ketone bodies in urine periodically.

#### METHODS

In ketonuria, acetoacetic acid, acetone, and betahydroxy butyric acid are all excreted in the urine. Consequently, a test procedure which principally determines one of these three components is generally satisfactory for the diagnosis of ketonuria. Specific tests do exist for the determination of each of these substances but they are not generally used because the methods are more cumbersome and they are less reliable and less sensitive. Many of the tests for ketone bodies in urine are based on the nitroprusside reaction (Rothera's test).

#### The Nitroprusside Reaction

Nitroprusside generally reacts with both acetone and acetoacetic acid in the presence of alkali to produce a purple-coloured compound.

#### Rothera's Test

Make 5mls of urine, saturate with Rothera's reagent (ammonium sulphate, sodium nitroprusside mixture (99:1) and add 1ml of concentrated ammonia solution. Shake well and allow to stand. A characteristic permanganate colour indicates presence of acetone, or acetoacetic acid or both.

**Acetest**

In this reaction procedure, ammonium sulfate used in the Rothera's reagent is replaced with glycine as the nitrogen containing solid state reagent. To carry out this test, place an acetest tablet on a clean white surface (e.g. filter paper), and put a drop of urine on the tablet. Compare the colour of the tablet after 30 secs with the Maker's colour scale. This test shows positive results for all the three ketone bodies, when present in urine. A purple colour is observed in positive cases.

**Dip-stick Strips**

Also a number of dip-stick strips, based on the nitroprusside reactions that are now available commercially, include Ketostix (single reagent strip) and multistix (multiple reagents strip).

**7**

## Gross Blood in Urine (Gross Haematuria)

Gross haematuria is a condition where blood visible to the naked eye is present in large enough amounts (more than 0.5ml blood per litre of urine) to give the urine specimen a definite red or "smoky" appearance on inspection. On the other hand, occult blood (micro-haematuria) is defined as blood in urine detectable only by some chemical or physical procedures. Chemical reactions detect both intact red blood cells and free haemoglobin as well as myoglobin. In contrast, microscopic examination of urine sediments will detect only intact erythrocytes. Haemoglobinuria is the presence of free haemoglobin, while haematuria is theoretically, the presence of intact blood cells in the urine. However, in practical terms, both cells and free haemoglobin are found in haematuria.

### GROSS AND OCCULT BLOOD IN URINE

When haemolysis occurs, free haemoglobin (Hb) is released into the surrounding medium. If haemolysis occurs in the circulation, e.g. in haemolytic anaemia, free Hb is present in the blood; when present in sufficient quantity, significant amount is filtered through the glomerulus and this appears in urine.

### CLINICAL APPLICATION

Haemolysis that produces haemoglobinuria may have occurred

in the blood stream, in a particular body organ, in the kidney or lower urinary tract, or in the sample itself. Thus haemoglobinuria may indicate an haematologic disorder, such as haemolytic anaemia, haemolytic transfusion reactions, nocturnal haemoglobinuria, paroxysmal (cold) haemoglobinuria, or favism (a form of G-6-P-D deficiency). Haemoglobinuria is found in severe infectious diseases such as yellow fever, smallpox, and malaria, in poisoning with strong acids or after ingestion of certain poisonous mushrooms; following extensive burns and renal infarction. A significant amount of free Hb may be found in the urine whenever red blood cells are present in excessive numbers as a result of frank occult bleeding that may accompany various renal disorders, infections, neoplastic disease or trauma affecting any part of the urinary tract.

In fact, it has been suggested that haematuria is one of the four critical findings in patients with rapidly progressing glomerulonephritis, indeed, absence of occult blood abnormalities of the urinary sediment in patients with renal failure should arouse suspicion of obstructive uropathy. Tables 11 and 12 show conditions where haematuria or haemoglobinuria may be constant features. In addition positive screening tests for haematuria may reveal cancer of the prostate or bladder in patients without symptoms.

Table 13 indicates the clinical utility of tests for occult blood in urine as related to screening, diagnosing and monitoring purposes.

#### METHOD

The reagent strip test is the simplest and most direct test for the detection of haemoglobin in urine. The reagent area is impregnated with tetramethylbenzidine, benzedrine and buffered organic peroxide. Tetramethylbenzedrine forms a

**Table 11**  
**Renal Diseases in which there may be Urinary Blood (Haematuria or Haemoglobinuria)**

Acute pyelonephritis	Intercapillary glomerulosclerosis
Chronic pyelonephritis	Scleroderma of the kidney
Papillary necrosis	Systematic lupus erythematosus
Acute renal failure	Angioseratoma corpus diffusum
Chronic renal failure	Hypercalcaemic nephropathy
Nephrolithiasis	The kidney of gout
Eclampsia	Obstructive uropathy
Preeclampsia	Grawitz's tumour
Lipoid nephrosis	Multiple myeloma
Renal vein thrombosis	Sickle cell nephropathy
Polycystic kidney	Acute glomerulonephritis
Hereditary nephritis	Chronic glomerulonephritis
Amyloid kidney	Malignant nephrosclerosis
Chyluria	Renal arterial occlusion
Nephrosis	Kimmelstiel-Wilson Syndrome
Wilm's tumour	Franconi Syndrome
Radiation nephritis	Periarteritis

**Table 12**  
**Possible Causes of Haematuria**

Urinary tract related	Non-urinary tract related
Acute pyelitis	Acute fevers
Benign tumour	Appendicitis
Calculi	Arteriosclerosis
Cystitis	Carcinoma of uterus, vagina or rectum

Drugs or toxic substances	Diverticulosis of colon
Hydronephrosis	Endocarditis
Injury	Excessive exercise
Nephritis	Haemophilia
Malignant tumour	Leukemia
Renal artery aneurysm	Prostatic complications
Renal purpura	Salpingitis
Tuberculosis	Shistosomiasis
Urethritis	Scurvy
	Ulceration of the intestine

Table 13  
Clinical Utility of Tests for Occult Blood in Urine

**Screening**

- Health examinations
- Pregnancy
- Athletes
- School children
- Hospital admissions
- Initial diagnostic workup
- Physician office examinations
- Elderly patients
- Newborn babies
- Employment examinations
- Insurance examinations
- Initial screening for follow-up of exfoliative cytology study

**Diagnosis**

- Glomerulonephritis
- Glomerulosclerosis
- Nephrosis

Pyelonephritis  
Renal lithiasis  
Fanconi syndrome  
Eclampsia  
Haemolytic diseases  
Transfusion reaction  
Muscle diseases  
Diabetes mellitus  
Hypertension

