Ethiopian TVET-System

MEDICAL LABORATORY

Level -III

Based on Apr.2018G.C. Occupational Standard

Module Title:- **Performing Urinalysis and body fluid analysis**

TTLM Code:-HLT MLT3 0919v1

This module includes the following Learning Guides

LG49: Identify concepts of urinalysis

LG50:Process samples and associated request details

LG51: Perform testing

LG52: Maintain a safe environment

LG53: Maintain laboratory records

Welcome to the module “Performing Urine and Body Fluid analysis”. This learner’s guide was prepared to help you achieve the required competence in “**Medical laboratory services Level-III**

This will be the source of information for you to acquire knowledge and skills in this particular occupation with minimum supervision or help from your trainer.

Summary of Learning Outcomes

After completing this learning guide, you should be able to:

LO1. Identify concepts of urinalysis

1.1. Anatomy and physiology of urinary system.

1.2. Metabolic products in urine

1.3. Testing methodology of urinalysis.

Learning-instructions

1. Read the contents of this Learning Guide. It is divided into sections that cover all the skills and knowledge that you need.
2. Read the information written in the “Information Sheet #1, #2, and # 3”.
3. Accomplish the “Self-check #1on page 15 &16, #2 on page 20, and #3 on page 23
4. If you earned a satisfactory evaluation on self-check proceed to next learning Guide. However, if your rating is unsatisfactory, see your teacher for further instructions.
5. Read the “Operation Sheet” and try to understand the procedures discussed.
6. Practice the steps or procedures as illustrated in the operation sheet. Go to your teacher if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedures

Instruction Sheet #1	LG49: Identify concepts of urinalysis

This learning guide is developed to provide you the necessary information regarding the

Following content coverage and topics –

LO1. Identify concepts of urinalysis

- Concept of renal physiology and anatomy are identified
- Metabolic products in urine are identified
- Testing methodology of urinalysis is identified

Learning Activities

1. Read the information written in the “Information Sheets”.
2. If you earned a satisfactory evaluation proceed to next module. However, if your rating is unsatisfactory, see your teacher for further instructions.
3. Read the “Operation Sheet” and try to understand the procedures discussed.
4. Practice the steps or procedures as illustrated in the operation sheet. Go to your teacher if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedure

1.1. Introduction to Basic Concepts in Urinalysis

Introduction: Dear trainees, this learning guide tries to explain to you that the basic concept and principles of Urineformationandurineanalysis,startingfromanatomyandphysiologyofkidneyuptotherenalClearance and threshold analysis.

Objectives: At the end of this learning guide you will be able to:

- Describe anatomy of the kidney.
- Explain the physiology of the kidney and formation of urine.
- List composition of urine.
- Identify factors affecting composition of urine.
- Discuss clinical significance of urine analysis.
- Describe renal clearance and renal threshold.

Urinalysis

Urinalysisisagroupoftestsperformedmostfrequentlyonrandomspecimen.Itisoneofthemosthelpful indicators of health and disease.

Uses:

Itisusefulasascreeningtestforthedetectionofvariousendocrineormetabolicabnormalities.

Itisalsousedtodetectintrinsicconditionsthatmayadverselyaffectthekidneysorurinarytract.

Generally,urinalysisprovidesusefulinformationconcerningthepresenceorabsenceofrenalandotherdiseases.

- Itisaverysimplemethodformonitoringthecourseofadiseaseaswellasthe efficacy of treatment

1.1.1. Urinary system

Theurinarysystemisalsocalledtheexcretorysystemofthebodybecauseoneofitsfunctionsistoremove waste products from the blood and eliminate them from the body.

- Composed of two kidneys, two ureters, one bladder and one urethra.
- The two human kidneys are the main structural part of urinary system, responsible for the formation of urine.
- Each kidney contains about a million filter units, called nephrons, designed for

the synthesis of urine in our body.

- The urinary system consists of

•**Two kidneys**: this organ extracts wastes from the blood, balance body fluids and form urine.

•**Two ureters**: this tube conducts urine from the kidneys to the urinary bladder.

- ✓ Ureters are two tubes stretched from kidney to bladder.
- ✓ Function of ureters is to transport urine from the kidney to the bladder.
- ✓ The transport methods in ureters are by gravity and peristalsis (a rhythmic squeezing) of smooth muscle of ureters.

•**Theurinarybladder**:thisreservoirreceivesandstorestheurinebroughttoitbythetwo ureters.

•**Theurethra**:thistubeconductsurinefromthebladdertotheoutsideofthebodyfor elimination.

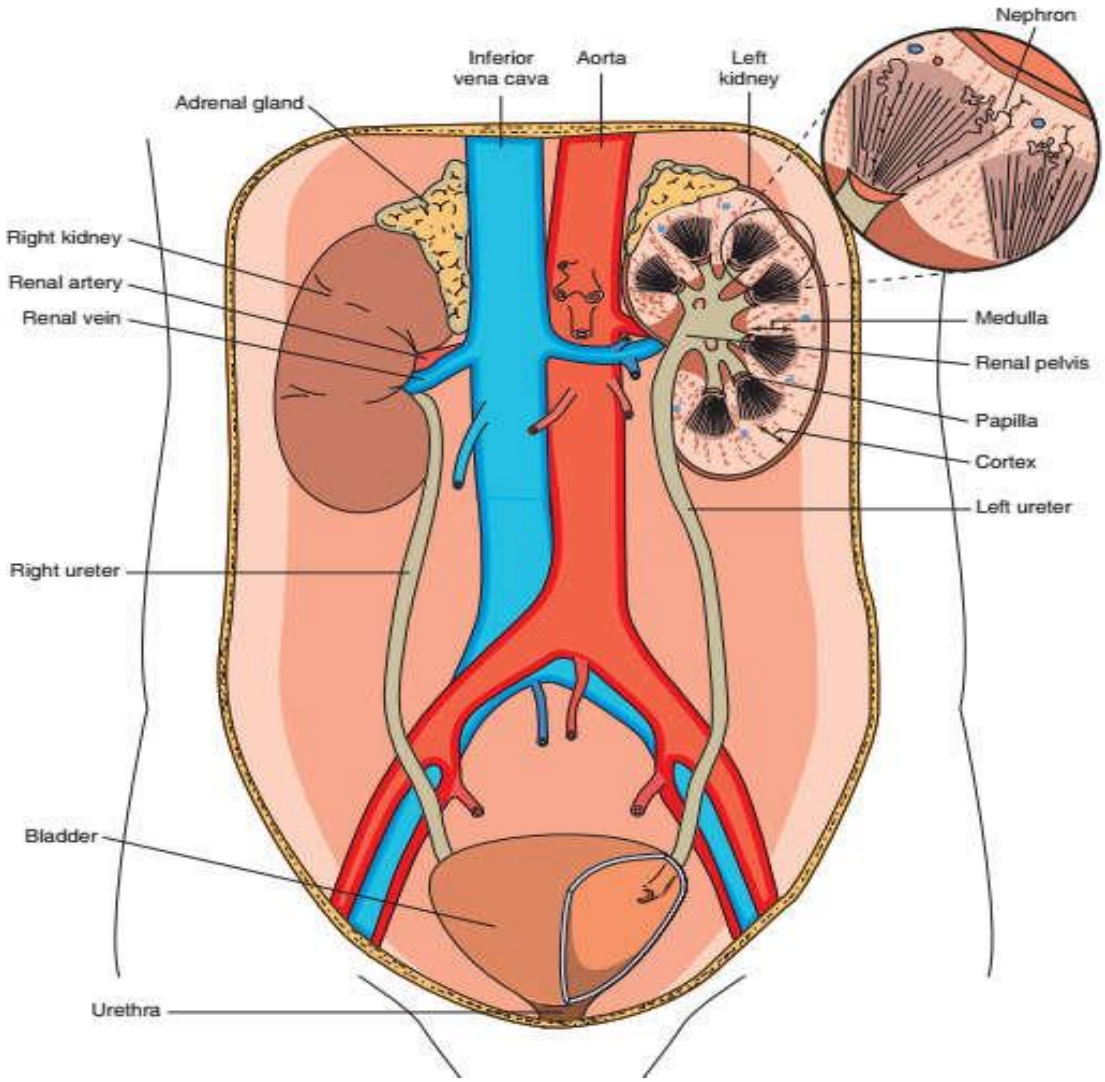

Figure 1.1:- Anatomy of the urinary system.

1.1.2.Anatomy of the kidney

Definitions:

- **Anatomy**:thewordanatomyisderivedfromaGreekword"*Anatome*"meaningtocutup.
  - ✓ Itisthe study of structures that make up the body and how those structures relate with each other.
- **Kidneys**: are two bean shaped organs; it weighs about 150 gm each.
- **Location:**Thekidneysareapairoforgansfoundalongtheposteriormuscularwalloftheabdominalcavity.Unliketheotherabdominalorgans,thekidneysliebehindtheperitoneum.Theribsandmuscles of the back protect the kidneys from external damage.
- **Structure**:Thekidneysarebean-

shapedwiththeconvexsideofeachorganlocatedlaterallyandtheconcave side media.

A. External Anatomy of the kidney

Apairofreddishbrown,beanshapedorganlocatedintheposteriorwalloftheabdominalregion,on eineachsideofthevertebralcolumn.They are protected atleastpartiallybythelastpairofribsand cappedbytheadrenalgland.Thebeanshapeofthekidneyismediallyconcaveandlaterallyconvex .

Onthemedialconcaveborderisthe***hilus***(smallindentedarea)wherebloodvessels,nerves&uret ersenter and leave the kidney.

- kidneys are two bean shaped organs, about 150 gm each
- Urine forming units:
  - ☐ Cortex
  - ☐ Medulla (lobed: renal pyramids)
  - ☐ Cortex and medulla composed chiefly of nephrons and blood vessels
- About 25% of cardial out put Supplied to kidney through renal arteries (branches of descending aorta)
- Returns back by renal veins (branches of inferior vena cava)

Covering and supporting each kidney are three layers of tissue:

•**Renal capsule** – innermost, tough, fibrous layer

•**Adiposecapsule** – the middle layer composed of fat, giving the kidney protective cushion.

•**Renal fascia** – is outer sub-serous membrane, connective tissue layer.

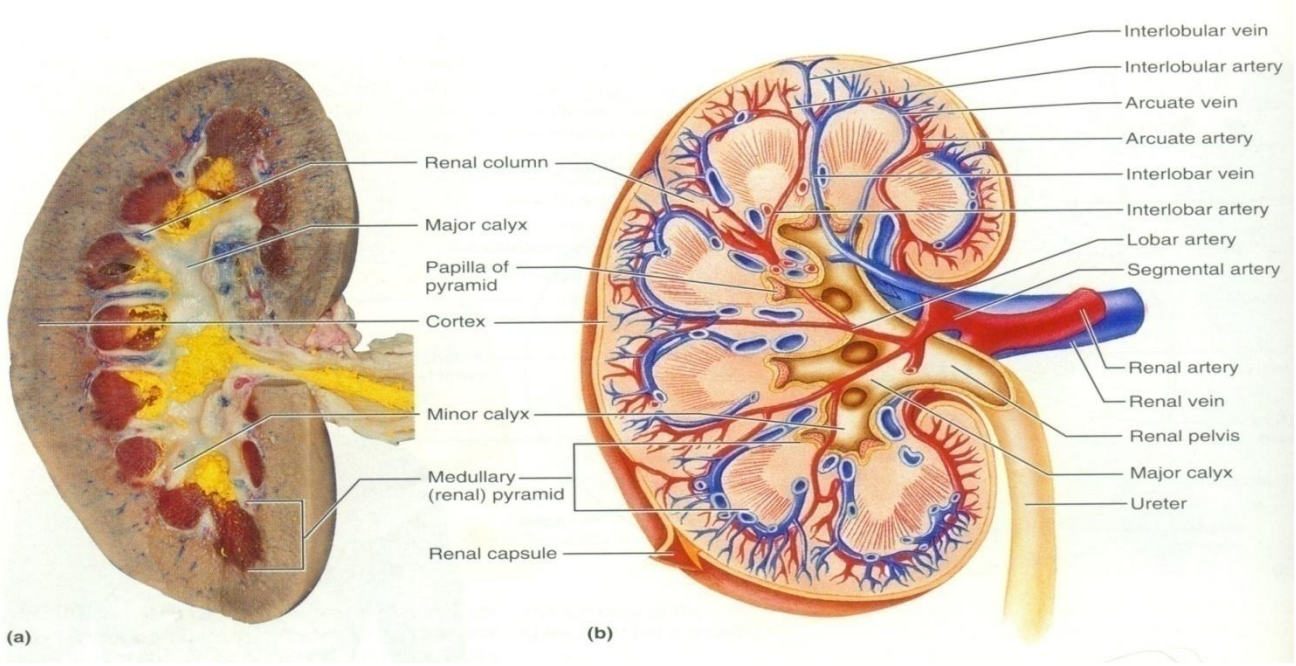

Figure 1.2 external and internal anatomy of kidney

B . Internal Anatomy of the kidney

- Asagitalsectionofthekidneyrevealsthreedistinctregionscalled**pelvis**,**medulla**and**cortex**(frominside out).
- The**Renalpelvis**isthelargecollectingspacewithinthekidneyformedfromtheexpandedupper portion of the ureters
- The**Renalmedulla**isthemiddleportionofthekidney.Itconsistsof8to18renalpyramids.
- Thebaseofeachpyramidisadjacenttotheoutercortex.Pyramidscontaintubulesand collecting ductsofthenephron. Tubulesinvolved intransportationandre-absorptionoffiltered materials.
- The**Renalcortex**istheoutermostportionofthekidney.Ithastworegionstheoutercortical andtheinnerjuxtamedullaryregion.
- Thecorticaltissuethatpenetratesbetweenpyramids forms***Renal Columns***. The renal columns composed of mainly collecting tubules

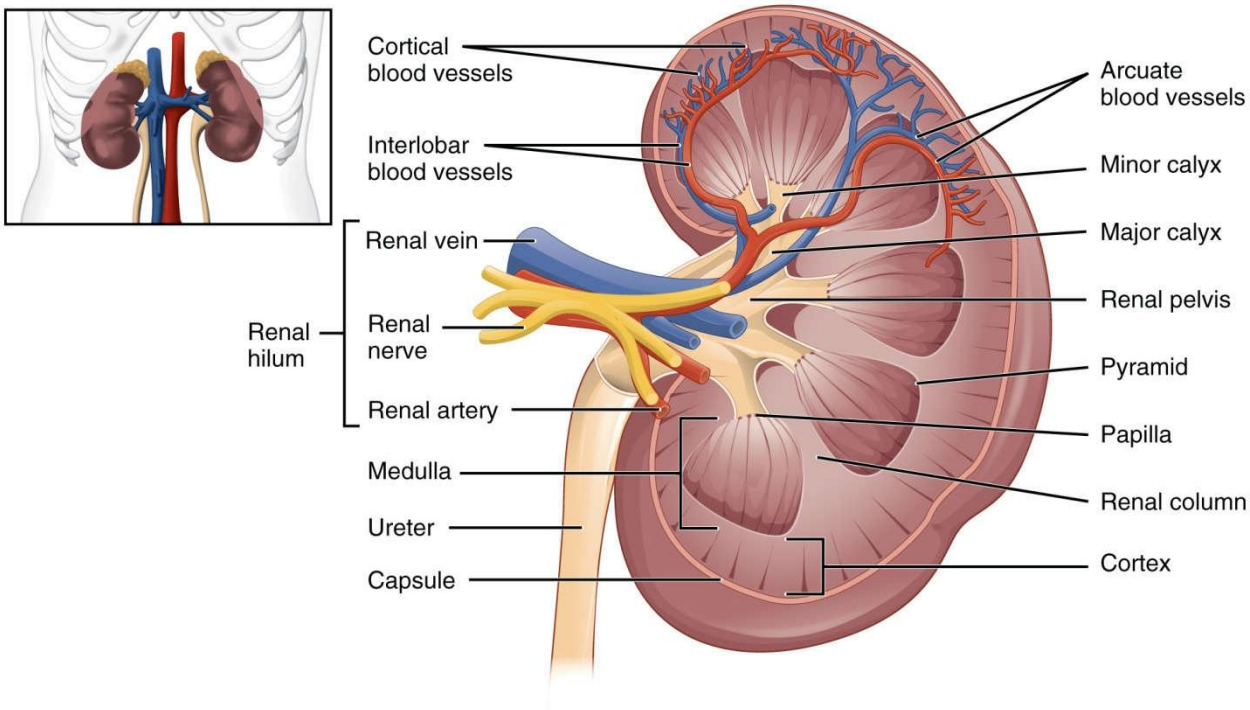

Figure 2:Internal anatomy of the kidney

The Nephron:

- It isthe basic functional unit of the kidney.
- Each kidney contains approximately one million nephrons.
- Each nephron is an independent urine-forming unit.
- Each nephron consists of two parts:**renal corpuscle** and**renal tubule**
- **Arenalcorpuscle** (where blood plasma is filtered), has two components:
- The **glomerulus** (capillary network) and
- The**glomerular(Bowman's)capsule,**adouble-walledepithelialcupthatsurroundsthe glomerulus.
- **Arenal tubule:** a tubuleinto which the filtered fluid passes. It consists of:

a. **Proximal convolutedtubule**isthe part of the tubule attached to the glomerular capsule and

b. **Loop of Henle/nephron loop:** the tubule is tightly coiled.

c. **Distal convoluted tubule:**it is the parts that further away from the glomerular capsule.