**Monitoring**

Pregnancy  
Hypertension  
Renal disease  
Diabetes mellitus  
Eclampsia  
Lithiasis  
Kidney transplant

green to dark blue compound when Hb catalyzes the oxidation reaction of tetramethylbenzidine with a peroxide. Development of green spots indicate non-haemolysed (intact) erythrocytes. The colour of the strip is compared with a colour chart, 40 secs after the strip is dipped into the urine. The resulting colour ranges from orange through green to blue; indicating (trace, +, ++ and +++) respectively as the case may be. Myoglobin, if present in large enough concentrations, will give a positive reaction. Myoglobin is very rapidly excreted from plasma into the urine, probably because of its lower molecular weight compared with Hb (1/4 of Hb's Mol. wt). Myoglobinuria is the excretion of myoglobin into the urine from traumatic muscle injury, such as road traffic accidents or football tussles or struggles. The differentiation of haemoglobinuria and

myoglobinuria is not readily accomplished but can be achieved by immunochemical techniques.

#### **Procedure**

*Occultest:* Place one drop of mixed uncentrifuged urine on a special test paper which is provided. Place an Occultest tablet in the centre of the moist area of the paper. Allow one drop of water to flow on the tablet, wait for 5-10 seconds for the reaction to take place 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 is shown by an area of the test paper turning blue, or bluish green.

*Interference:* Large amounts of ascorbic acid (vitamin C) in the urine may inhibit or retard the reaction.

## **8**

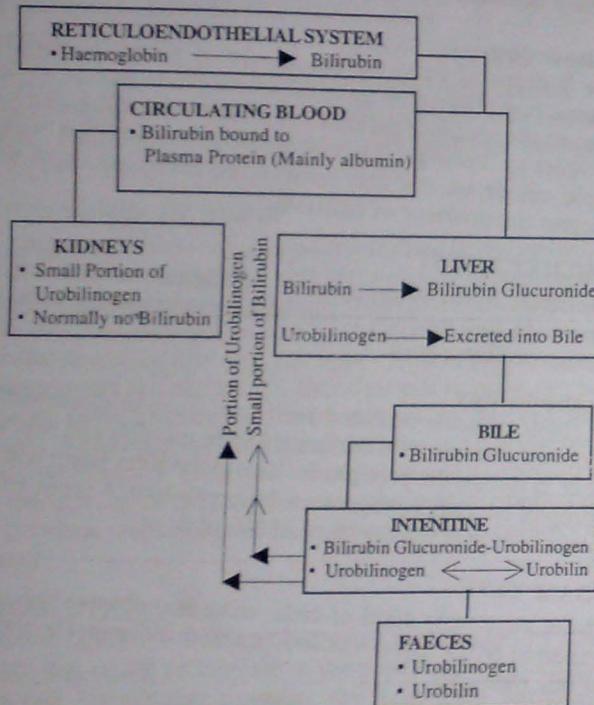
# **Urinalysis in Liver Disease and Hypatobiliary Disorders**

### **BILIRUBIN IN URINE**

Bilirubin is formed in the reticuloendothelial cells of the spleen and bone marrow from the breakdown of haemoglobin. A schematic diagram for normal bile pigment metabolism is shown in Figure 4 below.

In summary, bilirubin, a product of haemoglobin catabolism, is formed in the reticuloendothelial system, the spleen, and Kupffer's cells in the liver. It is conjugated primarily with glucuronic acid by the action of the enzyme glucuronyl transferase in the microsomes of the liver cells. The unconjugated bilirubin is lipid-soluble and water insoluble. Conjugation with glucuronic acid converts the unconjugated ("Primary", "indirect"), water-insoluble bilirubin to conjugated ("secondary", "direct") water-soluble bilirubin. Conjugated bilirubin, being water soluble, can be excreted by the kidneys, although normally its level in the blood is not sufficiently high to cause significant amounts to appear in the urine. If this normal pathway of bilirubin metabolism is interfered with, excess quantities of bilirubin may appear in the blood. The presence of bilirubin in urine indicates the presence of hepatocellular disease or intra- or extra-hepatic biliary obstruction. It is an early indicator of the presence of these disorders and therefore, a very useful diagnostic tool in liver dysfunction.

**Figure 4**  
**Schematic Interrelations of normal bile Pigment Metabolism**



Bilirubin present in urine is approximately 0.02mg/dl, reflecting the normally low levels of conjugated bilirubin. This amount is not detectable by routine semi quantitative techniques in urinalysis, and is therefore interpreted as a negative result.

#### CLINICAL UTILITY

Bilirubin excretion in the urine will reach significant levels in any disease process that increases the amount of conjugated bilirubin in the blood stream. In many liver diseases due to infections or hepatotoxic agents, liver cells are unable to secrete all of the conjugated bilirubin in the bile. Resultantly, large amounts are returned to the blood to elevate blood levels of conjugated bilirubin and cause significant bilirubinuria. In effect, hyperbilirubinemia encountered in intra- and extrahepatic obstructive jaundice, hepatocellular jaundice, acute and chronic hepatitis, and hepatocirrhosis, as well as in the icteric stages of the Dubin-Johnson and Rotor syndromes causes bilirubinuria.

In obstructive biliary tract disease, biliary stasis interferes with the normal excretion of conjugated bilirubin via the intestinal tract, thus causing a build-up in the bloodstream with resulting bilirubinuria. Since bilirubin may often appear in the urine before other signs of liver dysfunction (jaundice; clinical manifestation of increased bilirubin in blood) are apparent, bilirubinuria is an important diagnostic sign of liver disease and testing for bilirubin should be a part of common routine urinalysis.

A rare increase in unconjugated bilirubin occurs in haemolytic anaemias because the greater release of haemoglobin leads to greater production of albumin bound bilirubin. However, a normal, non-diseased liver can conjugate all the excess bilirubin and secrete the entire amount into the

biliary tract. Normally, bilirubinuria will be absent in such conditions.

#### **DIFFERENTIAL DIAGNOSIS**

- (i) Hepatocellular disease;
- (ii) Cholestasis

#### **FURTHER BIOCHEMICAL TESTS**

Liver function tests –

Urine urobilinogen

Serum total bilirubin

Serum conjugated bilirubin

AST activity

ALT activity

Alkaline phosphatase activity

GGT activity

Serum total protein

Serum protein electrophoresis

Prothrombin time determination

#### **Methods**

Many methods are available for detection of bilirubin in urine.

The principles involved include:

- (1) dye dilution procedures involving the blending of the yellow bilirubin colour with a dye such as methylene blue or methylviolet.
- (2) Oxidation of bilirubin to give coloured derivatives – most often green biliverdin
- (3) Diazotisation procedures in which bilirubin is coupled to a reagent nitrous oxide to produce a bright coloured compound.
- (4) Simple foam test – where a yellow-tinted froth appears when the urine is vigorously shaken.

#### **ICTOTEST TEST**

**Principle:** Ictotest tablet contains a stable diazonium salt which can couple with bilirubin on a mat moistened with urine to give a characteristic purple colour.

#### **Methodology:**

Place 3 drops of urine on the centre of a special asbestos cellulose mat referred to as 'Ictotest Mat'. Then put an Ictotest tablet on the centre of the mat and dissolve a small quantity of the tablet by flowing 2 drops of water on the tablet. A bluish-purple colour on the mat around the tablet after 30 secs indicates the presence of bilirubin.

#### **FOUCHET'S TEST**

**Principle:** Bilirubin precipitated by addition of 10% barium chloride in acid medium reacts with Fouchet's reagent to give a green or blue colour.

#### **Methodology**

To 10mls of urine acidified with few drops of dilute sulfuric acid, add 5mls of 10% barium chloride solution and mix well. Filter to isolate the precipitate. Unfold the filter paper and add a drop of Fouchet's reagent on the precipitate. A green or blue colour indicates the presence of bilirubin.

#### **FOAM TEST**

Shake vigorously 10ml of clear urine and observe the colour imparted on the foam. A yellow-tint froth indicates the presence of bile pigments.

#### **DIP-Stick Strips**

Commercially available dip-and-read test strips for bilirubin in urine include Ictostix, Bili-Labstix and Multistix reagent strips. The principle involved is based on the coupling of bilirubin with

a stable diazonium compound to give a brown colour.

To perform the test, the reagent strip is dipped into the urine specimen, and the bilirubin portion is compared to the Maker's colour chart at 20 secs. Although the dip-and-read test is less sensitive than Ictotest, comparative results agree well and it is much more convenient for routine urinalysis.

### **Precautions**

Fresh urine must be employed with bilirubin tests because on standing there is hydrolysis of the glucuronide with loss of reactivity. Oxidation of bilirubin may also occur on standing, and the resulting biliverdin is non-reactive.

### **UROBILINOPEN IN URINE**

Urobilinogen is a colourless compound formed in the intestine from bilirubin as a result of dehydrogenated reduction by bacteria in the intestines. It is partially reabsorbed into the blood stream. It is then excreted, in part by the kidney into urine and to a major degree by the liver. The upper limit of physiological urobilinogenuria is 1mg/100ml. Urobilinogen in urine can be measured quite readily and information so derived are important in assessing the function of the liver and bile pigment metabolism. Urinary excretion of urobilinogen is increased when the capacity of the liver for enterohepatic circulation of bile pigments is restricted or overburdened, or when the liver is by-passed.

### **CLINICAL UTILITY**

**Increased Levels:** Urinary urobilinogen is increased by any condition that causes an increase in the production of bilirubin and by any disease that prevents the liver from normally removing the reabsorbed urobilinogen from the portal circulation. Urinary urobilinogen is increased whenever there is an excessive destruction of red blood cells as in haemolytic

anaemias (including SCD), pernicious anaemia, and malaria. It may be the first biochemical constituent to show an increase. Also in infectious hepatitis, toxic hepatitis, portal cirrhosis, congestive heart failure (due to impaired circulation to the liver) and infectious mononucleosis the excretion of urobilinogen is increased. Increased excretion of urobilinogen in the urine is thus a finding that points in two directions:

#### **1. Hepatic dysfunction**

- (a) due to primary hepatopathy or
- (b) secondary to hepatic involvement in other diseases

#### **2. Increased haemoglobin catabolism**

- (a) due to primary haemolytic disease, or
- (b) secondary to other diseases.

Determination of urinary urobilinogen is a useful procedure in routine urinalysis because it serves as a guide in detecting and differentiating between liver disease, haemolytic disease and biliary obstruction.

Serial determinations of urinary urobilinogen in patients undergoing treatment also assist in evaluating progress of the disease and response to management.

**Decreased Levels:** Urobilinogen is absent from the urine when the liver fails to produce bile, biliary flow is impeded, or enteric reduction of bilirubin is arrested. Urinary urobilinogen is decreased or absent when normal amounts of bilirubin are not excreted into the intestinal tract. This usually indicates partial or complete obstruction of the bile ducts such as may occur in cholelithiasis, gallstone, severe inflammatory disease, or neoplastic disease (cancer of the head of the pancreas for instance). Also during antibiotic therapy, suppression of normal intestinal flora may prevent the conversion of bilirubin to urobilinogen, leading to the absence of urobilinogen in urine.

More clinically useful information can be obtained when test results for urinary bilirubin and those of urobilinogen are correlated. As indicated in the table below, the two findings, considered together, provide more helpful information for differential diagnosis than either finding alone.

**Table 14**  
**Urinary Urobilinogen and Bilirubin Levels in health and disease**

Parameters	In Health	In Haemolytic Disease	In Hepatic Disease	In Biliary Obstruction
Urine Urobilinogen	Normal	Increased	Increased	Low or absent
Urine Bilirubin	Negative	Negative	Positive or Negative	Positive

#### FURTHER BIOCHEMICAL TESTS

As for bilirubin above.

#### METHODS

The common method for the determination of urobilinogen is based on the principle that acidified paradimethyl aminobenzaldehyde reacts with urobilinogen and structurally related compounds like porphobilinogen and paraminosalicylic acid to form coloured compounds.

**Notes:** A freshly voided specimen of urine, preferably a sample collected over a 2-hour period in the early afternoon when urobilinogen excretion is thought to be at the highest rate for the day is necessary for a good result. Reagent strips will not accurately detect a decrease or absence of urobilinogen. There

are no substances known to clearly inhibit the reaction, but strongly alkaline urines show highest urine urobilinogen values and more acid urines show lower urobilinogen levels. Additionally, drugs containing a mixture of azo dyes will have a masking effect on colour development.

#### THE QUALITATIVE EHRLICH'S TEST

Prior to the introduction of the semi-quantitative reagent strips, Ehrlich's test was the routine procedure for determining urinary urobilinogen.

#### PROCEDURE

- (1) Place 10ml urine in a test tube. Allow to warm to room temperature.
- (2) Add 1ml Ehrlich's reagent, and
- (3) Allow to stand for 3 to 5 min.
- (4) Normal amounts of urobilinogen present in the urine sample will change the solution to pink colour, observable when viewed from top of the test against a white background placed beneath the bottom of the test tube.