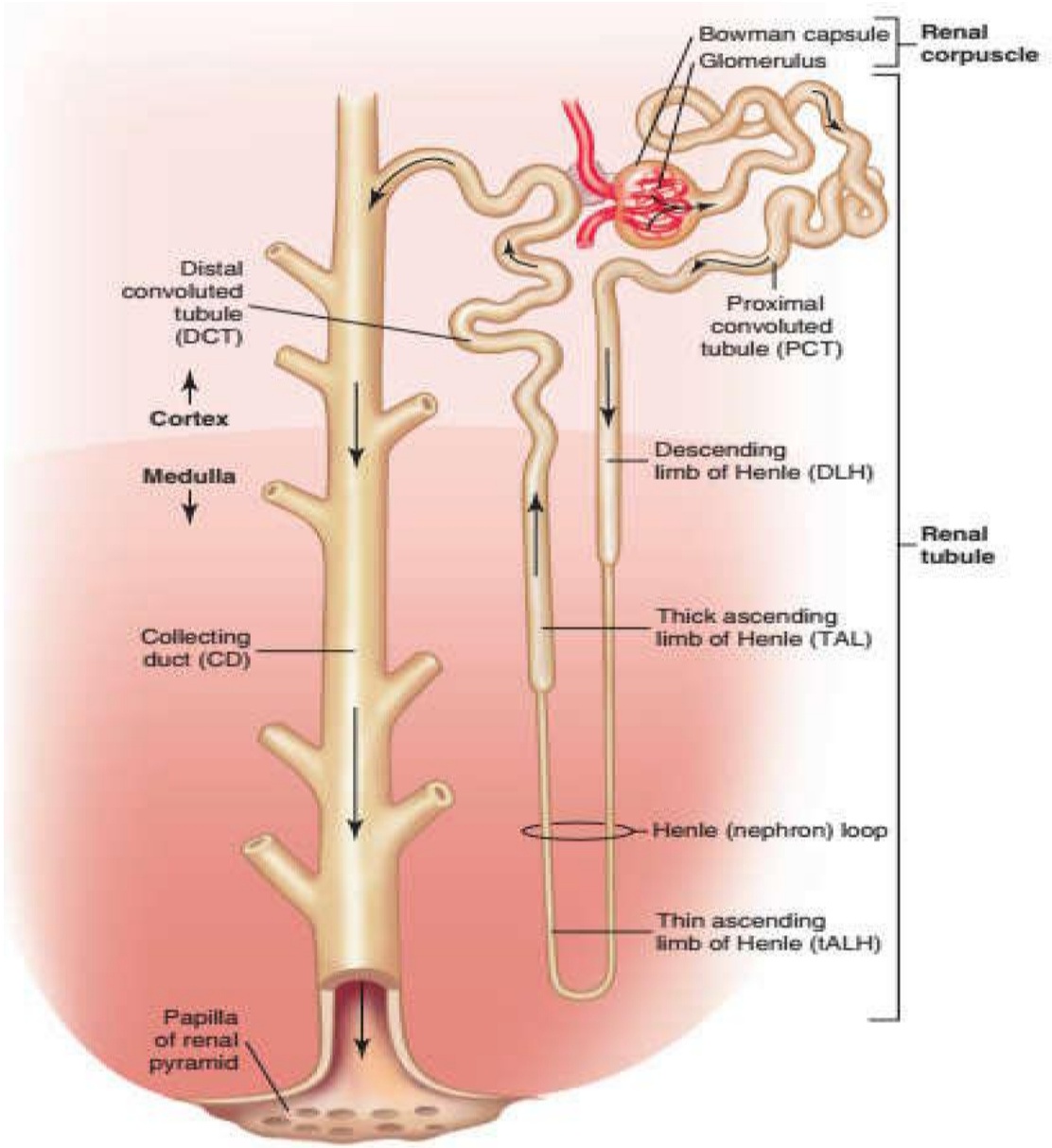

Figure 1.3: A diagram of a nephron tubules.

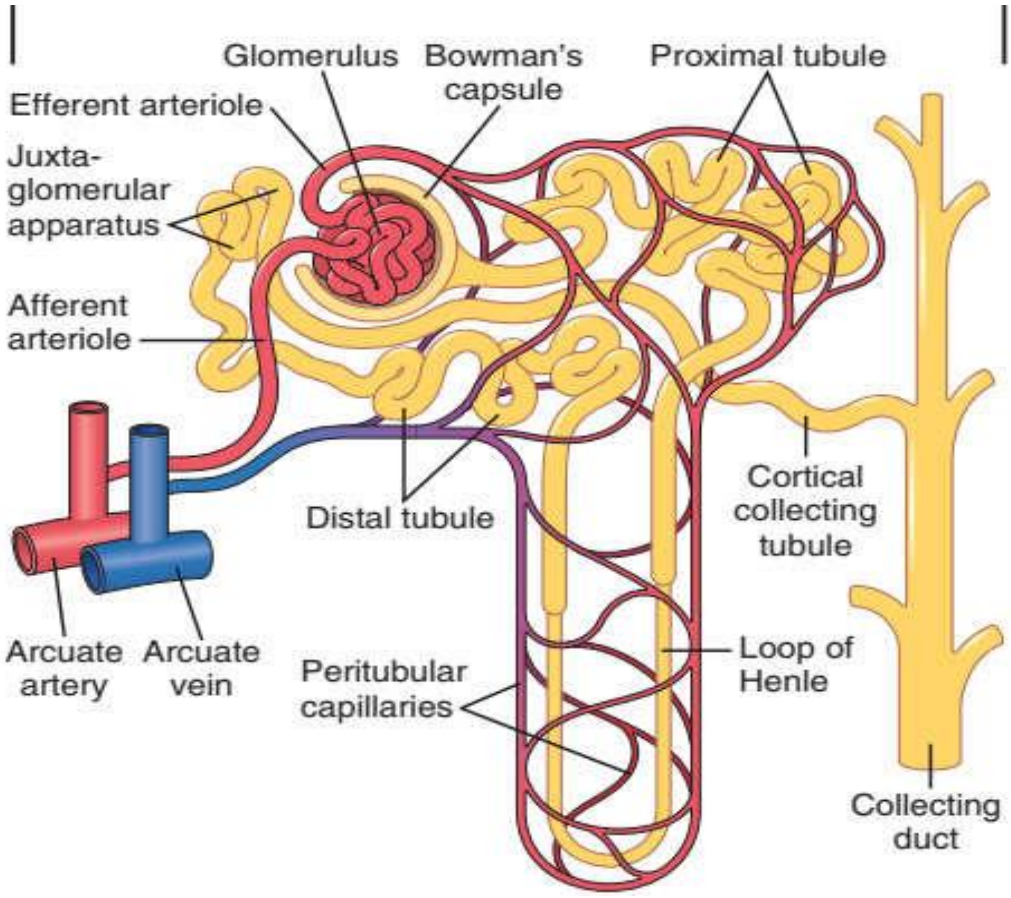

Figure 1.4: Basic tubular segments and blood supply of the nephron.

Blood Supply: Blood is supplied to the kidneys by renal artery and drainage is by renal vein.

1. Therenalarteriesbranchdirectlyfromtheabdominalaortaandenterthekidneysthroughthe renal hilus.
2. Inside our kidneys, the renal arteries diverge into the smaller afferent arterioles of the kidneys.
3. Eachafferentarteriolecarriesbloodintotherenalcortex,whereitseparatesintoabundleof capillaries known as a glomerulus.
4. Fromtheglomerulus,thebloodrecollectsintosmallerefferentarteriolesthatdescendintothe renal medulla.
5. The efferent arterioles separate into the peritubular capillaries that surround the renal tubules.
6. Next,theperitubularcapillariesmergetoformveinsthatmergeagaintoformthelarge renal vein.

7. Finally,therenalveinexitsthekidneyandjoinswiththe inferiorvenacava,whichcarries blood back to the heart.

1.1.3.Physiology of the Kidney and FormationofUrine

1.1.3.1.Physiology of the Kidney

Thekidneysperformtheirmostimportantfunctionsbyfilteringtheplasmaandremovingsubstances fromthefiltrate.Thekidneysclearunwantedsubstancesfromthefiltratebyexcretingtheminthe urine while returning substances that are needed back to the blood.

Kidneys homeostatic functions:

- Excretion of metabolic waste products and foreign chemicals
- Regulation of water and electrolyte balances
- Regulation of body fluid osmolality and electrolyte concentrations
- Regulation of arterial pressure
- Regulation of acid-base balance
- Regulation of erythrocyte production
- Secretion,metabolism, and excretion of hormones
- Gluconeogenesis

Group Discussion Point

- Discussonphysiologicroleofthekidneyexcretionof**wastematerialsandforeign chemicals**.

Hint:

Excretion of metabolic waste products, foreign chemicals, drugs, and hormone metabolites:

Thekidneysaretheprimarymeansforeliminatingwasteproductsofmetabolismthatareno longer needed by the body.Theseproductsincludeurea(fromthemetabolismofaminoacids), creatinine(frommusclecreatine),uricacid(fromnucleicacids),endproductsofhemoglobin breakdown (such as bilirubin), and metabolites of various hormones.

Regulation of Erythrocyte Production:

Thekidneyssecreteerythropoietin,whichstimulatestheproductionofredbloodcellsby hematopoietic stem cellsin the bone marrow

Group Activity•

Discuss how our kidney produce and excrete urine.

1.1.4.Formation of Urine

Urine is formed by the three physiological processes that are:

- ✓ Glomerular filtration
- ✓ Tubular re-absorption
- ✓ Tubular secretion

And is collected by the collecting duct and passes into bladder through uretersand then comes out through urethra.

The blood enters the glomerulus of each nephron by passing through the afferent arteriole into the glomerular capillaries. The capillary walls in the glomerulus are highly permeable to water and the low molecular-weight components of the plasma.

They filter through the capillary walls and the closely adhering membrane of Bowman's capsule into Bowman's Space from where the plasma ultrafiltrate passes into the tubule where re-absorption of some substances, secretion of others, and the concentration of urine occur.

Many components of the plasma filtrate such as glucose, water, and amino acids, are partially or completely reabsorbed by the capillaries surrounding the proximal tubules. In the distal tubules, more water is reabsorbed and potassium and hydrogen ions are secreted.

The Loop of Henle and the system of collecting tubules are the principal sites where the urine is concentrated as a mechanism for conserving body water.

- One of the main function of kidney:
  - selective absorption of substances necessary for our body
  - Removal of waste products and surplus substances, that would be harmful to our body in the form of urine.
- The formation of urine by the kidneys achieved by three phase processes:
  - Simple filtration
  - active and passive reabsorption
  - secretion

Discussion question?

List urine constituents that indicate abnormality of the kidney function.

1. _____
2. _____
3. _____

self-check #1	Written Test

Multiple Choices: Choose the correct answer from the given choices.

1. Which one of the following is the functional unit of the kidney?
   A.Bowman's capsule B.Cortex C. Nephron D. Medulla
2. The urinary system has the following functions except
   A. Synthesis of proteins
   B. Excretion of metabolic waste product
   C. Regulation of water and electrolyte balance
   D. Regulation of red blood cell production
3. ---------Tube conducts urine from the kidneys to the urinary bladder?
   A. Urethra B. Ureter C. Proximal tube D. Collecting duct
4. Identify wrong statement about external anatomy of the kidney?
   A. Located in the posterior wall of the abdominal region
   B. The bean shape of the kidney is medially convex and laterally concave

C. Capped by the adrenal gland

D. They are protected at least partially by the last pair of ribs

5. The site in the external portion of the kidneys where blood vessels, nerves & ureters enter and leave the kidney are--------?

   A. Renal fascia B. Hilus C. Renal corpuscle D. Renal pelvis

6. ------- is the large collecting space within the kidney formed from the expanded upper portion of the ureters?

   A. Renal cortex B. Renal medulla **C.** Renal pelvis D. Renal capsule

7. ----------Is a double-walled epithelial cup that surrounds the glomerulus?

   A. Bowman's capsule C. The glomerulus

   B. renal corpuscleD. capillary network

8. A sagital/inner section of the kidney reveals three distinct regions. Which alternatives show those regions from outer to inner in sequences?

   A. Pelvis→Cortex→Medulla C. Pelvis→ Medulla → Cortex

   B. Cortex→Medulla→ Pelvis D. Medulla→ Pelvis→ Cortex

Note:- Satisfactory point is above four (>4)

Not-satisfactory point is below four (<4)

Name————————————————————————Date————————————

1.2.1.Composition of Urine

- Urine
  - A fluid extracted by the kidneys, pass through the ureters, stored in the bladder, and discharge through the urethra.
  - in the presence of disease conditions, depending on the abnormality, the urine will have abnormal constituents.

Normal urine

- Freshly voided urine from healthy individuals is clear and pale yellow in color
- Having aromatic odor from volatile organic acids, and specific gravity about 1.024
- .It is slightly acidic (pH 5.0 to 6.0) and contains 95 % water.
- Normal urine contains
  - Creatinine, uric acid, urea, few epithelial cells, 2-3 leukocytes/HPF and amorphous urates (in acidic urine) and amorphous phosphates (in alkaline urines).
  - Urine also have electrolytes like sodium, chlorine; and hormones, like aldosterones, vitamins and drug metabolic products in a very small quantities.
- Those substances considered as normal components of urine because they are waste product of our body metabolism, and their means of elimination from the body is mainly through urine.
- Abnormal compositions of urine
  - Sugar, Proteins, Bilirubin, ketone bodies, different hormones & electrolytes in higher concentration.
  - Urine sediments, such as high number of leukocytes, red blood cells, different kind of Casts, parasites, bacteria's, and yeasts.

Table:- Summary of composition of urine

Normal Urine Constituents	**Abnormal Urine Constituents**
Water (about 95% of urine)	Glucose
Urea	Blood cells
Creatinine	Bile pigments
Uric acid	Protein, nitrite
Electrolytes	Cast, Crystals
Normal Urine Constituents	Parasites
	Microorganisms

1.2.2.The Factors Affecting the composition of Urine

- Diet and nutritional status
- Condition of body metabolism
- Ability of kidney function
- Levelofcontaminationwithpathogenicmicroorganismorevennon-pathogenic microorganism.

Group Activity:

Explain how the above factors affect the composition of urine

1.2.3.RenalClearanceandRenalThreshold

RenalClearance: RenalClearancevalueindicatesthedegreetowhichasubstanceisremoved from the blood by excretionintheurine.

- Clearanceisusuallydefinedasthebloodvolumethatcontainsthe

quantity of a substance excreted in the urine per minute.

- **Renal Clearancevalue**:
  - indicates the degree to which a substance is removed from the blood by excretion in the urine.

- **Clearance** :
  - the blood volume that contains the quantity of a substance excreted in the urine per minute.

GFR : Rate at which glomerular filtrate is formed

- About 120 ml of glomerular filtrate is produced per minute.
- Therateatwhichtheglomerularfiltrateisformedisknownasthe**glomerularfiltrationrate**(GFR).

❑ CREATININE CLEARENCES

- Creatinineisasubstancepresentinthefiltrate,whichisnotreabsorbed(however,thisissom etubular secretionofcreatinine).
- Thereforetheclearanceofcreatininefromtheplasmais120mlperminute.
- Hencecreatinineclearanceisusedclinicallytogiveanapproximateindicationofglomerular filtrate rateand,therefore,asatestofkidneyfunction.Whenthefiltrationratefalls,theconcentratio nof creatinineintheplasmarises.
- Thecreatinineclearancetestexpressesthevolumeofbloodcontaining the amount of creatinine excreted by the kidney in one minute.
  - ➢ Thecreatinineclearance(Crcl)iscalculatedbycollectinga24hrsurinespecimenanda blood sample as well within the urine collection time.
  - ➢ Creatinineisthendeterminedinbothurineandserumandthecreatinineclearancecalc ulated in milliliters per minute (ml/minute).

Crcl (ml/minute) = $\frac{U \times V}{S}$

- ✓ Where, U= Urine Creatinine Concentration in mol/l
- ✓ V= Volume of urine in ml per 24 hrs
- ✓ S= Serum Creatinine Concentration in mol/l
- ✓ **Normal Range:**The normal Crcl value usually ranges between110 – 140 ml/minute.

❑ N.B

- — Why is creatinine clearance most often used to monitor GFR?

- — Creatinine freely filtered by glomerulus
- — Creatinine not ‘rehandled’ by tubules
- — Creatinine is an endogenous substance
- — Amount of creatinine produced per day is constant
- — Amount of creatinine produced is proportional to muscle mass

❖ **Renal Threshold:**

✓ Therenalthresholdofasubstancereferstothehighestconcentrationofasubstance,whichispresent inthebloodbeforeitisfoundintheurine.

✓ Asubstancesuchasglucoseisahighthreshold(160-180ml/dl),becauseitiscompletelyabsorbedfromtheglomerularfiltrateandisonlyfound inthe urine,whenthebloodglucoselevelismarkedlyraised.

✓ Ureaandcreatinine,however,arealways presentintheurineindependentofthebloodlevelbecauseverylittle,ifany,ofthesesubstanceis reabsorbed.

Self check #2	Written exam

Instruction: Choose the correct answer from the given choices.

1. Which substance is used to evaluate renal clearance?
   A.Blood cell B.Cast C. Creatinine D.Protein
2. One of the following substances is not found in normal urine?
   A.Creatinine B.Electrolyte
   C.Protein D.Urea
3. Among the following alternatives which one contains normal constituents of urine only?
   A. Glucose, Electrolytes, Blood cells C. Uric acid, Urea, Creatinine
   B. Creatinine, Urea, Parasites D.Protein, nitrite,Bile pigments
4. If water intake is decreased, the kidney will protect the body from excessive retention of water by eliminating a larger volume of urine than normal and viseversa.
   A. True B. False C. Uknown
5. Which substance is used to evaluate renal clearance?
   A. Blood cell B. Cast C. Creatinine D. Protein

Note:- Satisfactory point is above three (>3)
Not-satisfactory point is below three (<3)

Name————————————————ID. No————————Date————————

1.3. Testing methodology of urinalysis.

In the diagnostic medical laboratory urine specimens can be analyzed by using different methods of examnation. Some of this methods may includes

- Phyical examination
- Chemical examination
- Microscopic examination
- Microbiological examination (Cultiring methods) and…etc.