Abnormally high amounts of urobilinogen will change the solution to a clearly discernible cherry red colour.

The composition of Ehrlich's reagent is as follows:

- Paradimethylaminobenzaldehyde - 10g
- Concentrated HCl ..... 75ml
- Water ..... 75ml

#### QUALITATIVE SCHLESSINGER'S TEST

This is particularly suitable for detection of urobin, the oxidised form of urobilinogen:

- (1) Place 10ml urine in a test tube.

- (2) Add 10ml of a saturated alcoholic solution of zinc acetate (10g zinc acetate suspended in 100ml ethanol).
- (3) Mix and filter.
- (4) Separate filtrate equally into 2 test tubes.
- (5) Add 1 drop of concentrated HCl to one.
- (6) Add 2 drops of Lugol's solution (5g iodine, 10g potassium iodide dissolved in 100ml water) to each tube. Mix by inversion.
- (7) View tubes for fluorescence with a Wood's (uv) light. Urobilinogen will produce a green-yellow fluorescence in the unacidified tube alone. Porphyrins produce red fluorescence.

Quantitative urobilinogen determinations are complex chemical procedures requiring the use of a colourimeter or spectrophotometer. For most routine chemical and diagnostic purposes, the semi quantitative test with the reagent strips described above is adequate. The 2-hour test for urobilinogen by Watson may be undertaken in special cases.

## 9

## Urinalysis in In-born Errors of Metabolism and Lead Poisoning: Phenylpyruvic Acid and Porphyrins in Urine

Various conditions with a genetic defect have been classified as inborn errors of metabolism. Each of these appears to be due to the absence of a specific enzyme which blocks the normal metabolism of intermediary compounds.

**Table 15**  
**Inborn Errors of Metabolism and Enzyme defects**

Disease	Deficient enzyme
1. Alkaptonuria	Homogentisate Oxygenase
2. Argininosuccinic aciduria	Argininosuccinase
3. Citrullinuria	Arginino succinic acid synthetase
4. Cystathioninuria	Cystathione cleavage enzyme
5. Galactosemia	Galactose-1-phosphate uridyl transferase
6. Gaucher	Beta-glucosidase
7. G-6-PD deficiency	Glucose-6-phosphate dehydrogenase
8. Glycogen storage disease	Glucosidase
9. Hartnup's disease	Tryptophan pyrolase
10. Histidinemia	Histidine alpha-deaminase

11. Homocystinuria	Cystathione synthetase
12. Ketotic hyperglycinemia	Propionyl CoA Carboxylase (Propionicacidemia)
13. Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyl transferase
14. Maple syrup urine disease	Ketoacid decarboxylase
15. Methylmalonic aciduria	Methylmalonyl CoA isomerase
16. Mucopolysaccharidoses	Beta-galactosidase
17. Niemann-Pick	Sphingomyelinase
18. Orotic aciduria	Orotidyl Pyrophosphorylase
19. Phenyl ketonuria (PKU)	Phenylalanine hydroxylase
20. Tay-Sachs	Hexosaminidase
21. Xanthinuria	Xanthine oxidase

For example, phenylketonuria or PKU derives its name from the presence of phenylketones in urine, and this is one of the most studied conditions of Inborn Error of Metabolism in Urinalysis.

**Clinical Application:** The disease known as phenylketonuria, (PKU), is due to hereditary deficiency of the enzyme phenylalanine hydroxylase, required for the conversion of phenylalanine to tyrosine in normal amino acid metabolism in man. Consequently, the phenylalanine ingested in milk and other foods accumulates in the tissues and blood. By the age of four weeks and frequently much earlier, intermediate metabolites of phenylalanine, particularly phenylpyruvic acid, start appearing in the infant urine. If untreated, PKU results in brain damage and severe mental retardation. However, when detected early and treated with a diet low in phenylalanine, the prognosis is good for normal mental development. Therefore testing the blood or urine of infants for the presence of phenylketones frequently during early life is one of the most pragmatic ways of detecting phenylketonuria.

## METHODS

Screening methods used for detecting PKU are based on the reaction between phenylpyruvic acid and ferric ion to yield a specific blue-grey-green colour. The intensity of this colour is proportional to the amount of phenylpyruvic acid in the urine specimen. The proprietary reagent strip is a commercial strip based on this principle, and this is called Phenistix.

The Phenistix reagent strips are impregnated with a solution containing ferric ions and are usually buffered to prevent interference from phosphates.

## PROCEDURE

The strip is either dipped into fresh sample of urine or pressed against a wet diaper. After exactly 30 secs, it is compared with a colour chart ranging in concentrations from 0, to 100 mg phenylpyruvic acid per 100ml urine. The Maker's Colour scale is graduated as 0, negative; 15, 40 and 100mg/100ml urine respectively.

## INTERFERENCE

The Phenistix strip test must be performed on a fresh urine specimen. For example, alkaline specimens, which are obtained when bacterial decomposition occurs in urine on standing, react with the ferric ions to produce ferric hydroxide, giving an interfering orange colour.

Phenistix strip turns pink to purple colour in urine containing high concentrations of salicylate, or phenothiazine metabolites. Similarly, high concentrations of bilirubin or ammonia in the urine may also alter the normal colour reaction developed with phenylpyruvic acid. These interferences have to be excluded to avoid experimental errors due to false positives. Also the presence of phosphate may interfere with ferric ions leading to false negative results.

### **WET REACTION FOR PKU**

As already indicated above, the ferric ion test forms colour reactions that can be used to detect PKU. The wet (or test tube) reaction is as outlined in the procedure below:

- (1) Add 2 drops of dilute HCl to 5ml urine to acidify the urine.
- (2) Add 2 drops of 10% of ferric chloride solution and record colour change.

**Result:** A dark green transient colour that fades to yellow indicates presence of phenylpyruvic acid. A red to purple colour occurs with salicylates, acetylpenditines, phenol, and phenothiazine metabolites. The presence of phosphate ions in the urine may cause a false negative result and therefore the process must ensure complete elimination of the effect of urinary phosphate.

### **ELIMINATION OF PHOSPHATE INTERFERENCE**

Phosphate may be removed from the urine before the ferric chloride reaction step as shown below:

- (1) 1ml of magnesium reagent prepared from  $MgCl_2$  (11g),  $NH_4Cl$  (14g) and concentrated  $NH_4OH$  (20ml) diluted to a total volume of 1 litre with water.
- (2) Add to 4.0ml urine and mix.
- (3) Allow to stand for 5 minutes and filter.
- (4) Then carry out the  $FeCl_3$  test as above.

## **10 | Porphyrins and Other Related Compounds**

Porphyrins are cyclic tetrapyrrole compounds which are formed from an intermediate in haemoglobin synthesis. They exist as different isomers, and a few easily identifiable specific porphyrins include coproporphyrin and uroporphyrin. Porphobilinogen is a basic condensation product of two molecules of delta-aminolevulinic acid, while porphyrinogens are fully hydrogenated, colourless tetrapyrrol methanes which contain six more hydrogen atoms than the porphyrins. These are precursors of haem and the body uses some of these to synthesise the respiratory enzymes and cytochromes. Porphyrinuria is defined as the presence of an elevated quantity of porphyrin in the urine. Most of the porphyrias (inborn error of porphyrin metabolism) manifest as porphyrinuria, but porphyrinuria may also be due to other causes (e.g. lead poisoning). These compounds are pigments or precursors of pigments and their presence turns urine to a pink-red colour. The urine of patients with porphyria is usually deep red in colour, but may vary from pale pink to almost dark red. Some patients excrete urine of normal colour which subsequently turns dark after exposure to light.

### **REFERENCE VALUE**

Coproporphyrins are normally excreted in the urine in amounts ranging from 70 to 250  $\mu g/1$  per day; porphobilinogen

excretion does not exceed 2 mg per day, and delta-amino levulinic acid excretion is between 1.0 and 7.0 mg per day.

### **CLINICAL APPLICATIONS**

The porphyrias are a group of rare disorders caused by enzyme defects in the haem-biosynthetic pathway. Although, most of the porphyrias are inherited, porphyria can be induced by chemical insult in normal individuals. In effect, porphyrias may primarily relate to defects in the liver or in the bones where excessive production of porphyrins leads to increased urinary and faecal excretion of porphyrin. In acute intermittent porphyria, the liver produces an excessive amount of porphyrin precursors, primarily porphobilinogen and delta-aminolevulinic acid (d-ALA) which will be excreted in large amounts in the urine. Clinical symptoms include intermittent severe abdominal pain and neurologic manifestations such as peripheral neuropathy, bulbar symptoms, psychotic alterations in personality, and involvement of the autonomic nervous system. The disease is usually first seen after puberty.

The urine from such patients may darken on standing due to conversion of porphobilinogen to porphobilin and uroporphyrin. Acute exacerbations may be precipitated by alcohol, barbiturates and hepatotoxins.

Porphyria cutanea tarda symptomatica is caused by defects in porphyrin metabolism in the liver. It is characterised by attacks of acute abdominal colic, cutaneous lesions, liver dysfunction and hyper pigmentation of the skin beginning between the ages of 10 and 30 years. Excretion of porphobilinogen and delta-aminolevulinic acid in the urine of these patients is increased during times of acute exacerbations.

Also coproporphyrin and uroporphyrin excretion in the urine is increased during acute attacks and coproporphyrin and protoporphyrin excretion, in faeces is elevated at all times during

acute attacks.

In diseases involving erythrocyte metabolism, excessive amounts of porphyrins are synthesised in erythrocytes in the bone marrow.

In congenital erythropoietic porphyria, a disease that becomes manifest in infancy with the onset of sensitivity to sunlight, formation of large bullous lesions over the exposed skin areas, and increased haemolysis and erythropoiesis, the colour of urine varies from pink to red due to increased amounts of uroporphyrin and coproporphyrin in urine.

### **AQUIRED DISORDERS OF PORPHYRIN METABOLISM: LIVER DISEASE AND LEAD POISONING**

Acquired porphyria cutanea tarda symptomatica occurs in patients with disorders of liver metabolism, such as alcoholic and nutritional liver cirrhosis, exposure to certain hepatotoxic chemical agents and liver malignancies. Clinical manifestations (viz, sensitivity to light, bullous lesion in exposed skin area, increased haemolysis and erythropoiesis) are the same as found in congenital porphyrias, and increased urinary excretion of uroporphyrin and coproporphyrin also occurs.

Elevated coproporphyrin excretion in the urine also occurs in many diseases: infections including certain malignancies, alcoholic cirrhosis, infectious hepatitis and obstructive jaundice.

### **LEAD POISONING**

Lead poisoning has no pathognomonic signs by which it can be recognised clinically. It is likely to increase with increasing level of industrialisation in different communities. A simple screening test for lead poisoning is useful. In lead poisoning, coproporphyrin III excretion in the urine is markedly elevated beyond the levels seen in other diseases enumerated above.

Coproporphyrin excretion may rise from a normal level of 250 mg/day to as high as 40 times the upper normal concentration (10,000 mg) (10g/day). Measurement of coproporphyrin III levels is especially important for the diagnosis and daily clinical management of patients with lead poisoning. This is however only a screening procedure. (See Table 16 for overall clinical utility.)

**Table 16**  
**Clinical Utility of Porphyrin Tests**

#### General Usage

Routine urinalysis of children for lead poisoning.  
Part of hospital admission urine studies of undiagnosed mental cases.  
Routine test in undefined disoriented states.

#### Diagnosis

Lead intoxication  
All porphyrias  
Severe liver cirrhosis.

#### Monitoring

Treatment of lead intoxication  
Treatment of intermittent porphyrias

#### Research

Identification of drug sensitivities.

Widespread screening tests available for porphobilinogen and coproporphyrin are the Watson-Schwartz test and coproporphyrin test to detect lead poisoning respectively. These tests are suitable as screening tests for most diagnostic purposes and are best performed on early morning specimens.

#### (A) Porphobilinogen-Watson-Schwartz Test

- (1) Place 2.5ml fresh urine in a test tube.
- (2) Add 2.5ml Ehrlich reagent and mix.
- (3) Add 5ml saturated sodium acetate (1 kg sodium acetate dissolved in 1 litre of water at 69°C) and mix well.
- (4) A pink-red colour appearing at this time indicate porphobilinogen or other Ehrlich reacting substances such as urobilinogen.
- (5) Add 5ml chloroform, shake, and either centrifuge or allow to stand and settle out.
- (6) The chloroform layer goes to the bottom, carrying with it urobilinogen and other Ehrlich reacting substances.
- (7) If porphobilinogen is present, the upper layer will turn a deep red or red-purple colour.