1.3.1. Type of Examination in Routine Urinalysis

1.3.1.1. Physical Examination of Urine includes

- ✓ □ Volume _ PH
- ✓ □ Color _ Appearances
- ✓ □ Odor _ specific gravity

1.3.1.2. Chemical Examination of Urine

- Glucose Ketones Urobilinogen blood
- Protein Bilirubin nitrite leukocyte esterase

1.3.1.3. Microscopic Examination of Urine

- RBCs _ Yeasts
- WBCs _ parasites
- Epithelial cells _ crystals
- Casts
- Bacteria

1.3.1.4. Microbiological examination (Cultiring methods)

- Possible pathogens may includes

✓ Gram positive Gram negative
✓ Staphylococcus Escherichia coli
✓ saprophyticus Proteus species
✓ Haemolytic streptococci Pseudomonas aeruginosa
Klebsiella strains
*Salmonella Typhi
*Salmonella Paratyphi
*Neisseria gonorrhoeae

1.3.2. Categories of Urine Tests

According to their degree of accuracy urine tests are grouped into three broad categories:

- Screening tests
- Qualitative tests
- Quantitative tests

1.3.2.1. **Screening tests** tell only whether a substance is present or absent, and the results are reported as positive or negative. They are done on random specimen.

1.3.2.2. **Qualitative tests** give rough estimate of the amount of substance present. They are also called semi-quantitative tests. The results of qualitative tests can be graded as negative, trace, +1, +2, +3 or +4.

1.3.2.3. **Quantitative tests** determine accurately the amount of the substances to be tested. However, since they are time consuming, they are not included in routine urinalysis. Most common quantitative tests performed in urinalysis laboratory are those for sugar and for protein.

The results of a quantitative test are usually reported in milligrams per deciliter, gram per deciliter, and per liter. For quantitative test, a complete 24-hour urine specimen is needed. An appropriate preservative should be added to the container or the specimen should be stored in refrigerator.

Self check #3	Written exam

Instruction 1:- Say true or false for each of the following questions

1. Screening tests give rough estimate of the amount of substance present.
2. Most common quantitative tests performed in urinalysis laboratory are those for sugar and for protein.
3. Qualitative tests tell only whether a substance is present or absent, and the results are reported as positive or negative.
4. Quantitative tests determine accurately the amount of the substances to be tested.
5. The results of qualitative tests can be graded as negative, trace, +1, +2, +3 or +4.

Instruction #2:- Choose the best possible answer among the given alternatives

1. Physical Examination of Urine includes all of the following except?
   A. Volume C. Specific gravity
   B. Color D. Leukocyte esterase
2. Which of the following alternatives includes only Chemical Examination of Urine?
   A. Glucose, Ketones, Specific gravity C. Urobilinogen, blood, Odor
   B. Protein, Bilirubin, nitrite D. Volume, PH, leukocyte esterase
3. Microscopic Examination of Urine may include all of the following except?
   A. RBCs and WBCs C. Yeasts & Crystals
   B. Parasites & Epithelial cells D. PH, & leukocyte esterase
4. If Microbiological examination is performed on urine specimenpossible outcome mayincludes?
   A. Staphylococcus & Escherichia coli C. saprophyticus & Proteus species
   B. Hemolytic streptococci & Pseudomonas aeruginosa D. All

Note:- Satisfactory point is above five (>5)
Not-satisfactory point is below five (<5)

Name————————————ID. No————————Date————————

Welcome to the module “Performing Urine and Body Fluid analysis”. This learner’s guide was prepared to help you achieve the required competence in “**Medical laboratory services Level-III**

This will be the source of information for you to acquire knowledge and skills in this particular occupation with minimum supervision or help from your trainer.

Summary of Learning Outcomes

After completing this learning guide, you should be able to:

LO2. Process samples and associated request details.

2.1 Collecting urine specimens

2.2 Sorting of Specimens according to ***tests*** requested, urgent status and volume.

2.3 Sample acceptance/rejection criteria

2.4 log accepted samples and request forms

2.5 processing of Sample

2.6 Storage of Samples and sample components.

How to Use this TTLM

- Read through the Learning Guide carefully. It is divided into sections that cover all the skills and knowledge that you need.
- Read Information Sheets and complete the Self-Check at the end of each section to check your progress
- Read and make sure to Practice the activities in the Operation Sheets. Ask your trainer to show you the correct way to do things or talk to more experienced person for guidance.
- When you are ready, ask your trainer for institutional assessment and provide you with feedback from your performance.

Instruction Sheet #1	LG50: Process samples and associated request details

This learning guide is developed to provide you the necessary information regarding the

Following content coverage and topics –

LO2. Process samples and associated request details

2.1. Collecting urine specimens

2.2. Sorting of Specimens according to ***tests*** requested, urgent status and volume.

2.3. Sample acceptance/rejection criteria

2.4. Log accepted samples and request forms

2.5. Processing of Sample

2.6. Storage of Samples and sample components.

Learning Activities

1. Read the information written in the “Information Sheets”.
2. If you earned a satisfactory evaluation proceed to next module. However, if your rating is unsatisfactory, see your teacher for further instructions.
3. Read the “Operation Sheet” and try to understand the procedures discussed.
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LO2.Process samples and associated request details

2.1. Collection of Urine specimen

Urine specimen is collected for – physical, chemical and microscopic examinations

- Urine specimen should be collected in the correct way and in suitable containers.
- The collecting containers must be cleaned, dried, link proof and It may plastic or glass
- and special poly ethylene bag for infants and children
- Urine collecting containers are used in collecting, storing and testing specimen of the urine
- The urine containers should be large wide mouthed plastic or glass containers with screw tope for cumulative of urine over a long period of time (24hrs of urine)

- The urine specimen must be obtained under septic condition and a sterile container to culture
- A fresh voided urine specimen is adequate for most urinalysis except ***microbiological culture***
- Avoid contaminated urine specimen collection. E.g. vaginal discharges and menstrual blood
- The specimen should be examined immediately after collection

1. First morning urine specimen

- A specimen obtained during the first urination of the day
- Most concentrated urine
- Bladder incubated
- Best used for specific gravity, Protein, Nitrite and Microscopic examination
- prevents FN of pregnancy test
- recommended for detection of formed elements

2. **Random urine specimen**

- Obtained at any time during examination
- Most convenient and Most common
- **Good for**: Chemical screen and Microscopic examination

3. **Second voided urine specimen**

- First morning urine specimen is discarded and the 2nd urine specimen is collected and test
- This specimen is **good for**: -
- Reflection blood glucose and Keeping of formed elements intact

4. **Post prandial urine specimen**

- A specimen obtained 2 hours after meal and It is good for glucose determination

5. Urine specimen of 24 hours: A specimen obtained within 24 hours and Necessary for quantitative tests, especially for ***quantitative determination of protein***.

6. Mid-stream urine specimen collection

- A specimen obtained from the middle part of the first urine
- It is commonly used for routine urinalysis
- It is also important for ***bacteriological urine culture.***

7. Clean catch urine specimen collection

- Used for microbial culture
- Used for routine urinalysis
- For bacteriological examination urine should be Collected by the clean catch method by catheterization in to sterilized container
- Catheterization is the process of passing a tube through the urethra to the bladder for withdrawal of urine

8. Urine specimen from infants

- urine collected in plastic bag with adhesive mouth
- bag is fixed in the genitalia & left for 1-3 hrs

9. Three glass collection

- Determines prostatic infection
- The 1^{st} , middle & 3^{rd} are collected in three d/t containers
- Culture is performed for all specimens
- The 1^{st} & 3^{rd} are examined microscopically
- 3^{rd} specimen have high WBCS count/HPF &
- 10 times bacterial count than the 1^{st} specime

9. Glucose tolerance specimen

✓ Collected correspond with blood samples the number of specimen varies with length of the GTT(1,2,3,4,... hrs)urine is tested for glucose & ketone

Instruction 1:- Choose the best possible answer for each of the following questions?

1. Which of the following alternative is/are false about Mid-stream urine specimen?
   A. A specimen obtained from the middle part of the first urine
   B. It is commonly used for routine urinalysis
   C. It is also important for bacteriological urine culture.
   D. None
2. Which statement is false about Random urine specimen?
   A. Obtained at any time during examination
   B. Most convenient and Most common
   C. Good for Chemical screen and Microscopic examination
   D. Used for microbial culture
3. Which of the following is true about Clean catch urine specimen collection
   A. Used for microbial culture C. Used for routine urinalysis
   B. Should be collected by catheterization in to sterilized container D. All
4. First morning urine specimen is discarded and the 2nd urine specimen is collected and test is performed. This type of specimen is termed as?
   A. Urine specimen of 24 hour C. Second voided urine specimen
   B. Post prandial urine specimen D. Mid-stream urine specimen
5. A specimen obtained 2 hours after meal which is good for glucose determination
   B. Urine specimen of 24 hour C. Second voided urine specimen
   C. Post prandial urine specimen D. Mid-stream urine specimen

Instruction 1:- Say true or false for each of the following questions?

1. Urine specimen of 24 hoursA specimen obtained within 24 hours and Necessary for quantitative tests, especially for quantitative determination of protein.
2. First morning urine specimen recommended for microbiological culture
3. The urine specimen must be obtained under septic condition and a sterile container to culture
4. A fresh voided urine specimen is adequate for most urinalysis except ***microbiological culture***
5. Post prandial urine specimen is recommended for detection of formed elements

Note:- Satisfactory point is above five (>5) Not-satisfactory point is below five (<5)

2.2.1. Checking of request papers and samples

Pre-analytical variables refer to any and all procedures that occur during sample collection, prior to sample analysis.

- This involves patient identification, physical sample collection, sample transportation to the testing site and sample preparation. Patient samples are sometimes collected by the patient themselves, for example, Urine specimen from conscious patients. It is important that the laboratories have set protocols to ensure that appropriate collection kits with instructions for collection, safety precautions, and labeling are available for their patients.
- It is suggested that instructions for the patients be in the languages for the community the laboratory is serving or presented as simple easy-to-understand graphics.

Proper patient identification is mandatory to produce quality test result in the laboratory. Some of the pre-analytical activities in the lab are the following.

- **Patient preparation:** Some tests require that the patient be fasting. There may also be special timing issues for tests such as early morning urine,post-prandial urine sample, blood glucose, drug levels, and hormone tests. The client from whom sample is to be taken has right to know the type of the sample to be collected, the reasons why we collect the sample and the procedure applied to collect the sample.
- **Patient identification:** we have to properly check whether we have collected a sample from appropriate patient and the request paper and sample must be labeled correctly with some informations such as; name of the patient, age, sex, ward address of the patient, required test. The person collecting the sample must accurately identify the patient. This might be done by questioning the patient, by questioning an accompanying family member, or by the use of an identifying wrist band or other device.
- **Sample collection:** appropriate procedures must be applied to collect the sample. Some of the important information to be considered here are:
    - Specimen container
    - Volume of the specimen
    - Time of collection
    - Type of specimen
    - Type of anticoagulants for blood specimens

- Preservatives to be considered etc.…

- **Sample transportation:** specimens can be transported to reference laboratories for more specialized tests or for quality control purpose.
- Here proper labeling, packing, and correct preservative selection are mandatory.

➢ Generally the pre-analytical phase is the phase where the laboratory has no direct control on the process. Pre-analytical factors that can affect results include: sample type, sampling time, sample handling, patient's preparation and the nutritional status of the patient.

2.2.2. Sorting of specimens according to its urgency

- **Sample:** is a part which represents a characteristic of the whole.
- **Urgent test:** a laboratory test requested & need priority
- It is only those tests should be requested urgently that are required for the immediate care of a patient or to manage a serious public health situation.
- **The laboratory test request format:** is a Paper or electronic format in which the physicians or clinicians order a Laboratory test.
  - **Essential information on Test Request form includes**

- Patient‘s Identification such asFull name, Age, and sex,Address or village of patient (valuable epidemiological data).In patient, or outpatient identification number.

➢ Relevant clinical information regarding patient’s condition
➢ Tests requested; Specific test(s) required.
➢ Time and date of the sample collection;
➢ Origin of request, (requesting Unit).
➢ Name of the medical officer, community health worker, or midwife requesting the test and to whom the report should be sent.
➢ Specimen provided type of specimen for requested test.
➢ Source of the sample, when appropriate ,anatomical site where the sample is collected
➢ Clinical data, when indicated;
➢ Contact information for the health care provider requesting the test and others.
➢ Once a sample enters the laboratory, there are a number of steps needed prior to testing.

- These pre-examination steps include:
- Verifying the sample is properly labeled, adequate in quantity, in good condition, and appropriate for the test requested.

- The test request must be complete and include all necessary information;
- Recording sample information into a register or log;
- Enforcing procedures for handling sub-optimum samples, including sample rejection, when necessary

Fig .2.2 labeling specimen container

The objectives of the sorting concepts are to monitor and control the sample flow regarding the whole laboratory cycle.

The advantages of sorting sample are:

- Control and monitor sample material from delivery to disposal
- Documentation of know-how and regulations in the system
- Flexible assignment of the expert staff and quick integration of new employees.
- Defined & structured process
- Continuous sample cycle time
- Continuity regarding sample flow and capacity utilization by recursive sample sorting and defined buffer zones (to smooth peaks)
- Preparation of sample material for automation
- Automated handling of standard samples

- Sorting of special material on manual work places
- Programming of special rules by the maintenance personnel
- Special workflows can be configured
- Daily analysis of the order data

Collection of sufficient quantity is important to permit detection of organisms and to prevent rapid drying. The urine specimen should contain at least 15-30ml.

Process & examine urine specimen immediately after collection, if not, preserve urine specimen by using appropriate preservative method.

Collect approximately 15-33ml urine in a clean, dry container without preservatives for routine urinalysis. A screw-cupped brown container labeled with full information identifies the patient is most suitable.

2.3.1. UrinespecimenacceptanceandRejectioncriteria

2.3.1. Acceptancecriteria

•Inordertoprovidethemostreliablepatientresultspossible,laboratoriesmustadheretostrict guidelines for accepting patient specimens and requisitions.

2.3.2. **Rejectioncriteria**

- Unlabelled, illegibly-labeled, mislabeled, or inadequately labeled specimens.
- Specimens received with incomplete requisition information or without a requisition.
- Discrepancies between specimen label and requisition information.
- Improper patient preparation (e.g. not fasting when required) prior to collection.
- Incorrect time of collection where timing impacts result interpretation.
- Incorrect container for test requested
- Visibly contaminated, leaking, damaged or inappropriate collection containers.
- Inadequate sample size or volume, including over-filled samples.
- Presence of interfering substances: Lipemic, hemolyzed, icteric or clotted specimens.
- Obviously incomplete 24 hour urine collection.
- Syringe specimen with needle attached (attach supplied cap prior to transporting to lab).
- Improper specimen storage or transport temperature.
- Transportation delays to the lab which may adversely affect the test result.
- Bacteriologically or chemically contaminated specimen
- Wrong type /amount of preservative
- Partial loss of specimen or inclusion of two-morning specimen in the 24hrs collection
- Inadequate mixing of specimen before examination
- Careless measuring of the 24 hrs volume
- Mixing of specimen

Role Play: Urine Specimen Collection

Instruction:Deartraineesplaythisroleplaybytakingtheroleofthethreepatientsandthelabpersonnel,onebyone.Attheenddiscusswithyourtrainerabouturinesampleacceptanceand rejection criteria.

Allotted time: 1hrs

a. A 25 year's old female patient came to the laboratory with urinalysis request form. After taking urine cup, she brought urine sample which is mixed with fresh blood. When she was asked she informed to the lab personnel as she was on menstrual cycle.
b. Another patient also brought a urine sample which is turbid. When he was asked, he told to the lab personnel as he mixed small amount of stool for examination.
c. The third patient brought a normal color urine sample with bottle of syrup from his home.

2.4. Sample log and labeling

The label must contain the following legible information**:**

- Patient name.
- Patient medical record number,
- Patient location.
- Collection date and time.
- Specimen type and/or source.
- The initials of the person collecting the sample.
- Test required (note any special handling required)
- Ordering physician.

Potential outcomes of collection and labeling errors:

- Delays in reporting test results
- Unnecessary re-draws/re-tests
- Decreased customer satisfaction
- Increased costs
- Incorrect diagnosis / treatment
- Injury
- Death.

During Labeling:

- Make sure that container label & the requisition match.
- Label should be on the container not on the lid, since the lid can be mistakenly placed on a different container.
- Ensure the labels on the containers are adherent under refrigerated conditions.

Fig, 2.2. Labeling specimen

The laboratory should keep a register (log) of all incoming samples. A master register may be kept, or each specialty laboratory may keep its own sample register.

Assign the sample a laboratory identification number – write the number on the sample and the requisition form. If computers are used for reports, enter the information into the computer.

The register should include:

- Date and time of collection;
- Date and time the sample was received in laboratory;
- Sample type;
- Patient name and demographics, as required;
- Laboratory assigned identification
- Tests to be performed

The laboratory needs a system to allow for tracking a sample throughout the laboratory from the time it is received until results are reported.

This can be done manually by careful keeping of records.

- Confirm receipt of samples, include date and time;
- Label samples appropriately; keep with the test requisition until laboratory Id is assigned;
- Track aliquots–traceable to the original sample**.**

If computers are available, maintain a database for tracking. The following information about each sample should be entered into the database:

- Identification number;
- Patient information;
- Collection date and time;
- Type of sample: for example, urine, throat, cerebrospinal fluid for culture;
- Tests to be performed;
- Name of ordering physician (or other health care provider);
- Location of patient, such as ward, clinic, outpatient;
- Diagnostic test results;
- Time and date results are reported.

Information sheet #5	Preservation and storage of urine specimen

2.5.1. Preservation of urine specimen

Urine should be examined immediately much as possible after it is passed because some urinary components are unstable.

If urine specimen cannot be examined immediately it must be refrigerated or preserved by using different chemical preservatives

The maximum time that urinary contents to be maintained is ***one hour***

Long standing of urine at room temperature can cause

- Growth of bacteria
- Break down of urea to ammonia by bacteria and leading to
  - An increase in the PH of the urine
  - The precipitation of calcium and phosphates
- Oxidation of urobilinogen to urobilin
- Destruction of glucose by bacteria and Lysis of Cells (RBCs, WBCs and Casts) and parasites (T.vaginalis)

Method of preservation of urine specimen

Urine should be examined immediately as much as possible after it is collected, because some urinary components are unstable.

If urine specimen cannot be examined immediately, it must be refrigerated or preserved by using different chemical preservatives.

A. Physical method: Refrigeration and Freezing

B. Chemical method: Use preservation chemicals such as: Thymol, Toluene, Formaldehyde, Hydrochloric acid (HCL), Chloroform, Boric acid, Chlorhexidin.

Urine Preservatives and Methods of Preservation		
Physical methods		
Preservatives	**Advantage**	**Disadvantage**
•Refrigeration	•Chemical Interference	•Use for a short period of time (3-6 hrs)
•Freezing	•Forspecimentransport	•Maydestroyformedelements
Chemical Methods		
Preservatives	**Advantage**	**Disadvantage**
Thymol	Preserves,acetone,Reducing, Substances, protein	Flammable
Toluene	Preserves most constituents	Can cause false positives for proteins
Formaldehyde	Preserves urine aldosterole level	Settles to the bottom of the urine containers
Hydrochloric acid	Preserves formed elements	Interferes with glucose evaluation
Chloroform	Stabilize steroids, catecholamine's	Formed elements are destroyed
Boric acid	Preserves,chemicals,and formed elements	Precipitate uric acid
Sodium carbonate	Preserves,porphyrinesand urobilinogen	

2.5.2. Storage of sample and its components

Sample storage:

Written policies should be developed that include:

- Description of what samples should be stored;
- Retention time;
- Location–consider ease of access;
- Conditions for storage, such as atmospheric and temperature requirements;
- System for storage organization, one method being to store samples by day of receipt or accession number.

Sample retention:

Set a laboratory policy for retention of each type of sample. Some samples can be quickly discarded, and others may need to be retained for longer periods.

Monitor stored samples, and do not keep for longer than necessary, as refrigerator and freezer space may be limited.

Sample freeze/thaw cycles must be monitored, as samples may deteriorate with these conditions.

Planning is required for samples that may need long-term storage.

An organized, accessible system using computer tracking would be useful for these samples.

The inventory of stored samples should be reviewed at specified intervals to determine when they should be discarded.

Sample referral:

When referring samples to other laboratories for testing:

- obtain a laboratory handbook with detailed procedures from each laboratory;
- ensure the sample is labelled correctly, in the correct container, accompanied by a requisition form that specifies the required test(s), and includes the sending laboratory's contact information;
- carefully monitor samples that are referred:
  - keep a record of all tests / samples referred, date of referral, name of person referring the test;
  - record and report results received for each referred sample;
  - Monitor turnaround times and record any problems encountered.

Sample disposal:

The laboratory is responsible for ensuring that disposal of all laboratory waste is handled in a safe manner.

- To ensure proper disposal of patient samples:
- To develop a policy for sample disposal; apply local, as well as country regulations for disposal of medical waste;
- Establish and follow procedures to disinfect samples prior to disposal

Self-check #5	Written exam

Instruction 1:- Matching

Column A	**Column B**
1. Formaldehyde	A. Physical method of preservation
2. Hydrochloric acid	B. Chemical method of preservation
3. Refrigeration and Freezing	C. Preserves urine aldosterole level
4. Unlabelled, illegibly-labeled	D. Interferes with glucose evaluation
5. First morning urine specimen	E. Rejection criteria
	F. Acceptance criteria
	G. Most concentrated urine

Note: -Satisfactory point is above three (>3)

Not-satisfactory point is below three (<3)

Score————————————

Remark————————————————————————

Name————————————————ID. No————————————Date————

Operation sheet #1	Procedures for collecting Random specimen

1.1. Collecting Random Urine Sample

Purpose: Thepurposeofthisactivityistoenablethetraineetopracticeanddevelopskillonhowto **collect random urine specimen** based on the check list provided.

Procedures for collecting random urine specimens

1. Wear gown, glove and other PPE
2. Clean the working bench
3. Assemble the required materials
4. Greet the patient and Receive the request form
5. Checkthe request form for its legality
6. Label the urine collection material
7. Instruct the patient kindly to bring enough volume of urine
8. Receive the sample and Cross check the label on the container and request form
9. Observe the urine sample for acceptance/rejection
10. Observe the urine sample for acceptance/rejection
11. Log (register) the sample on specimen log book (if accepted) or on rejection log book (if rejected)
12. Perform physical examination of urine

Critical aspect of the competency:

- Able to assemble the required equipment
- Able to instruct patient kindly during urine sample collection
- Accept or reject urine sample

1.2. Collecting 24-hrs Urine Sample

Purpose: Thepurposeofthisactivityistoenablethetraineetopracticeanddevelopskillonhowto **collect 24-hrsurine specimen** based on the check list provided.

Procedure for collection of 24 hours urine specimen

1. Wear gown, glove and other PPE

2. Clean the working bench
3. Assemble the required materials
4. Greet the patient and Receive the request form
5. Check the request form for its legality
6. Labeltheurinecollectionmaterial(bottlethatcancontain2literswith preservative)
7. Direct the patient to completely empty his/her bladder and discard his/her urine at the beginning of the 24 hrs time collections,andCollect all urine voided during the following 24 hour
8. Receive the sample and Cross check the label on the container and request form
9. Observe the urine sample for acceptance/rejection
10. Observe the urine sample for acceptance/rejection
11. Log (register) the sample on specimen log book (if accepted) or on rejection log book (if rejected)
12. Performphysicalexaminationofurine(measurevolume,protein concentration)

Critical aspect of the competency:

- Able to assemble the required equipment
- Able to instruct patient kindly duringurine sample collection
- Able to accept or reject urine sample based on the criteria

1.3: Collecting Clean-Catch Urine Sample

Purpose: Thepurposeofthisactivityistoenablethetraineetopracticeanddevelopskillonhowto **collect**clean-catch**urine specimen** based on the check list provided.

Procedures for collecting clean catch urine specimen

1. Direct the patient to clean the genital area with soap and water and rinsed well
2. The patient should urinate a small amount and this is discarded
3. The mid-stream specimen should be collected in to sterile container of 30-50ml
4. After obtaining the specimen the patient continuous to urinate and this is discarded**.**
5. Receive the sample and Cross check the label on the container and request form
6. Observe the urine sample for acceptance/rejection

7. Observe the urine sample for acceptance/rejection
8. Log (register) the sample on specimen log book (if accepted) or on rejection log book (if rejected)
9. Performphysicalexaminationofurine(measurevolume,protein concentration)
10. Store the sample accordingly

Critical aspect of the competency:

•Able to assemble the required equipment

•Able to instruct patient kindly duringurine sample collection

•Accept or reject urine sample based on the criteria

LAP TEST	Practical Demonstration

Instruction: - perform each of the following activities

Project 1: - Collecting urine medical sample

Task1:- Collect random urine sample

Task2:- Collect 24hr urine specimen

Task3:- Perform clean-catch urine sample collection

LG51. Perform urinalysis tests

Welcome to the module “Performing Urine and Body Fluid analysis”. This learner’s guide was prepared to help you achieve the required competence in “**Medical laboratory services Level-III** this will be the source of information for you to acquire knowledge and skills in this particular occupation with minimum supervision or help from your trainer.

Summary of Learning Outcomes

After completing this learning guide, you should be able to:

3.1. Assembling required ***equipment ,materials and systems***

3.2. Selection of the authorized tests

3.3. conduct Individual tests according to standards

3.3.1Physical Examination of Urine

3.3.2Chemical Examination of Urine

3.3.3Microscopic Examination of Urine

3.3.4Body Fluid Analysis

3.3.4.1 CSF Analysis

3.3.4.2 Semen Analysis

3.3.5Applying required quality control procedures

3.4 Recording interpretation of results

3.5 discussing of Colleagues with when result interpretation is outside parameters

3.6 verifying of Results before releasing for clinician/client

3.7 storage of Tested Samples and sample components forretesting when requested

Learning-instructions

1. Read the contents of this Learning Guide. It is divided into sections that cover all the skills and knowledge that you need.

2. Read the information written in the “Information Sheet #1, #2, #3, #4, #5, #6, #7, and # 8”.

3. Accomplish the “Self-check #1on page 6, #2 on page 10, #3 on page 19, #4 on page 30, #5 on page 48, #6 on age 56, #7 on page 61

4. If you earned a satisfactory evaluation on self-check proceed to next learning Guide. However, if your rating is unsatisfactory, see your teacher for further instructions.

5. Read the “Operation Sheet” on page #31, #50, and #57, and try to understand the procedures discussed.

6. Practice the steps or procedures as illustrated in the operation sheet. Go to your teacher if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedures

LO3. Perform urinalysis tests

3.1. Assembling required ***equipment ,materials and systems***

1. The necessary materials used for the collection, centrifugation and examination of urine specimens are:

- ✓ Clean dry plastic or Glass containers, which enable to collect at least up to 15 ml of urine for routine urinalysis.
- ✓ Hand (manual) or electrical centrifuge.
- ✓ Conical centrifuge tubes, or regular test tubes.
- ✓ Pasture pipette with rubber fit or automatic pipettes if possible.
- ✓ Slides and cover slides 20 x 20 mm.
- ✓ Electrical or solar microscope, which has 10x and 40x objectives.

2. Preparation of patient

- Explain the purpose of the test by using simple language. Do not use medical terms or try to explain details of the procedure.
- Advise the patient how to collect the specimen. The first morning urine or mid-stream urine specimen is more preferable, because it is more concentrated.
- If the patient is female, advice her to wash her genital organ before giving the specimen. This is because bacteria that are normally found on the genital tract may contaminate the sample and affect the result.
- Advise the patient to collect at least 15 ml of urine in to the clean, sterilize and dry urine cup that is supplied from the laboratory.

5.1.2. Source of Errors in the Microscopic Examination of Urine

Possible errors that may encounter during microscopical examination ofurine include:

- Drying of the specimen on the slide.
- During trial of observing 2 specimens in a single slide by putting ateach side of slide, (mix up of the specimens).
- If the supernatant fluid after centrifugation is not poured off properly,that is if some drop is left in the tube, it may decrease concentrationof urine sediments and false result may be reported

- If the whole sediment with supernatant is discarded during invertingdown the tube for long period, the whole sediments will be discardedand so again false negative result will be reported.

Self-check #1	**Written tests**

Instruction 1:- Say true or false for each of the following question

1. Explaining the purpose of the test by using simple language is better than using medical terms or try to explain details of the procedure.
2. If the whole sediment with supernatant is discarded during inverting down the tube for long period, may cause false negative result to be reported.
3. Drying of the specimen on the slide is the possible sources of error.

Instruction 2:- Answer the following question appropriately

1. List at least 3 sources of error during urine analysis?
2. List at least 3 materials used for examination of urine?

Score————————————

Remark————————————————————————

Name————————————————ID.no————————Date————————

3.2. Type of Examination in Routine Urinalysis

Physical Examination of Urine

- Volume
- Color
- Odor
- PH
- Appearances
- specific gravity

Chemical Examination of Urine

- Glucose
- Protein
- Ketones
- Bilirubin
- Urobilinogen
- blood
- nitrite
- leukocyte esterase
- Indican
- Melanin

Microscopic Examination of Urine

- RBCs
- WBCs
- Epithelial cells
- Casts
- Bacteria
- Yeasts
- parasites
- crystals

Categories of Urine Tests

According to their degree of accuracy urine tests are grouped into three broad categories:

- Screening tests
- Qualitative tests
- Quantitative test

Methods for Examining Urine Sediments

A. Unstained Urine Sediment

1. Bright field microscopy of the unstained urine sediment

Traditionally, the urinary sediment has been examined microscopically by placing a drop of urine sediment on a microscopic slide, cover with cover slide and observing the preparation with the lower and high power, objective of the bright field microscope.

When the sediment is examined under the bright field microscope, correct light adjustment is essential, and the light must be sufficiently reduced, by the correct positioning of the condenser

and the irisdiaphragm to give contrast between the unstained structures and theback ground liquid.

2. Phase Contrasts (PC)

P.C. illumination is useful in the examination of unstained urinarysediment, particularly for translucent elements such as hyaline castsand mucus threads, which have a refractive index similar to that of urinein which they are suspended. Phase contrast has the advantage ofhardening the outlines even the most ephemeral formed elements.

B. Stained Preparation

Cellular detail is best seen with stained preparation

The following stains are commonly used:

1. A crystal violet safranin stain (sternheimer and malbin) is useful in the identification of cellular elements.

Procedure

Add 1 or 2 drops of crystal violet safranin stain to approximately 1 ml ofconcentrated urine sediment. Mix and place a drop of this suspensionon a slide and cover with cover slide.

Staining reaction to crystal – violet safranin stain:

RBC – Purple to dark purple.

WBC – Cytoplasm -violet to blue.

Nucleus – reddish purple.

Glitter cells – blue.

Cells	**Nucleus**	**Cytoplasm**
Squameous epithelial cells	**Purple**	**Pink to violet**
Euro epithelial	**Dark blue**	**Blue**
Renal tubular cells	**Dark purple**	**Orange purple**

2. Methyl blue (Loffler's stain)

3. CytoDiachrome stains

When such stains are used, it is recommended that both the stained andunstained sediment be mounted and observed, as the stain may causeprecipitation of some constituents. This is especially the problem withalkaline urine specimens, because the precipitated materials mayobscure important pathological constituents.

***II. Table 3. Relationship between Physiochemical and Microscopic Findings of Urine in Selected Disease States*.**

Physical Findings	Chemical Finding	Microscopic	Observation
Colored brown Turbidity Specific gravity	Protein + Blood +	WBC, RBC Hyaline or Granular or Cellular casts	Acute Glomerulonephritis
Urine volume Turbidity Odor pH	Protein + Blood + Nitrite +	- RBC, WBC - Cellular casts, - Bacteria	Acute tubular Necrosis Or lower Nephrosis
Specific gravity Urine volume Specific gravity	Protein Blood Protein + Blood +	Colorless Hexagonal Plate crystals	Cystinosis
Specific gravity Odor- sweet	Protein Glucose Ketone	Yeasts Some times Present	Diabetes Mellitus
Color darker	Glucose + Ketone + Blood + Bilirubin + Urobilinogen+	Pigment laden Prussian blue Casts	Hemochromatosis
Turbidity	Portion + Blood +	Casts Oval fat Bodies	Nephrotic Syndrome
Specific gravity	Protein + Blood +	Sickled RBC	SickleCell Syndrome
Turbidity	Protein +	RBC, WBC Casts	Systemic lupus Erythematosus

III. Table 4: Correct and Incorrect Approach in Urine Testing

Correct approach	**Incorrect approach**
Use fresh urine	Delay in the testing of urine without Preservation
Make quality control of reagents	Using expired reagents
Be aware of normal as well as abnormal results which are significant	Believing urine results have little significance in the overall diagnostic picture of the patient
Follow the directions carefully	Being careless
Accept only clear and proper collection bottles	Using any container.
Be familiar with interfering Substances	Not giving due attention to cross reaction and artifacts
Mix Urine properly	Not mixing well
Record results accurately	Not checking the results recorded during the training of new personnel
Give proper training to Professionals	New personnel always jumping into urinalysis because it is the easiest to do and least significant

Self-check #2	Written tests

Instruction 1:- choose the best possible answer for each of the following questions?

1. Which of the following is the possible source of error in the microscopic examination of urine?
   A. Inadequate centrifugation C. Inadequate centrifugation
   B. Drying of the specimen on the slide D. All
2. Identify the factors that may not results in falsely increase in high number of RBCs?
   A. Menstrual bleeding C. Renal stone
   B. Vaginal bleeding D. Aspirin ingestion or over dose
3. Which one is not included in the chemical examination of urine?
   A. Ketones B. Leukocyte Esterase C. Crystals D. Nitrite
4. Creatinine clearance is most often used to monitor GFR because of the following reasons except?
   A. Creatinine is an endogenous substance C. Creatinine freely filtered by glomerulus
   B. Creatinine is reabsorbed by tubules D. None
5. The correct method for labeling urine specimen containers is to.
   A. Attach the label to the lid C. Attach the label to the container
   B. Attach the label to the bottom D. Use only a wax pencil for labeling
6. A urine specimen for routine urinalysis would be rejected by the laboratory because:
   A. The specimen had been refrigerated C. The label was placed on the side of the container
   B. More than 50 ml was in the container D. The specimen and its requisition did not match
7. A sagital/inner section of the kidney reveals three distinct regions. Which alternatives show those regions from outer to inner in sequences?
   C. Pelvis→Cortex→Medulla C. Pelvis→ Medulla → Cortex
   D. Cortex→Medulla→ Pelvis D. Medulla→ Pelvis→ Cortex
8. Which of the following specimen type is/are commonly used for microbiological tests?
   A. Random urine specimen C. Midstream urine specimen
   B. Terminal urine specimen D. 24-Hour urine specimen
9. A sagital/inner section of the kidney reveals three distinct regions. Which alternatives show those regions from outer to inner in sequences?