#### REAGENTS

- |                                   |       |
|-----------------------------------|-------|
| (1) Paradimethylaminobenzaldehyde | 3.5g  |
| (2) Concentrated HCl              | 750ml |
| (3) Distilled water               | 500ml |

#### (B) Coproporphyrin Test to Detect Lead Poisoning

- (1) Place 5ml urine in a test tube.
- (2) Add 1.0ml glacial acetic acid.
- (3) Then add 5ml diethyl ether.
- (4) Add 3 drops of fresh 3% hydrogen peroxide.
- (5) Stopper test tube, and mix by inversion 8 to 12 times.
- (6) Allow to stand for 10 to 15 minutes until the two layers separate.
- (7) Examine tube in a darkened room with ultraviolet reflected light (Wood's light) to detect fluorescence in the top most, ether layer.

Pale blue fluorescence signified negative or normal amounts of coproporphyrins in urine. Fluorescence ranging in colour

from violet and pink to light rose and then dark rose indicates successively, a 1+ through 4+ concentration of coproporphyrin.

**Table 17**  
**Maker's Colour Chart for Quantitation of Urinary Coproporphyrin**

Observed Colour	Quantity
Violet	+
Pink	2+
Light Rose	3+
Dark Rose	4+

Sem-quantitation of coproporphyrin in urine reflects the severity of lead poisoning.

#### URINALYSIS IN DRUG SCREENING

Urine can contribute important information relative to drug use and abuse. Salicylate poisoning used to be one of the most frequent intoxications either from over dosage or intentional over dosage from suicide attempts.

Salicylate is the active compound in aspirin, one of the most widely used drugs either alone or in combination with other drugs. When aspirin (acetyl salicylic acid) is absorbed, it is metabolized (essentially by hydrolysis) to free salicylate which becomes elevated in blood and consequently excreted in urine. Detection of salicylate in urine is readily achieved by using the ferric ion ( $\text{FeCl}_3$ ) test. This can also be used in the strip form 'Phenistix' which is more popularly used for detection of PKU. Phenistix will react with urine containing salicylate to give a reddish brown to purple colour. When a positive result is obtained it is important to exclude acetoacetic acid or pyruvic acid from PKU patients.

Salicylate intoxications may be ameliorated by alkaline diuresis. When the kidney excretes alkaline urine the rate of salicylate excretion is a lot greater than when the urine is acidic. This is the basis of urine alkalinisation in the management of salicylate intoxication.

#### THE DRUG ABUSE PHENOMENON

Drug abuse, similar to the problems of the AIDS syndrome, is one of the greatest calamity that has afflicted contemporary mankind. This is particularly noticeable in the youth who responds to a stressful and ostensibly uncaring society by recourse to drugs. The youth should be admonished by all concerned – parents, religious groups, teachers and advocacy groups – that recourse to drugs is a wrong solution to adverse situations.

Urine examination contributes significantly to the recognition, management and monitoring of the drug abuse syndrome. Urine examination may provide information regarding recent ingestion of a number of drugs. Metabolites of heroin or morphenol for instance are readily detected in urine by means of thin layer chromatography (TLC) or the commercial system known as the Toxilab System, based on the same principle.

Barbiturates and amphetamine metabolites are excreted in the urine and urinalysis may be useful in determining whether or not these drugs have been ingested. There is a considerable number of drugs which may be subject to abuse which do not yield recognisable metabolites readily detectable by routine urinalysis in urine. These may be detectable by other more sensitive methods such as high performance liquid chromatography (HPLC) or immuno assay techniques. These may also become more easily detectable by application of recent advances in analytical techniques. In the past it was widely believed that use of marijuana and ingestion of lysergic diethylamide (LSD),

a drug of such great potency that only microgram amounts may elicit tremendous psychotic effects belongs to these groups, were thought not to be easily detectable by existing procedures.

Recent advances in biomedical procedures have debunked such claims as the urine is now more widely used to provide information relating to drug abuse than assessing drug derivatives or metabolites in serum.

### **ALCOHOL**

Alcohol though not often regarded as a drug, but a social toxicant has wrecked havoc in families and the society at large. It leads to behavioural disorders and wrong judgments that may lead to accident with far reaching implications such as loss of life and morbidity. Alcohol can be easily detected in urine. Its detection in urine is an indicator (a biomarker) of alcohol ingestion. In chronic cases when hepatic decompensation has set in, excessive alcohol ingestion leads to cirrhosis of the liver (alcoholic liver cirrhosis) which impairs the enterohepatic circulation and leads to excretion of excessive amounts of urobilinogen in urine. Usually, only very small amounts of urobilinogen are found in urine of normal people. Thus in a patient with ascites with current or a past history of persistent alcohol ingestion, a raised urobilinogen level in urine may be a useful pointer to support the diagnosis of alcoholic liver cirrhosis in such subjects.

### **FURTHER BIOCHEMICAL TESTS**

These may include assay of:

- plasma gamma glutamyl transferase determination (often raised but not completely specific for this condition).
- plasma uric acid concentration (increased).
- plasma mean corpuscular volume (MCV) of

erythrocytes (increased).

- plasma albumin concentration (decreased).
- serum protein electrophoresis with characteristic beta (b)- gamma fusion.
- different isoforms of carbohydrate deficient transferrins (CDT).
- (highly sensitive and specific assay for alcohol ingestion.)

### **Other drugs**

Morphine, methadone, chlorpromazine, codeine, quinine, amphetamines, barbiturates and glutethimide are other recognisable drugs that may be revealed by urinalysis, applying the methods indicated above.

## 11 Urinalysis in Nutritional Disorders

### NUTRITIONAL DRUGS

There are a number of substances which fulfill both nutritional and drug roles. The most common ones include vitamin C, vitamin B12, Folic acid and vitamin B6 or pyridoxines as well as a number of others. These drugs or nutrients or their metabolites can be studied in urine and their levels provide useful information in therapeutic monitoring or the recognition of deficiency states.

Ascorbic acid is one of the essential vitamins for human nutrition and one of the most abundant water soluble anti-oxidants. It regulates the levels of other potent anti-oxidants such as the master anti-oxidant  $\alpha$ -tocopherol (vitamin E). It has protective properties on other anti-oxidants, like beta-carotene and vitamin A, by preventing oxidation of their polyunsaturated bonds. It also plays a role in the metabolic synthesis of collagen through its involvement in the hydroxylation of proline to hydroxyproline and lysine to hydroxyl lysine. It also plays a role in the activation of tetrahydrofolate through its effect on the activity of folate reductase, a major enzyme in purine synthesis. Thus, this drug or nutrient which was earlier recognised for its role in scurvy plays a very important role in health and metabolism.