E. Pelvis→Cortex→Medulla C. Pelvis→ Medulla → Cortex

F. Cortex→Medulla→ Pelvis D. Medulla→ Pelvis→ Cortex

10. The type of urine specimen that taken at any time of the day that the pts attend the diagnostic laboratory is termed as--------?

A. Early morning specimen C. Random urine specimen

B. Midstream urine specimen D. Clean catch urine specimen

Note:- Satisfactory point is above five (>5) Not- Satisfactory point is below five (<5)

Answer sheet

Instruction 1

1. ———
2. ———
3. ———
4. ———
5. ———
6. ———
7. ———
8. ———
9. ———
10. ———

Score———————————— Remark————————————————————

Name————————————————ID.no————————Date————————

3.1. Physical Examination of Urine

- Physical examination of urine is the first part of routine urinalysis.
- It is the simplest procedure of all urine examination, but this simplicity does not mean that anyone can do it without any background knowledge and experience.
- Physical examination of urine usually gives hint for the subsequent urinalysis.
- For example, white turbid urine sample may suggest to the technician the presence of Leukocytes (pus cells) and/or
- Epithelial cells in microscopic examination, and in chemical examination, with positive result of Nitrite.

3.1.1. Volume

Normally, 600 – 2000 ml of urine is voided per 24 hr.

Volume of urine excreted is related to:

- ✓ Individual fluid intake
- ✓ Body temperature
- ✓ Climate
- ✓ Individual's health status

Abnormally higher amount (greater than 2000 ml/24) or very low amount i.e. less than 600 ml/24hr occur mostly due to some pathological conditions.

For the measurement of the volume of urine, the patient should collect 24 hr urine specimen.

Clinical Significance

The Measurement of the volume of urine indicates the evaluation of fluidbalance and kidney function.

When an individual excretes more than 2000 ml of urine/24 hr, consistently (for long period) it is called **Polyuria.**

It may occur due to:

- ✓ Diabetic mellitus
- ✓ Diabetic insipidus
- ✓ Certain tumors of brain and spinal cord
- ✓ Acromegaly
- ✓ Myxedema
- ✓ Some type of tubular necrosis (improper function of urine tubules)

Diuresis: Any increased amount of urine volume, even if for short period. It is usually due to excessive fluid intake.

Oliguria: Excretion of constantly small amount of urine, i.e. below 400 ml of urine/24 hr. It may occur due to:

- ✓ Dehydration or poor blood supply to kidney that may be due toprolonged vomiting, diarrhea, etc.
- ✓ Obstruction of some area of the urinary tract/system (mechanical)
- ✓ Cardiac insufficiency
- ✓ Various renal diseases such as glomerulonephritis, etc.
- ✓ Fasting
- ✓ Excessive salt intake etc.

Anuria :Complete absence of urine excretion. It is less than 100 ml of urine per 24 hr. It may occur due to:

- ✓ Complete urinary tract obstruction
- ✓ Acute renal failure
- ✓ Acute glomerulonephritis
- ✓ Hemolytic transfusion reaction, etc

Polyuria: may result physiologically after consumption of

- ✓ Intravenous glucose or saline
- ✓ Coffee, alcohol, tea, caffeine
- ✓ Pharmacological agent, such as thiazides and other diuretics

2. Odor

Normally fresh voided urine from healthy individuals has faint aromaticodor, which comes from volatile acids, normally found in urine, mostly, ammonia.

The test is conducted by smelling of urine and the result is based on the perception of the technician.

Clinical Significance

Abnormal urine odor may result from aging of urine, disease and diet.

If the urine specimen is old, i.e. after collection, left on the bench without preservative for more than 2 hrs, it will have ammonical (pungent) odor.

The ammonical odor result is due to break down and conversion of urea in the urine into ammonia by the action of bacteria.

Cystinuria and homocystinuria (type of amino acids, voided fromabnormal metabolism) have sulfurous odor.

Oasthouse urine disease has a smell associated with the smell of abrewery (yeast).

Tyrosenemia is characterized by cabbage like or **“fishy”** urine odor.

The presence of ketone bodies in the urine, that may be due todiabetes mellitus, vomiting, starvation, strenuous exercise,characterized by **“sweet fruity”** odor.

Butyric / hexanoicacidemia produce a urine odor resembling that ofsweat.

Urine of infants, which has inherited amino acid metabolismdisorder, smells like **“burnt sugar”** or maple, hence the name, ***“maple sugar urine disease”.***

Also due to some food stuff such as asparagus, characteristic, urineodor is produced, which has no clinical significance.

3. Foam

Normally when urine specimen is voided in a container, it produces smallamount of white foam. But during certain abnormal physiological andmetabolic conditions, the color and amount of foam may be changed.

- ✓ For example, when there is high bile pigment in the urine, the amount offoam increases, and the color of foam becomes yellowish. This mayindicate the presence of bilirubin in the urine.
- ✓ But the presence ofyellowish foam should not be taken as a confirmatory test for thepresence of bilirubin in urine. Chemical analysis of urine for bilirubinshould be done.

4. Color

Normally color of urine may vary within a day; in the morning it has darkyellow color, while in the afternoon or evening, the color ranges fromlight yellow to colorless.

Normal urine color varies from straw (lightyellow color) to dark amber (dark yellow).

- ✓ Light yellow indicate that the urine is more diluted, and has lowspecific gravity. Such exceptional condition occurs in case ofdiabetic mellitus. In this condition the color of urine is mostly lightyellow, but because of having high glucose content, its specificgravity is high.
- ✓ On the other hand, dark amber (dark yellow) color mostly indicatesthat the urine is concentrated, and has high specific gravity. Thistype of urine is seen normally in the first morning urination.
- ✓ Normal urine color results from three pigments. They are:

1.**Urochrome**, responsible for yellow color formation. This pigments found in high proportion than the other two.

2.**Uroerythrin**, – responsible for red color formation.

3. **Urobilin**, – responsible for the orange-yellow color formation.

Thus, normal urine gets its color from a combination of theabove-mentioned three pigments.

Procedure of the Test

Urine color is recorded, after looking at freshly voided urine specimen. Ifthe urine sample color is not recorded within 30 minutes after collection,chemical changes will occur in it, and so its color will change, and willresult in false report.

Clinical Implication

By observing the color of freshly voided urine, an experienced laboratorytechnician can forecast the possible findings in the chemical andmicroscopical examination of urine. Depending up on the constituents ofurine, the abnormal color of urine varies as follows:

 Pale to colorless urine may indicate:

- Large fluid intake
- Diabetic mellitus
- Diabetic insipidus
- Alcohol consumption
- Nervousness

 Dark yellow or brown red urine may indicate:

- Concentrated urine
- Decreased fluid consumption
- Dehydration
- Fever
- Certain urinary tract medication (e.g. phenazophyridine)

• Yellow brown or “beer brown” color may indicate the presence ofbilirubin.

This is also confirmed:

- By looking at the yellow foam or green foam by shaking thesample.
- By letting it to stand for more than 30 minutes and looking atthe change of color into green, because of oxidation ofbilirubin into biliverdin.
- Due to bilirubin crystals, as mentioned in urine segment, theurine samples have opalescent appearance.
- By doing chemical tests for bilirubin.

 Clear red may indicate presence of Hemoglobinuria (presence ofhemoglobin in the urine).

This hemoglobinuria may result from:

- Incompatible blood transfusion.

- Increased red blood cell destruction (intravascularhaemolysis) due to different hemoparasites, e.g. Malaria.
- Glucose – 6-phosphate dehydrogenase deficiency.
- Certain infections or disease.

 Cloudy red / smoky red color may indicate hematuria (presence ofred blood cell in the urine).It differs from clear red by the presence ofRBC rather than Hgb alone. It is important to differentiate hemoglobinuria from hematuria, because the cause of this abnormalurine differs. On standing the red cells in hematuria may hemolyze and settle, and so the urine becomes clear red (hemoglobin in urine).

To differentiate this definition; specific gravity is important.

- ✓ Hematuria has high specific gravity than hemoglobinuria.

 Dark brown colored urine may contain porphyrines, melanin,homogenstic acid, which is associated with an abnormal metabolismof tyrosine. Milky urine may contain fat, cystine crystals, and manyWBC or amorphous phosphates.

Dark reddish color may indicate myoglobin (muscle Hgb), usually associated with extensive muscleinjury, hemoglobinuria and porphyrine.

Interfering Factors

It is usually important to consider, that on standing of urine for more than 30 minutes, the urobilinogen that is found in urine will oxidize andchange to urobilin. Thus due to this process, the color of urine becomesdark. Therefore, the physical examination of urine should be doneimmediately after the delivery of urine to the laboratory.

Other interfering factors that result in abnormal urine color formation arecertain foodstuff, and medications.

- ✓ Food stuff, such as beets will give white red color.
- ✓ Drugs such as Vitamin B12 and riboflavin will give bright yellow colorto urine.
- ✓ Rifampicilin will give red color to urine.
- ✓ Iron salt will give dark color to urine.
- ✓ Sulfonamides will give rusty yellow or brownish color.

Therefore, when abnormal colored urine is observed, it is important toask the patient, what kind of food he consumed in the last 36-24 hrs, andalso whether he used drugs or not. If so, it is important to know whatfood and what drug he used.

5. Appearance (Transparency)

Fresh voided urine specimen is normally clear and transparent. On longstanding, due to chemical changes that occur in normal constituents ofurine through time, it becomes turbid.

Procedure of the Test

 Appearance (transparency) of urine can be measured only byobservation of fresh voided urine specimen.

 Degree of cloudiness of the urine is described by using commonterms, starting by clear to turbid i.e. clear, hazy, cloudy, very cloudyand turbid.

Clinical Implications

Freshly voided urine specimen appearance may indicate the presence ofsome abnormal constituents in it. Causes of turbid urine, as it is freshly voided include:

- White blood cells (pus cells) that occur due to UTI
- Kidney stones
- RBC's
- Yeast cells,
- High number of bacteria cells
- High number of epithelial cells
- Fat droplets in urine, which give opalescent appearance (rarecondition).
- Amorphous urates, in case of gout and leukemia.
- High number of mucus trades.

All the above findings are confirmed by urine microscopic examination.

Interfering Factors

High consumption of foodstuff that contains urates and phosphates mayproduce cloudy urine. This is because of the precipitation of urates andphosphates in the form of amorphousurate and phosphates respectively.

Semen, or vaginal discharge mixed with urine is other common causesof urine turbidity. Urine specimen, stood for long period in the bench, willbecome hazy or cloudy due to precipitation of crystals, mucus tradesetc., which normally occur in urine. The settlements of crystal andmucus trades seen in urine sample are to be preserved in refrigerator.

Amorphous urates have **"Brice red"** precipitation, while amorphousphosphates have **white** precipitations.

Clinical Significance

As indicated in the chapter one, one of the functions of renal system is toregulate pH of blood i.e. keeps pH of blood at 7.4 + 0.05. This is done byabsorption or release of hydrogen ion, especially at distal convolutedtubules of the nephron, depending on the pH of blood, i.e. hydrogen ionabsorbed from surrounding blood capillaries of nephron when pH isacidic (below 7.35), and release from nephron to the surrounding bloodvessels when pH of blood is alkaline (above 7.45).pH measurement of urine, like other physical tests of urine, may indicatethe on-going process in body, mostly about the renal system.

Normal pH of urine is 5-6.

***Persistent alkaline urine (pH > 6) may be caused by**:

- ✓ □ UTI
- ✓ □Renal failure
- ✓ □ Vomiting
- ✓ □ Anorexia nervosa
- ✓ □ Alkalosis (metabolic or respiratory e.g. due to accumulation CO2 inour body.
- ✓ □ Alkalizing drugs i.e. during intake of drugs such as streptomycin, kanamycin etc.
- ✓ □It should also important to bear in mind that certain vegetables,citrus fruits, and milk products also may cause alkaline urine, whichis not pathological

*** Persistent acid urine (pH < 6) may be caused by:**

- □ Diarrhea
- □Malabsorption syndromes
- □ Diabetic ketoacidosis
- □ Dehydration
- □ Fever
- □ Starvation
- □And also certain drugs such as – Phenacetic
- □Here it is important to bear in mind that high protein diet may alsoresult in acidic urine, but this is not a pathological condition.
- □ pH measurement is also important in the management of renal stonepatients, who are being treated for renal calculi and who arefrequently given diets or medications to change the pH of the urineso that kidney stone will not form.

□ Calcium phosphates, calcium carbonate, and magnesium phosphatestones develop in alkaline urine. In such instances the urine mustbe kept acidic (i.e. either by diet such as meat, or medication).

 Uric acid, cystine, and calcium oxalate stones are precipitated inacidic urine. Therefore, as part of treatment, the urine should bekept alkaline (either by diet e.g. leguminous plants, citrus fruits andmost vegetables or by medication).

Interfering Factors

If urine specimen is left on the bench for more than 2 hours, bacteria willgrow in it and by converting urea into ammonia, the pH will becomealkaline. This is false alkaline urine, and indicates the specimen in notfreshurine.

6. Specific Gravity of Urine

Specific gravity is defined as the ratio of the weight of a fixed volume ofsolution to that of the same volume of water at a specified temperature,usually 20o C (in some books 25oC). The specific gravity of urine hasbeen used for years as measure of the total amount of materialdissolved in it (total solids), and thus of the concentrating and excretorypower of the kidneys.

Measurement of Specific Gravity

The following methods are used to test the specific gravity of urine:

- Urinometer
- Refractometer
- Reagent strip
- Weighing technique

Specimen: It should be the first urine passed at the beginning of the daywith the patient having taken no fluid for 10 hours. The testing of randomurine specimen has little clinical value.

1. The Urinometer

The specific gravity of a urine specimen is often measured with urinometer.

The urinometer is a glass float weighted with mercury, withan air bulb above the weight and a graduated stem on the top.

It is weighted to float at the 1.000 graduations in distilled water whenplaced in a glass urinometer cylinder or appropriate sized test tube. It isimportant that the cylinder, or test tube, be of the correct size so that theurinometer can float freely. The specific gravity of the urine is readdirectly from the graduated scale in the urinometer stem.

The scale ofthe urinometer is calibrated from 1.000-1.060 with each division beingequal to 0.001.

Sources of Error:

- Temperature differences

- Proteinuria
- Glycosuria
- X-ray contrast media, it increases urine specific gravity
- Chemical preservatives

Urinometer Controls:

The following solutions can be used to check Urinometers:

Solutions Specific gravitypure water 1.000

Sodium chloride solution (2.5 g/dl) 1.018

“““(5 g/dl) 1.035

“““(7.5 g/dl) 1.051

2. **Refractometer**

It is an instrument, which reads the refractive index of the urine. There refractive index measurement depends on the number of dissolved particles in the urine.

The higher the concentration of the particles the greater the refractive index, and so the specific gravity.

3. **Reagent Strip Test of the Specific Gravity of Urine**

A test area to determine specific gravity in urine can be found in the multiple test strip of Ames called N-multistix. The reagent test area responds to the concentration of ions in the urine. It contains certain pretreated polyelectrolyte. The pKa of which changes depending up on the ionic concentration of the urine .The indicator bromothymol blue is used to detect the change.Colors ranges from deep blue when the urine is of low specific gravitythrough green to yellow- green when the urine is of high ionicconcentration.

Self-Check 3	Written Test

Instruction1:- Say True or False

1. Urine color and urine concentration commonly vary together.
2. The normal yellow color of the urine is due primarily to uroblin, uroerythrin and urochrome.
3. A turbid urine specimen always indicates a pathologic condition.
4. The incidence of turbidity of the urine increases following refrigeration..
5. The pH of the urine usually rises after collection due to the growth of urea splitting bacteria, which produce ammonia.

Answer sheet

a. ———
b. ———
c. ———
d. ———
e. ———

Score————————————
Remark————————————————————————

Name————————————————ID.no————————Date————————

3.3.2. Chemical analysis of urine

Chemical analysis of urine is an important procedure, in the detection of many diseases. Urine contains normalchemical compositions. But in abnormal conditions itscomposition varies in kind and quantities. So the chemical changesof urine can indicate disease at the early stage. The compositionof urine varies because it is the principal route for soluble wastematerial from body metabolism. Its composition therefore dependsgreatly on how much and what specific waste material is to beexcreted.

- Urea, creatinine, uric acid, ammonium salts, chlorides,sulphates and phosphates of sodium, potassium, calcium and magnesium are the **normal composition of urine.** They are excretedthrough the urine as a final body metabolism.
- Glucose, protein,ketone bodies, bilirubin , bile salts…etc are the **abnormal constituentsof urine.** Normally these substances do not appear in the urine indetectable amount. So their appearance in the urine shows thepathological condition.
- For example, glucose does not appear in theurine in detectable amount. But during diabetes mellitus it appears inthe urine. Protein also appears in the urine during renal disease.Generally the chemical examination of urine helps to investigate thehealth condition of individual.

1. Determination of Urinary Sugar (Glucose)

Glucose, a monosaccharide, is the principal sugar in blood, serving thetissues as a major metabolic fuel. It is mainly the end- product ofcarbohydrate digestion, which provides energy for life process. Whenbody requires energy glucose oxidized to pyruvate and then to acetyl-CoA and enter cycle Krebs (tricarboxilic acid, TCA cycle). Along thesemetabolic processes it gives energy in the form of adenosinetriphosphate (ATP). ATP is very important energetic organic compoundused for proper body function. When glucose is not required for thebody's immediate energy needs, it is converted to glycogen and storedin liver and muscles by the metabolic process called glycogenesis. Whenthere is an excess glucose in the blood (especially after carbohydratemeal), it can be also converted to fats. Glucose first oxidized to acetyl- CoA through glycolysis. The formed excess acetyl-CoA and thenconverted to fats to be stored in the tissue. When it is required tomaintain the blood glucose level, particularly during starvation, glycogenis converted to glucose by glycogenolysis. For maintaining the bloodglucose level, it can be synthesized from non-carbohydrate precursorslike amino acids, glycerol, lactate and etc. by the metabolic process,which is called gluconeogensis. The blood glucose level is controlled bya hormone, insulin, which is produced by the beta-islets of Langerhansof the pancreas.

Insulin lowers the content of the glucose in the bloodand increases its utilization and storage in the liver and muscle asglycogen. The absence or lower production of insulin resulted inDiabetes mellitus, which is characterized by an elevated blood glucoselevels (hyperglycemia) and accompanying glycosuria and may beaccompanied by changes in fat metabolism.

Glucose is the sugar most commonly found in the urine, althoughother sugars, such as lactose, fructose,galactose, and pentose, maybe found under certain condition. Normally, urine does not contain asufficient amount of sugar to react with any of the popular enzymeor reducing tests. When sugar appears in the urine, it shows theabnormality caused by disease diabetes mellitus.Hence urine sugartests are extremelyuseful in monitoring the treatment of diabetes.

Clinical Significance

The presence of detectable amount of glucose in the urine isknown as glycosuria**.** Normally almost all the glucose, which passesfrom the blood into the glomerular filtrate, is reabsorbed back into thecirculation by the kidney tubules(proximal convoluted tubules). Usually less than 15 - 20 mg/dl (0.8mmol) is excreted in the urine. But this amount cannot be detectedby the routine laboratory tests. The term glycosuria is usually used todescribe the presence of more than the normal amount (15- 20 mg /dl)of glucose in the urine.

The occurrence of glucose in the urine is not normal if more than 15 – 20mg/dl. The blood glucose concentration normally lies between 65 and110 mg/dl. After a meal it may increase to 120 - 160 mg/dl. If the bloodglucose concentration becomes too high (usually greater than 170 – 180mg/dl), the excess glucose will not be reabsorbed into the blood andglucose start appearing in urine. The lowest blood glucoseconcentration that will result in glycosuria is termed as the renalthreshold. The most common condition in which the renal threshold forglucose exceeds is diabetes mellitus.

Causes of Glycosuria

- Physiological
- Pathological

1. Physiological

Sometimes under physiological situations, glycosuria can occur

- ✓ After large ingestion of carbohydrates
- ✓ Anything that stimulates sympathetic nervous system such asexcitement, stress etc.
- ✓ 15 to 20% cases of pregnancy may be associated withphysiological glycosuria.

- ✓ Renal Glycosuria: In some persons, glycosuria is found whenblood glucose is in normal range. This is known as renalGlycosuria. This is again due to lowered renal threshold. Usuallythis is a benign condition.

2. Pathological Glycosuria

A. Diabetes mellitus

The most common condition for glycosuria is diabetes mellitus, ametabolic disorder due to deficiencies of insulin.

Glucose is not properlymetabolized and blood glucose concentration rises, and when it is inrange of 170 - 180 mg /dl , glucose starts appearing in urine.

B. Glycosuria due to other endocrine disorders

Deranged function of a number of endocrine disorders can causehyperglycemia and this may result in glycosuria.

e.g. - Hyperthyroidism
- Hyperadrenalism
- Hyperpitutarism

Types of Urinary Sugar (Glucose)Tests

- Test for urine sugar is used to detect diabetes mellitus and alsoused to monitor the effectiveness of diabetic control.
- There are various tests for glucose which may be applied tourine.

The most frequently used are:

A.**Non specific reduction tests**based on the reduction ofcertain metal ions by glucose;

B. specific (Enzymatic) testsbased on the action of glucose oxidase onglucose.

A. Non- Specific Tests for Glucose

These tests are based on the ability of glucose to act as reducingsubstances. Tests that are based on the reducing ability of glucose arenot specific for glucose. In these tests, glucose is acting as a reducingagent, and any compound with a free aldehyde or ketone group will givethe same reaction. Hence Glucose is not the only reducing substancethat may be found in urine. Urine contain non-glucose reducingsubstance (NGRS) such as: uric acid, creatinine, galactose, fructose,lactose, pentose, ascorbic acid, chloroform,and formaldehyde.

Commonly used non-specific tests for urinary sugar are **Benedict'sQualitative Test** and the **Clinitest Tablet Test.**

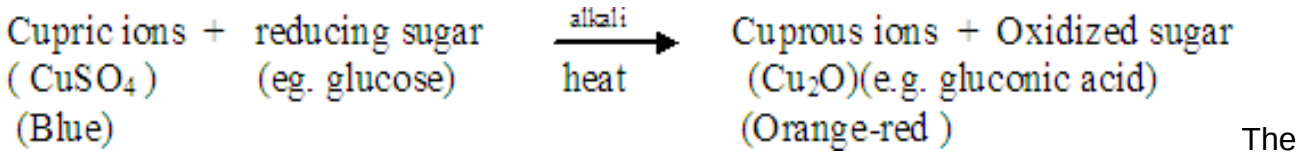
1. Benedict's Qualitative Test

Benedict is a very sensitive copper reduction test and may givepositive reactions with non-specific non-glucose reducing substancesnormally present in urine. Since glucose is the reducing agent, it isoxidized to gluconic acid. The positive reaction is indicated by a colorchange. It is a qualitative test in which the degree of color formation isproportional to the amount of reducing substance present in thespecimen and the results are graded as negative, trace 1+, 2+, 3+, and4+.

Principle

When boiled in an alkaline copper sulphate solution, glucose and otherreducing substances reduce (convert) the blue copper (II) in Benedict'squalitative reagent to copper (I) oxide (Cu2O), which is orange to red incolor. A positive reaction is graded as a change in color ranging fromblue to green, yellow, orange and finally red.

The overall reaction is*:*



The copper (II) ions are supplied in Benedict's qualitative reagent in theform of copper sulphate (CuS04). In the presence of a strong alkali this isconverted to copper (I) oxide (Cu2O). The heat is supplied by meansof a boiling-water (100Oc) bath. The tubes are brought back to roomtemperature, and the results are read when convenient.

Grade results according to the following criteria:

Negative: No change in the blue color of the reagent or the occurrenceof a white or green precipitate from phosphates in the urine.

Trace: Slight amount of yellow precipitate with a greenish blue to bluishgreen mixed solution. (This represents less than 500mg/dl ofsugar).

+ : Moderate amount of yellow precipitate with green, often referredto as apple green, mixed solution. (Approximately 500mg/dl ofsugar).

++: Large amount of yellow precipitate with a yellowish green, oftencalled muddy green mixed solution. (Appr. 750mg/dl of sugar).

+++: Large amount of yellow precipitate with green yellow, or muddyorange, mixed solution. Some blue color remains insupernatant.(Appr. 1000mg/dl of sugar)

++++: Large amount of yellow to red precipitate with reddish yellowto red mixed solution. No blue remains in the supernatant.(Appr. 2000mg/dl)

A .Specific (Enzymatic) Tests

Enzymatic tests are specific tests for glucose. They are reagent strips(dipsticks), which are impregnated with enzymes glucose oxidases.

Glucose oxidase catalyzes only the oxidation glucose to gluconic acidand hydrogen peroxide. The principle of all enzymatic, which is basedon the uses of glucose oxidase, is the same. They differ only on theuses of different type of chromogen (a color indicator).

1. Clinistix Reagent Strip Test

Principle

This is a specific test for glucose based on the use of the enzymeglucose oxidase, which is impregnated on a dip strip. In this test glucoseoxidase oxidizes glucose to gluconic acid and at the same timereduces atmospheric oxygen to hydrogen peroxide. The hydrogenperoxide formed, in the presence of the enzyme peroxidase,oxidizes the reduced form of o-toluidine(a chromogen) to oxidizedform of the indicator, which produces a color change proportional tothe amount of glucose in the urine.

A positive reaction is seen as a change of color from red to blue,depending on the amount of glucose present in the urine.

Step 1: Glucose + O_2 $\underline{\text{Glucoseoxidase}}$→Gluconicacid + H_2O_2

(In urine) (From air)

Step 2:H_2O_2 + reduced form of dye $\underline{\textbf{Peroxidase}}$→ **Oxidized form of dye + H_2O**

(o- toluidine) (Red) (Oxidized o- tolidine) (Bule)

Sensitivity: Clinistix is more sensitive to the presence of glucose than Benedict's Test or the Clinitest tablets and will detect 100mg/dl of glucose or less inthe urine.

Precautions:

 Observe the precautions in the literature supplied with the clinistixstrips. The test area must be completely moistened, but excessivecontact with the specimen will dissolve the reagents from the strip.

The result must be read within 10 seconds. Falsely positive resultsmay be obtained.

Large concentrations of ascorbic acid (vitamin C) cause falsenegative results or results that are delayed for 2 minutes or so, whilebleach or peroxide may cause falsely positive reactions.

2. Diastix Reagent Strip for Glucose

Principle

Diastix is a specific test for glucose based on the use of glucoseoxidase, which is impregnated on the reagent strip. The chemicalreaction is the same as for clinistix, the difference being the chromogensystem used to indicate the presence of glucose. The reagent areacontains glucose oxidase, peroxidase, a blue background dye, andpotassium iodide as the chromogen. In a positive reaction oxidation ofpotassium iodide results in the formation of free iodide, which blends with the blue background dye to give shades of green through brown (The Boeringer dip-strip Test is also based on the same principle). As withclinistix, large amounts of ascorbic acid may give falsely negative ordelayed results for glucose. This suppression is not as great as withclinistix, but it may cause problems.

Bleach and hydrogen peroxidemay cause falsely positive reactions, as with Clinistix.

Diastix has the advantage of being suitable as a screening test for thepresence of glucose in the urine, and giving a rough estimate of theamount of glucose present. It detects as little as 100 mg of glucose per100 ml of urine. However, urine specimens from pediatric patients mustbe subjected to a non-specific test for urinary sugar (Clinitest orBenedict's test) in addition to the specific glucose screening test in orderto detect the presence of sugars other than glucose.

Sensitivity

Diastix reagent strip detects as little as 100mg of glucose in 100 ml ofurine.

4.2 Determination of Ketone Bodies

Ketone bodies are normal products of fat metabolism. They are normallynot detectable in the blood or urine. In normal metabolism, fat is brokendown in the tissues to glycerol and fatty acids. The free fatty acids aretransported by the plasma albumin to the liver where they are brokendown to acetyl coenzyme A (acetyl Co-A) molecules. These condensewith oxaloacetate in the Krebs cycle to produce citrate. The citrate isthen oxidized to produce heat and energy. Whenever there isinadequate carbohydrate in the diet or a defect in carbohydratemetabolism or absorption, the body metabolizes increasing amounts offatty acids, which is then converted into excessive amount of acetyl-CoA.The extra acetyl-CoA molecules join up in pairs to form acetoacetic acid.Most of this is reduced to β-hydroxybutric acid while some isdecarboxylated to acetone. Acetoacetic and β-hydroxybutric acids aretransported in the blood to the peripheral tissues to serve as analternative fuel for cells. In the peripheral tissues these ketone bodiesare reconverted to acetyl- CoA, and oxidized by the tricarboxilic acidcycle to give energy. Acetone is excreted in the urine.

Clinical Significance

When the rate of formation of ketone bodies is greater than the rate oftheir use, their levels begin to rise in the blood, which is calledketonemia, and eventually in the urine, which is known as ketonuria.

These two conditions are seen most often in cases of starvation anddiabetes mellitus. Ketone bodies can be seen also in the urine duringprolonged vomiting, severe diarrhea, anesthesia, severe liver damage,high fat intake and low carbohydrate diet.

The excessive production and accumulation of ketone bodies may leadto ketosis.

Its physiological effect is serious because acetoacetic acid and β-hydroxybutyric acid contribute excess hydrogen ions to the blood,resulting in acidosis - a condition that tends to lower the blood pH. If notcorrected in time this may result in death.

Another physiological effect of ketone accumulation concerns thesubstance acetone and acetoacetic acid. Both have been found to betoxic to brain tissue when present in increased amounts in the blood.So this condition can result in permanent brain damage.

When ketones accumulate in the blood and urine, they do not occur inequal concentrations. B-hydroxybutric acid is present in the greatestconcentration and acetone in the smallest concentrations. Howevermost of the tests for ketonuria are most sensitive to the presence ofacetoacetate. There are no simple laboratory tests for β-hydroxy-butricacid. Most tests react with acetone and acetoacetate or both.

Types of Tests for Ketone Bodies

A test for ketone bodies should be done routinely on any urine that ispositive for glucose because they appear in the urine of diabetics. Testfor ketonesshould be done within 2 hours after collection

Some of the commonly used tests for ketone bodies are the following:-

- Acetest tablet test,
- Acetone powder test,
- Reagent strip tests (Ex. Ketostix),
- Lang's test,
- Rothera's test.

Principle of the Tests

Both **acetone** and **acetoacetate** give **a purplecolor** with ***alkalinesodium nitroprusside.*** This is the general principle for the testsmentioned above.

Results - Report the test as positive or negative

4.3 Determination of Urinary Protein

Protein is a macromolecule, composed of one or more polypeptidechains, each possessing a characteristic amino acid sequence andmolecular weight. It has many biologically important functions. Someof the functions are acting as enzyme(e.g. trypsin), transport protein (e.g. hemoglobin, myoglobin) nutrient and storage protein (e.g. ovalbumin(egg), casein (milk), contractile or motile protein (e.g. actin, myosin)structural protein (e.g keratin, fibroin, collagen), defense protein (e.g. antibodies, fibrinogen), and regulatory protein (e.g. insulin, growthhormone).

Test for urinary protein is one of the most important and valuable parts of the routine urinalysis. Albumin is one of the important proteins,which appears in urine during a pathological condition. It often occursas a symptom of renal disease. Globulins are excreted lessfrequently. Bence Jones protein is a specific type of globulin excretedin multiple myeloma.

Clinical Significance

The presence of protein in the urine is called Proteinuria. It is oneof the most important indicators of renal disease. Its presence in theurine depends on the nature of the clinical and pathological disorderand the severity of the specific disease.

Causes of Proteinuria

1. **Increased permeability of the glomerulus**

Normally, the glomerular membrane, the initial stage in the formation ofurine, is not permeable for protein molecules. If the glomerularmembrane is damaged these large protein molecules can pass through,and end up in the urine.

2. **A decrease in normal re-absorption in the tubules**

Under normal conditions, the small amount of protein (with lowermolecular weight), which does filter through the glomerulus, isreabsorbed back into the blood stream. Normal urine, therefore,contains only traces of protein, insufficient for detection by routinelaboratory tests. However, the concentration of protein that normallyfilters into the glomerular filtrate is extremely small, and only 1% of theglomerular filtrate is eliminated from the body as urine;the rest isreabsorbed. Failure to reabsorb any protein from this large volume ofglomerular filtrate will result in fairly large amounts of protein in the urine.

Types of Proteinuria

1. **Accidental or false proteinuria**

Accidental or False Proteinuria occurs when there is a mixture of urinewith a proteinous fluid such as pus, blood or vaginal discharge. Thesecan occur in infection of the kidney, bladder or vagina.

2. **Physiological or functional proteinuria.**

Physiological or functional proteinuria is protein excretion in associationwith fever, exposure to heat or cold, excessive exercise, emotionalstress, and later stage of pregnancy. The underlying physiologic mechanism that induces proteinuria in all of these, is renalvasoconstriction.

*3***. Postural (orthostatic) proteinuria**

Postural or orthostatic proteinuria is excretion of protein by patients, whoare standing or sitting for a longtime. The proteinuria is intermittent anddisappears when the individual lies down. It can also occur duringabnormal curvature of spinal cord.

*4***. Renal or true proteinuria**

Renal or true proteinuria occurs when protein passes from the blood intothe urine because of some malfunction in the filtering system, either inthe glomerulus or tubules.

Table .2 Proteins in Urine

Proteins	**Conditions**
Albumin	✓ **Strenuous Physical Exercise** ✓ **Emotional Stress** ✓ **Pregnancy** ✓ **Infections** ✓ **Glomerulonephritis** ✓ **Newborns (First Week)**
Globulin	✓ **Glomerulonephritis** ✓ **Tubular Dysfunction**
Hemoglobin	✓ **Hematuria** ✓ **Hemoglobinuria**
Fibrinogen	✓ **Severe renal disease**
Nucleoprotein	✓ **WBCs in Urine** ✓ **Epithelial Cells in Urine**
Bence jones	✓ **Multiple Myeloma** ✓ **Leukemia**

Tests for Urinary Protein

A. Precipitation or Turbidimetric Tests

Principle: The general principle of these tests is that protein is eitherprecipitated out of the urine specimen by means of a chemical, which isusually a strong acid, or it is coagulated out of solution with heat. Thesetests include:

- Robert's test
- Heller's test
- Sulphosalicylic Acid Test&Heat and Acetic Acid Test

Turbidimetric test based on acid reagents are non-specific since anyurine components, which is insoluble in acid, will give a positive result.