The status of this important drug can at least be screened by

urinalysis. The ascorbate saturation test is particularly well known. It is briefly described as follows:

Ascorbic acid will normally disappear from the urine after 4 or 5 days on diet without ascorbate (ascorbic acid free diet). Recognition of either increases or decreases in urinary ascorbic acid concentrations have useful diagnostic and therapeutic significance. A raised level may suggest favourable therapeutic outcome in a hitherto ascorbic acid deficient subject, while decreased concentrations suggest the ingestion of a nutritionally deficient diet. It should be noted that many diets inadequate in vitamin C content have other dietary inadequacies. This may lead to oxidative stress. C-stix (a strip marketed by Ames) is a dip-read strip for detection of ascorbic acid, and the principle is based on the colour reaction of molybdate with ascorbic acid. The 2, 6-dichlorophenolin-dophenol indicator is also useful in wet reactions.

**Caution:** Ascorbic acid is a potent reducing agent and as such usually employed as a preservative in several parenteral solutions. This may be an unanticipated source of urinary ascorbic acid that may lead to spurious results.

### ASCORBIC ACID SATURATION TEST

The ascorbic acid saturation test is fairly satisfactory as an index of the state of tissue saturation and it is superior to measurement of plasma or random urine ascorbate concentrations. This test is based on the assumption that the administered ascorbic acid will be taken up by the tissues. When these tissues are saturated and the plasma level of ascorbic acid reaches about 1.6mg/100ml, tubular reabsorption of ascorbic acid is inhibited and there will be a sharp rise in its urinary excretion. Ascorbic acid can be given intravenously (which eliminates absorption problems) in a single dose of 500mg, after which 200mg should be excreted in the urine in

the next two hours. The common practice, however, is that ascorbic acid is given orally. The test is simple to carry out and ascorbic acid in therapeutically useful amounts is administered during the conduct of the test. The procedure of Harris and Abbas (1937) is commonly adopted.

#### **PRINCIPLE OF REACTION**

Ascorbic acid reduces the blue dye 2, 6-dichlorophenolin-dophenol to a colourless substance. Ascorbic acid in urine can therefore be measured by titrating directly into a known amount of dye solution to the colourless end point.

#### **METHYLMALONIC ACID (MMA)**

Vitamin B12 (cyanocobalamin) is an important haematinic which is also involved in the processing of folate coenzymes. Thus B12 deficiency results in a megaloblastic anaemia in a similar manner to that seen in folate deficiency.

Deficiency of vitamin B12 can also present with neurobiological symptoms which may be irreversible if the deficiency is protracted. The pathophysiology of the neuropathy is not completely understood, it is however known that it may be linked to a decreased availability of methionine ( $\text{CH}_3\text{-S}(\text{CH}_2)_2\text{CH-COOH}$ ) an important methyl donor, from which some choline ( $\text{CH}_3\text{-NCH}_2\text{COOH}$ ) (needed for the synthesis of sphingomyelin and other phospholipids and other lipids used in myelin synthesis) is formed, or to accumulation of the toxic metabolite – homocysteine.

Evidence abound that elevated serum or urine methyl malonic acid (MMA) levels may be a more definitive indicator of early cobalamin (B12) deficiency. Methyl malonic acid is an intermediate which is broken down by the enzyme methyl malonyl CoA mutase requiring the presence of B12 as a coenzyme. In malonyl CoA mutase deficiency MMA accumulates in plasma.

An increase in serum Methylmalonic acid rather than that of cobalamin ,reflects decreased tissue concentration of cobalamine and this is an early indicator of B12 deficiency. Cobalamin dependent neurologic disease with normal haematological indices and B12 levels may be associated with significant elevation of serum and urine MMA. Gas chromatography – Mass spectrometry (GC-MS) methodology for MMA determinations is preferred (though not yet in wide spread use). To avoid dietary influence, assessment of serum level of MMA is preferred to that of MMA in urine in non-fasting patients. But in fasting patients urinalysis can provide valuable information about B12 metabolism.

#### **URINE AND PYRIDOXINE ASSESSMENT**

Pyridoxine (vitamin B6) is a very important vitamin. It has to be converted to the metabolically active form, pyridoxal phosphate (PLP) for metabolic transformation processes. Pyridoxal phosphate is biologically critical in amino acid and protein metabolic pathways (e.g. transamination and decarboxylation reactions are dependent upon B6 enzymes). Also, both forms of Glycogen phosphorylase require B6 enzymes and deltaaminolevulinic acid synthetase for normal activities. Pyridoxal phosphate is required for DNA synthesis through its role in one-carbon metabolism. Pyridoxine deficiency results in a decreased PLP concentration, which is required for catabolism of tryptophan . In B6 deficiency, an intermediate of the catabolic pathway 3-hydroxy kyneurenine and its precursors accumulate in the tissues or are excreted in greater quantity in urine. The excretion rate of a further intermediate, xanthurenic acid is measured in urine usually after a standard dose of tryptophan (the tryptophan-loading test). Urinalysis can provide valuable information about this nutrient loading.

### **URINALYSIS IN FOLATE DEFICIENCY**

Folate and its derivatives are involved in the transfer of single carbon units in mammalian metabolism. These transfers are important for DNA and RNA synthesis and amino acid metabolism. Measurement of folate is important for the evaluation of anaemias particularly in the differential diagnosis of megaloblastic anaemia and for the assessment of the remethylation of homocysteine to methionine ( $\text{HS}-(\text{CH}_2)_2\text{CH}_1-\text{NH}_2\text{CH}_3-\text{S}-(\text{CH}_2)_2-\text{CH}_1\text{NH}_2$ ). Poorer memory has been found to be more strongly associated with low folate level combined with high level of homocysteine. Hyperhomocysteinemia is suggested to contribute to clogged arteries, including arteries that lead to the brain. Many studies have confirmed that low folate and high homocysteine are linked to memory impairment because folate is crucial in the synthesis of neurotransmitters.

The active form of folic acid, tetrahydrofolate TH4 (THF) is required for the dissimilation of an intermediate in the catabolism of the amino acid, histidine; formi mino glutamic acid (FIGLU). In folate deficiency THF is unavailable and therefore FIGLU accumulates. It is excreted in urine in large amounts in folate deficiency. This was a very useful index for screening for folate deficiency in the past. Currently tests of folate status involving measurement of the urinary excretion of FIGLU following histidine load to enhance sensitivity of the test are now obsolete in industrialised countries but are still in use in poor communities. Urinalysis plays an important role in screening for deficiency of this important nutrient.