It requires large volumes (0.5 to 5 ml) and requires eitherdisposable tubes or glass tubes which must be cleaned for re-use.

The results of the precipitation tests are read in terms of the amount ofprecipitate or turbidity that is formed in a test tube (in case of Heat andacetic acid, and Sulphosalicylic acid tests) or in terms of the size ofring of contact between reagents in case of Robert`s and Heller's tests.

The amount of turbidity or precipitation is roughly proportional tothe amount of protein present in the urine specimen, and the results aregenerally graded as negative, trace, 1+, 2+, 3+, or 4+.

Since the result in precipitation tests is determined by the presence ofeither turbidity or a precipitate, it is important that the urine be free fromparticles or clear before the test is performed. To clear the urine, itshould be filtered or centrifuged. The clear filtrate is tested for thepresence of protein.

The **non-ring** precipitation is read and interpreted as follows:

Negative - no turbidity or no increase in turbidity (approximately 5mg/dL or less)

Trace - Perceptible turbidity (approximately 20 mg /dL).

1+ - Distinct turbidity, but no discrete granulation (approximately50 mg/dL).

2+ - Turbidity with granulation, but no flocculation (approximately200 mg/dL).

3+ - Turbidity with granulation and flocculation (approximately500 mg/dL).

4+ - Clumps of precipitated protein, or solid precipitate (approximately 1000mg/dL or >)

The Ring Test is read as follows:-

Negative - No cloudiness appears at the zone of contact

Trace - Ring is just perceptible against a black background

1+ - Ring is distinct against a black background, can barely beseen when held up to the light.

2+ - Ring is very definite against light, fairly visible when viewedfrom above

3+ - Ring is heavy against light, distinct cloudiness when viewedfrom above.

4+ - Ring is thick and dense against light, opaque when viewedfrom above.

The reading is interpreted as in the case of non-ring precipitationtest.

A. Robert's Test

Principle

The principle of this test is based on the precipitation of protein andformation of white compact ring using concentrated Nitric acid(HNO3).

C. Sulphosalicylic Acid Test

Principle

This test is based on the precipitation of protein (particularlyalbumin) by sulphosalicylic acid,

D. Heat and Acetic Acid Test

Principle

The test is based on the precipitation of protein by heat.

Sensitivity

This method is the most sensitive for small amount of protein and canreliably detect protein concentrations of 2 to 3 mg/dl.

II. Colorimetric Reagent Strip (Dipstick) Tests

The Colorimetric (dipstick) Protein Tests are more specific than Turbid metric Tests. They require only a drop of urine enough tomoisten the reagent area.The Colorimetric reagent strip test is basedon the ability of protein to alter the color of some acid-baseindicators without altering the pH. When an indicator, such astetrabromophenol blue is buffered at pH 3, it is yellow in solutions without protein but , in the presence of protein, the color willchange to green and then blue with increasing proteinconcentrations. In this case the pH of the urine is held constant bymeans of a buffer so that any change of color of the indicator willindicate the presence of protein.

The tests for urinary protein are all commercial ones that are availableas reagent strip, tests (Dipsticks) either alone or in combination withother tests. Example.Albustix, Uristix, N-Multistix, Combur3 orCombur9. Although the colorimetric tests are useful primarily asscreening tests for protein, these strip tests can be readsemi quantitatively as negative, trace, 1+, 2+, 3+, or 4+ to give a roughestimate of the amount of protein present. To do this, the resulting colormust be matched closely with the color chart provided with the teststrips.Thealbustix and other multiple-reagent strips produced by amesco. are plastic strips with protein test areas impregnated with citratebuffer and tetrabromphenol blue. The citrate buffer maintains the pH at3. At pH 3

tetrabromphenol blue is yellow in the absence of protein andyellow - green, or blue in its presence.

The shade of the color isdependent on the amount of protein present. Falsely positive reactionsmay occur when protein is absent, if the urine is exceptionally alkaline orhighly buffered.

Quantitative 24 hour Protein Determinations

Simple estimates of the protein content of urine are performed byquantitating the amount of precipitation formed following the addition of aspecific chemical to the urine. The precipitate is measured either bycomparison with known standards (sulphosalicylic acid turbidity test) orby recording the height of the column of precipitate in a speciallydesignedtube (Esbach's test).

4.4 Determination of Bilirubin

Bilirubin is a waste product that must be eliminated from the body. It isformed by the breakdown of hemoglobin in the reticulo-endothelial cellsof the spleen and bone marrow, and then transported to the liver.

On its way to the liver it is not water-soluble, and is carried through theblood stream linked to plasma albumin. This water insoluble form ofbilirubin is often referred to as free bilirubin or unconjugated bilirubin orindirect bilirubin. Since this albumin - bound form is insoluble in water; itdoes not appear in the urine.

In the liver bilirubin is converted to awatersolubleproduct by conjugation with glucuronic acid to form bilirubinglucuronide. The water-soluble form is called conjugated bilirubin. It isalso called direct bilirubin. The liver cells that form the conjugatedbilirubin excrete it into the bile and it is then excreted into the intestinaltract through the bile duct. In the small intestine this conjugatedbilirubin is converted by intestinal bacteria to urobilinogen orstercobilinogen.Even though normally the level of conjugated bilirubin in the blood is nothigh enough to cause significant amounts to appear in the urine, thiswater soluble and conjugated bilirubin can be excreted by the kidneys.

Normal Value: approximately up to 0.02 mg/dl (This amount is notdetected by routine qualitative or semi quantitative techniques).

Clinical Significance

Tests for urinary bilirubin and urobilinogen were normally performed onlyindicated by abnormal color of the urine or when liver disease or ahemolytic condition was suspected from the patient's history. Thepresence of bilirubin and urobilinogen in the urine is an early sign of livercell disease

(hepatocellular disease) and obstruction of the bile flow fromthe liver (Obstructive or post - hepatic jaundice).

Urine containing bilirubin will typically have been brown color andproduce a yellow foam when shaken. Bilirubin is not stable in solution,but will be oxidized to biliverdin, which is a green pigment. Thus urinecontainingbilirubin will typically be red-brown when voided, and will turngreen on standing, especially if exposed to light. Tests for bilirubin willnot be positive in the presence of biliverdin; so the urine must beexamined when fresh.

Tests for Bilirubin

Tests for bilirubin are based on the oxidation of bilirubin to biliverdin.

Specimen: Freshly passed urine is required. Urine containing bilirubinshould be analyzed immediately after collection (with in 2 hrs of voiding).If bilirubin exposed to sunlight, it will oxidize to biliverdin, which cannotbe detected by the reagents used in any of the tests. The following testsare used to detect bilirubin in the urine.

C. Diazotization Tests for Bilirubin

The tablet and reagent strip tests for bilirubin are based on the couplingof bilirubin with a diazonium salt in an acid medium to form azobilirubin,which gives a blue or purple color.

1. Icotest Tablet Test

The Ictotest tablet contains nitrobenzinediazonium, p-toluene sulfonate(bilazo), sulfosalicylic acid, and sodium bicarbonate. The mats are absorbentasbest as cellulose.

2. Reagent Strip Tests for Bilirubin (Ex. Multistix)

Principle

These tests for bilirubin are available only on multiple-reagent strips inconjugation with other tests. They are diazotization tests and are analogous to the Ecotest tablet test. The test area for bilirubin onMultistix and other Ames Co. reagent strip products is impregnated with2,4-dichloro-aniline diazonium salt.The reagent strip tests for bilirubin are difficult to read and the colorformed after reaction with urine must be carefully compared with colorchart supplied by the manufacturer.

Self-Check 4	Written Test

Answer the following questions

1. Discuss by comparison the Benedict's Qualitative and Glucose oxidase Tests.
2. List down the possible substances, which give false positive results in non-specific tests for glucose determination.
3. Mention the physiological effects of ketone accumulation in blood.
4. Write the principle of the test for determination of bilirubin and hemoglobin.
5. Write the general principles for the two types of determination of urinary protein.

Answer sheet

1. ——————————————————————
2. ——————————————————————
3. ——————————————————————
4. ——————————————————————
5. ——————————————————————

Score————————
Rate————————
Remark————————

Name————————————————Date————————

4.1. Procedure of performing chemical urinalysis by reagent strips

Observe the precautions and follow the instructions supplied by the manufacturer.

13. Wear gown, glove and other PPE
14. Clean the working bench
15. Assemble the required materials
16. Collect 15ml of urine into clean, dry container
17. Dip the reagent area of the strip briefly into the specimen.
18. Remove excess urine by tapping or drawing the edge of the strip along the rim of the urine container.
19. Compare the color that develops with the color chart supplied by the manufacturer and report as indicated on the chart.

4.2. Quantitative 24 hour Protein Determinations

Purposes

Simple estimates of the protein content of urine are performed by quantitating the amount of precipitation formed following the addition of a specific chemical to the urine. The precipitate is measured either by comparison with known standards (sulphosalicylic acid turbidity test) or by recording the height of the column of precipitate in a specially designed tube (Esbach's test).

Procedure

a. Pipette 2.5 ml of centrifuged urine into a test tube.
b. Add 7.5 ml of 3% sulphosalicylic acid.
c. Invert to mix
d. Let stand 30 minutes.
e. Compare the turbidity with known standards prepared from solutions containing 10, 20, 30, 40, 75 and 100mg albumin/dl, and estimate the concentration of the unknown. If the unknown urine contains more than 100mg/dl protein, dilute the urine and repeat the test.

LAP TEST	Practical Demonstration

Name————————————ID.No————————Date————————

Time started————————Time ended————————

Instruction1:- Demonstrate each of the following activities.

Project1:- Performing urine chemical examination

Task1:- Perform urine dipstick tests of glucose determination?

Task2:- Perform Quantitative 24 hour Protein Determinations?

5.0. Microscopic Examination of Urine

Microscopic examination of urine is one of the routine tests of urinalysis.

Urine containsmany substances in addition to water.

The amounts of solid substances,which are found in the urine, may indicate an individual's health status. i.e. whether one is healthy or sick.

Normally small amount of solid substances is found in the urine. But when their concentration become high, it may indicate the existence ofabnormal physiological function of our body.

Microscopic examination ofurine to some extent can be considered as ***"renal biopsy"*** because itreveals more about the function of the kidneys.

Repeated evaluation of urine sediment is frequently valuable in followingthe course and management of urinary tract disorders, because theappearance of cellular elements, and casts in the urine is a reflection ofchanges that take place in the kidney.

Urine sediments can grossly be categorized into organized and non-organizedsediments based on the substances they are composed of.

- **Urinary Sediments**

Classification of Urinary Sediments

Organized Elements

- Formed from Living Materials

Non-organized Elements

- Formed for Non-living Material (Crystals)

Organized (Formed) elements

- WBCs/HPF -Amorphous Urates,
- Epithelial cells / LPF -Uric acid crystals,
- Casts / LPF -Cystine crystals
- Parasites/LPF -Calcium Phosphate
- Bacteria / HPF -Cholesterol

-Ammonium Biurates

- Yeast Cells /`LPF -Tyrosine, Leucien, Bilirubin,
- Mucus trade/LPF -Calcium sulfates (urates)
- Spermatozoa - Calcium carbonate
- Miscellaneous substances (Common contaminants**)**

Non-organized (Non-living Material)

I. *Slightly acidic urine*

- ☐ **Triple phosphates**
- ☐ **Amorphous phosphate**
- ☐ **Calcium carbonate**
- ☐ **Calcium phosphate**

II. *Acidic, Neutral, or slightlyAlkaline Urine crystals*

- Calcium Oxalate crystals

III. ***Alkaline, Neutral, orSlightly acidic urine***

- Triple phosphates

IV. Alkaline Urine Crystals

- Amorphous phosphate
- Calcium carbonate
- Calcium phosphate

5.4 Organized Urinary Sediments

A. RED BLOOD CELLS

Appearance: Normally RBCs appear in the fresh sample as intact,small and faint yellowish discs, darker at the edges

- Measure 7-8 µm
- In concentrated urine may be crenated, and their size becamesmall (5-6 µm)
- In diluted urine, RBCs may be turgid and increase in size (9-10µm)
- In alkaline urine, they may be small or entirely destroyed formingmassive of brownish granules

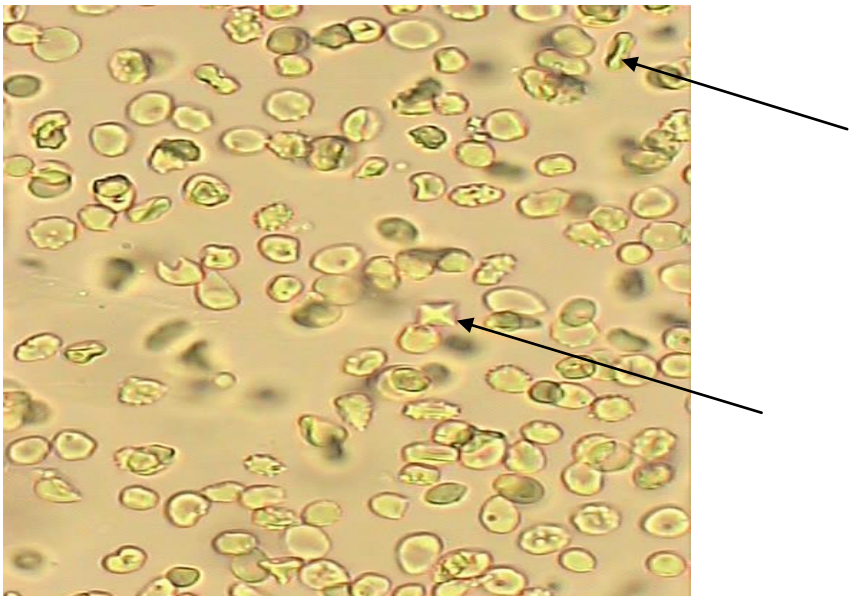


Fig. 3.1. shows RBCs and Calcium oxalate

Clinical Implications: When the number of RBCs is found more thantheir normal range, usually greater than 5 RBCs/HPF it may indicate:

 Presence of disease conditions in the urinary tract, such as:

• Acute and chronic glomerulonephritis

• Renal stone

• Cystitis

• Prostates

• Trauma of the kidney

• Presence of parasites, such as: schistosoma.

• Presence of bacterial infection, such as: renal tuberculosis

• Other disease conditions, such as hemophilia, malignanthypertension.

Temporarily (transient) increased RBC may be seen

- After strenuous exercise

- Exposure to cold temperature

Other substances confusing with RBCs

Yeast cells, and fat droplets may confuse with RBCsmorphologically. They may be differentiated by their morphology.

Red blood cells are somewhat round or disc shaped, and uniform insize: while yeast cells are oval in shape, and have budding at thesurface. On the other hand fat droplets are irregular in size and theyare shiny.

Another means of differentiating RBCs from yeast and fat droplets isthat, when 5% of acetic acid is added under the cover slide, RBCswill hemolize, while yeast cell and fat droplets will not show anychange.

How to report result:

• After looking RBCs under the 40x objective, they can be reported bymentioning the average number of RBCs/HPF.

Interfering factors:

Factors that may result falsely in high number of RBCs, i.e. without thepresence of actual renal or other normal physiological disturbancesincluded:

• Menstrual bleeding

• Vaginal bleeding

• Trauma to per anal area in female patients

• Following traumatic catheterization

• Due to some drugs, such as,

- Aspirin ingestion or over dose

- Anticoagulant therapy over dose

B. LEUKOCYTES (WBCs)

Normal range: 0-4 WBC/HPF.

Appearance: normally, clear granular disc shaped,

 Measure 10-15 µm, the nuclei may be visible.

In alkaline urine, they may increase their size and become irregular.

Predominantly, polymorph nuclear neutrophils are seen.

Sometimes because of predominance of neutrophils and theoccurrence of bacterial cell together with polymorph nuclear cells,WBCs are called pus cells.

 WBCs (pus cells) may be seen in clumps.

It is also possible to see single irregular nuclei and small round lobednuclei in the WBCs, that are seen in the urine sediment.