### **URINALYSIS: OTHER METABOLITES**

Tryptophan is an amino acid which requires B6 for its normal metabolism to form a precursor for the synthesis of thiamine (vitamin B<sub>1</sub>). Tryptophan supplements are used therapeutically to manipulate the level of serotonin which is formed from

tryptophan after decarboxylation in the presence of PLP (metabolic form of B6). Serotonin is a neurotransmitter in the brain that influences sleep and mood. Tryptophan supplements are being used clinically as antidepressants with effects comparable to those of the commonly prescribed drugs; imipramine and amitriptyline but without unwanted side effects of these well-known drugs.

As indicated above tryptophan can be decarboxylated to form tryptamine or first hydroxylated and then carboxylated to form 5-hydroxytryptamine (5-HT or serotonin), a local hormone. Serotonin or its derivative 5-hydroxyindo acetic acid (5-HIAA), is excreted in large quantities by subjects suffering from carcinoid syndrome, a tumour of the argentaffin cells of the intestinal mucosa. Estimation of these substances in urine (as surrogate tumour markers) is of great aid in the diagnosis of the disease.

## 12 A Quick Peep into Some Important Tips and Facts About Urine and More Facts

	I	II	III	IV
CRENAE	E	N	P	
URINE	N	O	A	
URINE	D	R	C	
URINE		E	F	
URINE			G	

- Urine** –
- Is the principal means of waste product excretion
  - Is the main route of water excretion
  - Is responsible for the excretion of more solids than any other route
  - Is not a standard fluid
  - Is a unique fluid
  - Study has a long historical heritage
  - Is a great source of information
  - Is extensively studied in almost all parts of the world
  - Provides information about many body processes
  - Tells about disease
  - Available in every part of the patient journey
  - Provides some information about disease that is not determinable by any other means

- May tell you something that will happen in future
- Formation involves glomerular filtration, and selective reabsorption in the tubules
- Is clear
- Is the regulatory mechanism for maintaining water and electrolytes
- Study is a major component of laboratory practice
- Collection is done by using clean containers
- Specimen must be properly identified
- Can be tested anywhere, laboratory, ward, office, house, etc.
- Testers frequently don't have much status
- Composition varies
- Volume may be increased in diabetes mellitus
- Volume may be decreased in diarrhea or vomiting
- Protein may be measured by either colorimetric or precipitation methods
- Sugar tests are useful in screening, diagnosing and monitoring diabetes mellitus
- May contain many nonglucose reducing substances
- Ketone levels become very high before serum levels are detectable.
- Which contains bilirubin may not always be dark in colour
- Bilirubin and urine urobilinogen together allow the differentiation of jaundice
- Chloride usually reflects dietary intake
- Sediment examination with the microscope is the only routine test to detect casts
- Is the substance most frequently used for analysis for drugs of abuse
- Always use fresh urine specimens for analysis
- Anuria, polyuria and oliguria relate to our daily urine

- volumes
- A clean urine container does not contain cleaning compound residues
- Ascorbic acid in very large quantities may interfere with both kinds of urine sugar tests
- As urine volume increases, specific gravity decreases
- N - Normally there is no detectable glucose in urine
- N - Nephrons as units do what the kidney does as a whole
- N - Normally there is no detectable bilirubin in urine
- N - Normally there is a small amount of urobilinogen in urine
- N - Negative test results on urine specimens have great value
- D - Detectable ketonuria may occur without glucosuria
- D - Deteriorated urine gives bad answers
- D - Detectable protein is not always present in urine which contains blood
- M - Most people can get along on only one kidney
- M - Much can be learned from the physical appearance of urine
- M - Myoglobin is excreted into urine after traumatic injury
- O - Most pregnancy tests today are immuno chemical
- O - One fourth of all blood from the left heart passes through the kidneys
- O - Only dummies control recognise red cells in urine sediment
- R - Routine urine study is part of each hospital admission
- R - Reagents strips used by moistening in the urine avoid collection container problems
- R - Reduction of one substance is accompanied by oxidation of another
- E - Elevated urine amylase concentration may mean

- pancreatitis
- Errors may occur with either precipitation or colorimetric methods for urine protein
- Every laboratory should use urine controls as unknowns or blind specimens
- F - For bilirubin test, holding the reacted strip to the colour chart is a must
- Fresh urine doesn't stink
- Ferric chloride is not a good test for bilirubin because it reacts with so many other compounds
- For good results in urine study, good specimens, good reagents, good methods, good testers, and good interpretation are all necessary
- A - Always use fresh urine specimens for analysis
- Anuria, polyuria, and oliguria relate to our daily urine volumes
- Ascorbic acid in very large quantities may interfere with both kinds of urine sugar tests
- As urine volume increases, specific gravity decreases
- A clean urine container does not contain cleaning compound residues
- C - Complete urinalysis involves several tests
- Copper reduction tests for urine sugar are non specific
- Chemical tests for blood and microscopic examination of urine complement each other
- Control urine specimens can be used in several ways
- Crystal identification in the sediment is unimportant most of the time
- Clinilab automates and records the same kind of urine results obtained with dip-and-read tests
- Certain peculiar odours of urine may signify disease

- T
- The relationship of urine examination to disease detection was referred to in 400BC by Hippocrates
  - Treat urine with respect
  - The colour of urine may mean something significant
  - Turbidity of urine has several causes
  - There are several methods for measuring specific gravity
  - The two kinds of tests for urine sugar are reduction and enzymatic
  - The 10-second glucose test involves a double sequential enzyme reaction
  - There is no point for having a very sensitive reduction test
  - The best urine specimen to use for glucose testing is a post prandial one
  - The amount of protein in the urine does not necessarily relate to the severity of the disease
  - The specificity of any urine test influences the results
  - The protein occurring most often in the urine in renal disease is albumin
  - The normal and abnormal pH ranges of urine are the same
  - There are chemical and culture tests for bacteriuria.
  - Some abnormal constituents of urine affect the colour of urine
  - Sodium chloride and urea have much influence on specific gravity
- S
- Specific gravity values have significance in combination with other information
  - Sensitive tests for sugar should also be specific
  - Sensitivity of urine tests depend on the method
  - Substances interfering with urine tests can alter results of such test - some times giving false positives

- and other times giving false negatives
- Severe, or moderate proteinuria may occur in most renal diseases
- Stale urine becomes alkaline because of bacterial decomposition of urea to ammonia
- Serial determinations of pH are more useful than single tests
- Susceptibility to bacteuria is more common in pregnant women and little girls
- Screening urine for PKU and mucopolysaccharidosis is worth while