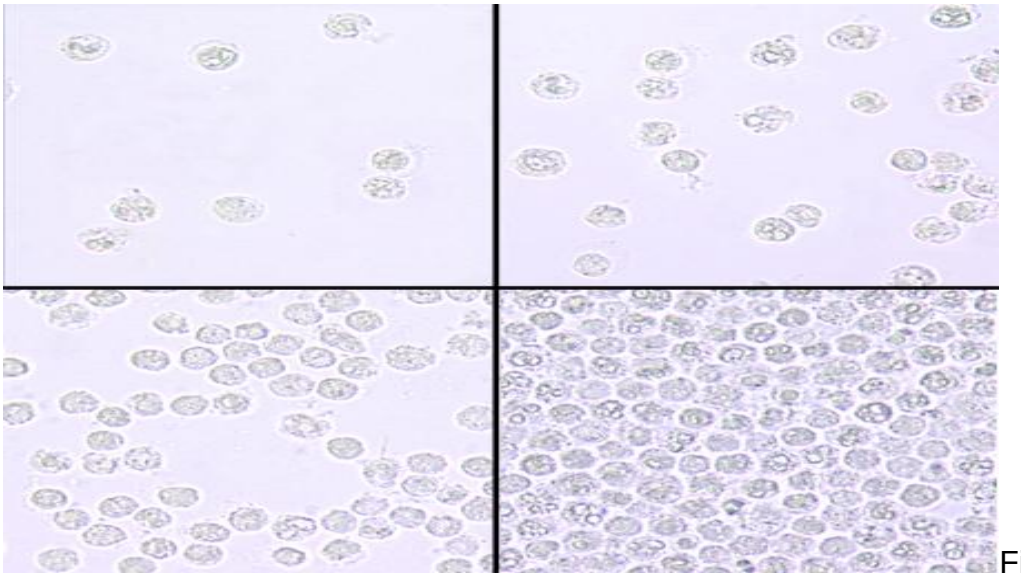


Fig. 3.2. WBCs

Clinical implication: Increased numbers of leukocyte urine are seen incase of:

 Urinary tract infection

 All renal disease

 Bladder tumor

 Cystitis

 Prostates

 Acute or chronic bacterial infection such as renal tuberculosis,temporarily increased numbers of leukocytes are also seen during: Fever and after strenuous exercise

How to report the result:

- After observing the distribution of leukocytes under 40 x objectives, atleast 10 fields of microscope, it is possible to report as: 0-5leukocytes / HPF, 20-39 leukocytes / HPF or

0-5 leukocytes / HPF are seen..... normal

5-10 leukocytes / HPF are seen..... few leukocytes / HPF

10-20 leukocytes/HPF are seen…….....moderate leukocytes/ HPF

20-30 leukocytes /HPF are seen …..... many leukocytes / HPF

Above 30 leukocytes / HPF / are seen …… full/field

C. EPITHELIAL CELLS

• Normally few epithelial cells (0-2 / HPF) can be found

• Appearance

Their size differs depending on the site from which theyoriginated.

a. renal cells

- Size is small as compared to other epithelial cells
- It measures 10µ to 18 µm in length, i.e., slightly larger thanleukocytes
- Very granular
- Have refractive and clearly visible nucleus
- Usually seen in association with proteins or casts (inrenal disease).

b. Cells from pelvis and urethra of the kidney

- Size is larger than renal epithelia's
- Those from pelvis area are granular with sort of tail, while thosefrom urethra are oval in shape
- Most of the time urethral epithelia is seen with together ofleukocytes and filaments (mucus trades and large in number)
- Pelvic epithelia's seen usually with no leukocyte and mucustrade, and are few in number

c. Bladder cells

- Are Squameous epithelial cells?
- Very large in size.
- Shape seems rectangular and often with irregular border.
- Have single nucleus.

* Here it is important to keep in mind that it is not expected from anexperienced Lab. technician after simply observing epithelialcells, to say that these are urethral cells, and of pelvic origin andreporting such a false result in the laboratory request form.

* Knowing the origin of the epithelial cells and reporting it, may havemore meaning when requested by the physician for specialpurpose, especially by the urologists.

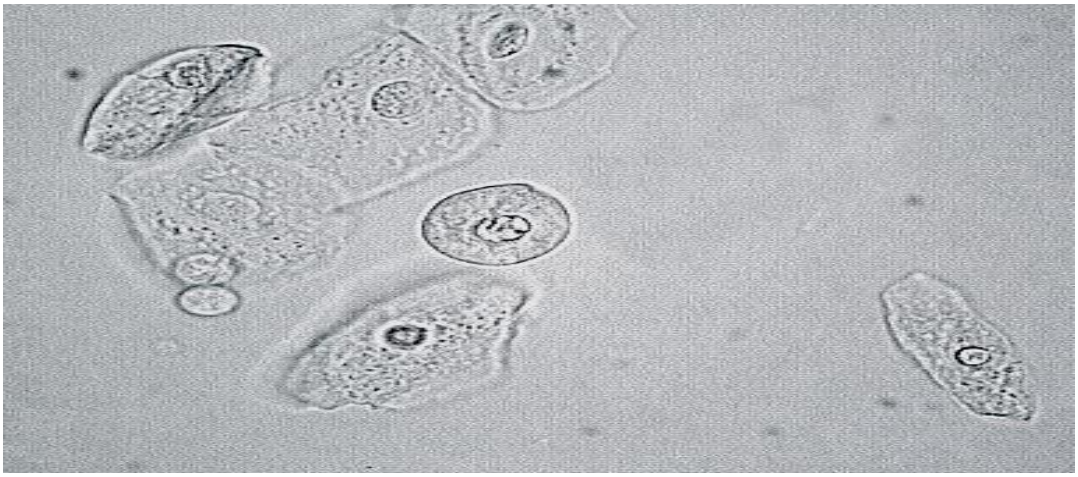


Fig. 3.3 epithelial cells

Clinical implication

Presence of epithelial cells in large number, mostly renal types mayindicate:

- Acute tubular damage
- Acute glomerulonephritis
- Silicate over dose

* The presence of large number of epithelial cells with large numberof Leukocytes and mucus trades (filaments) may indicate Urinary tract Infections (UTI).

Reporting of the result:

• Epithelial cells distribution reported after looking under 10x (lowpower objective) of the microscope.

• Usually they are reported semi quantitatively by saying

- Occasional epithelial cells /LPF ……1-3 epithelial cells seen inthe whole LPF
- Few epithelial cells / LPF..... 2-4 epithelial / LPF
- Moderate epithelial cells / LPF..... 6-14 epithelial / LPF
- Many epithelial cells / LPF..... 15-25 epithelial/ LPF
- Full of epithelial cells / LPF.....when the whole fieldof 10 x objective covered by epithelial cells.

CASTS

• Formed by precipitation of proteins, and aggregation of cells withinthe renal tubules. Most of them dissociate in alkaline urine, anddiluted urine (specific gravity ≤ 1.010) even in the presence ofProteinuria. Most of them are transparent. Thus to look themclearly, it is important to lower the

condenser and close (partially) thediaphragm. Look them under 10 x (low power objective) of themicroscope. There are different kinds of casts based on their shapeand content (morphologically) may be grouped in to the following.

A. Hyaline Casts

• Normal range: 0-2/HPF

Appearance

- Transparent (clear), cylindrical shape
- Have parallels side with slightly round ends
- Their appearance in urine depends on rate of urine flow, i.e.many hyaline casts are seen when the flow rate is slow, andare not seen in alkaline urine mostly; and as the degree of protein urea is high, there concentration also increase.

Clinical Implication

Presence of large number of hyaline casts may show possible damageof glomerular capillary membrane. This damage permits leakage ofprotein through glomerulus and result in precipitate and gel formation(i.e. hyaline casts) in the tubule. Thus this may indicate:

• Nephritis
• Meningitis
• Chronic renal disease
• Congenital heart failure
• Diabetic nephropathy

Hyaline casts may also be seen in moderate number temporarily in thecase of*:*

• Fever
• Postural orthostatic strain
• Emotional stress
• Strenuous exercise
• After anesthesia

B. Granular Casts

• More similar in appearance with hyaline casts and in whichhomogenous, course granules are seen. More dense (opaque)than hyaline cast, thus can be more easily seen than hyalinecasts. They are also shorter and broader than hyaline casts.May represent the first stage of epithelial cell cast degeneration.

Some other studies also suggest that, they are formedindependently from cellular cast degeneration, and stated thatthey result from aggregation of serum proteins into cast matrixof mucoproteins

• Based on the amount and type of granules, they can be furtherdivided into fine, and course granular casts.

Clinical implication

Granular casts may be seen in

• Acute tubular necrosis

• Advanced granulonephritis

• Pyelonephrites

• Malignant nephrosicosis

• Chronic lead poisoning

• In healthy individuals these casts may be seen after strenuousexercise

C. Cellular Casts

Cellular casts are casts, which contain

• Epithelial cells

• White blood cells

• Red blood cells

Normal range: normally not seen in normal individual

Appearance

• These are casts in which cellular elements are seen.

• Formed usually after accumulation of cellular element in the renaltubules

Clinical Significance

• Epithelial / renal / casts mostly seen in tubular degeneration.

• Red cell cast usually seen in acute glomerulonephritis cases.

• White blood cell casts seen mostly during pyelonephritesconditions.

NOTE: Casts are very significant findings of urine microscopicexamination. This is because their presence indicates the existence ofrenal disease. Sometimes it is possible to get a single cast havingcourse granules, fine granules and fat droplets, i.e. different substancesin a single cast, at the same time. At this time decision is made afterlooking and evaluation of other fields and based on the majorities.

Reporting of Laboratory Result

• Casts are examined under 10x objective of the microscope.

• Always the condenser should be lowered and at the same time inorder to have good contrast, the diaphragm should be partiallyclosed.

• Casts are reported quantitatively by saying:

o Occasional casts / LPF

o Few casts / LPF

o Moderate casts / LPF and

o Many casts / LPF

During reporting the type of cast that is seen should also be mentioned

Example: few hyaline casts / LPF are seen

PARASITES

Parasites that can be seen in urine microscopy are:

Trichomonasvaginalis

Schistosomahaematobium

Wuchereriabancroftie

* Other parasites also may occur due to contamination of the urine withstool.

A.TrichomonasVaginalis

It is a protozoa parasite that infects the genitourinary tract.

Appearance

- Size is about 15 µm.
- Shape is round, globular.
- Has vibratory, whirls and turns type of movement.
- Has also undulating membrane that is like the fin of a fish, on oneside very motile.
- Have 4 flagella.

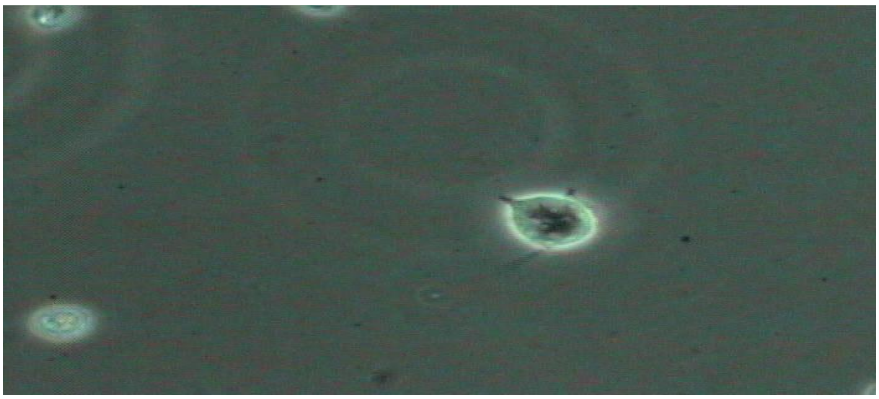


Figure 3.4. Trophozoites of T. vaginalis

B. SchistosomaHaematobium

It is fluke that infect venules of the bladder.

Appearance of the egg

- It is found in the urine sediment.
- Has pale yellow brown color.
- Large and oval in shape.
- Has characteristic small spine at one end (terminal spine).
- Measure about 145 x 55 µm.
- The egg contains a full-developed miracedium. Sometimes themiracedium hatch from the egg and can be seen swimming in the urine. The miracedium swim in the urine by the help of ciliates thatare surrounding it.High excretion of S. haematobium egg can be seen usual between 10.00a.m. and 2 p.m. It is also important to remember that even when personsare highly infected, eggs may not be present in the urine. Therefore thatis important to examine several specimens collected on different daysand examine carefully, that is due to the irregular pattern of eggexcretion.

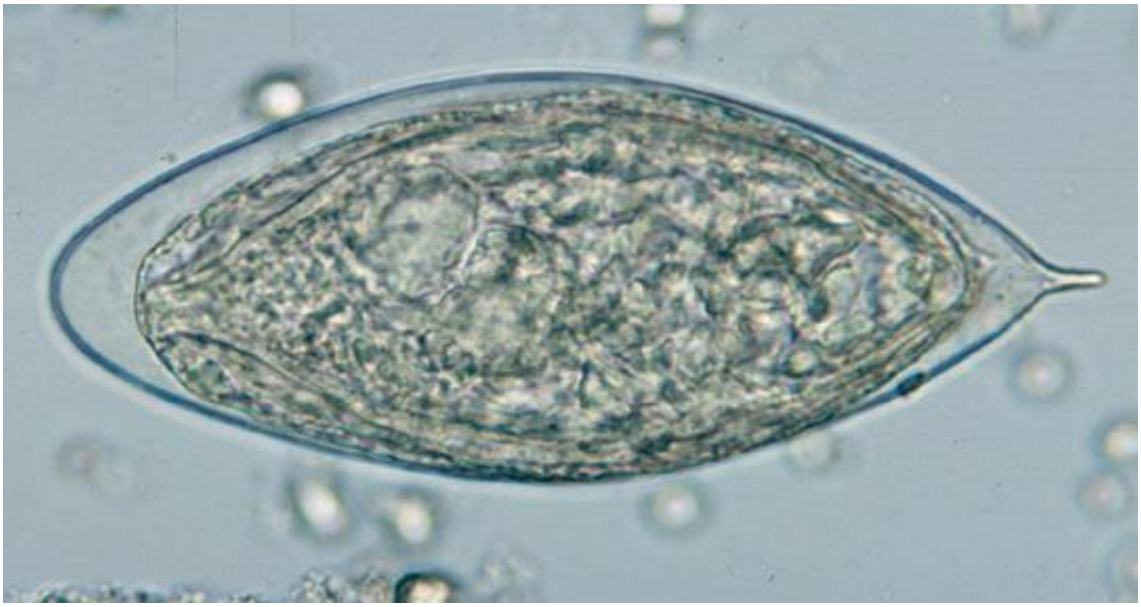


Figure 3.5. Egg of ***Schistosoma Haematobium***

C. WuchereriaBancroftie

• It is tissue nematode that invades lymph vessels. It is usually attacklower limb.

• In chronic bancroftiefilariasis, a condition called chyluria can occur. i.e. passing of chyle in the urine. It occurs when the urogenitallymphatic vessels, which are linked to those, that transport chylefrom the intestine became blocked and rupture.

• Chile consists of lymph and particles of digested fat (soluble inether).

• Urine containing chyle appears creamy white. When blood is alsopresent, the urine appears pinkish-white.

• Large, measuring 275-399 x 8-10 µm.

• Body curves are few, nuclei are distinct.

• Sheath stains pink with Giemsa and palely with haematoxylin.

• There is no nuclei in the tip of at the tail.

Other points that should be considered also

• The parasite usually found in high concentration during night from10:00 p.m. – 4:00 a.m. and i.e. it has nocturnal periodicity.

• Differentiate from B. malai and L. loa by its tail feature.

• Differentiate from Mansonella species by its large size and sheath.

YEAST CELL

Yeast cells are fungi that are not normally seen in health individuals.

Appearance

- Variable in size
- Colorless.
- Oval in shape, and usually form budding.
- Have high refractive index.
- Usually confused with Red Blood Cells. The way in which one candifferentiate yeast cells from RBC is discussed in detail under RedBlood Cells.

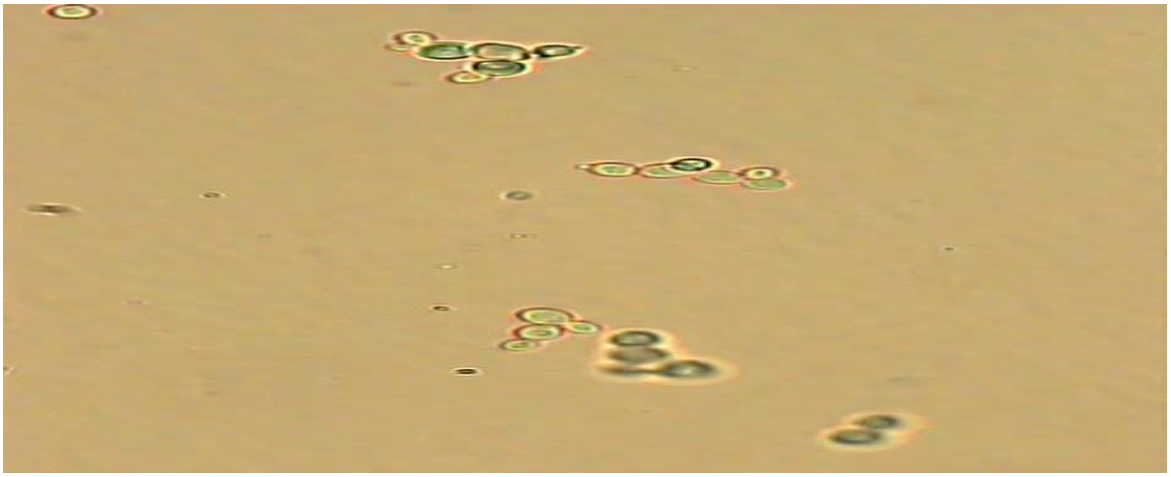


Figure 3.6. budding yeast

Figure 3.7. branching pseudohyphae

Clinical Significance

• They are usually of Candida species (Candidaalbicans) and arecommon in patients with

- Urinary tract infection
- Vaginites
- Diabetic mellitus
- Intensive antibiotic or immunosuppressive therapy.

BACTERIA

Bacteria are the most common cause of UTI and aerobic gram-negativebacilli, particularly, members of the enterobacteriacea, are the mostdominant agents. The Gram-positives account for proportionately largenumber of infections in hospital inpatients. Normally, bacteria are notseen in the healthy individual's urine.

To check the presence or absence of bacteria a technician can eithercheck for Nitrate that was formed in the urine after breakdown of nitriteinto nitrate by the metabolic action of bacteria. Hence, dipstick test cangive indirect clue. Or one can use urine microscopy test to check

thepresence of pus cells within the drop of urine or its sediment. Furtherthe observed bacterial cell can be identified by bacteriological culture.

Appearance

- Bacteria that are seen in the microscopic examination of the dropof urine sample. Their shape varies with the type of bacteriaobserved..
- Depending on the type of bacteria they can be either motile or nonmotile organisms.
- They can be observed when examined under less than 40 x(high power) objective of the microscope.

Clinical Significance

- Presence of bacteria may indicate the presence of UTI orcontamination by genital or intestinal micro flora.
- To confirm what type of bacteria they are and whether or not theyare the causes of the disease, it is important to culture them inappropriate media and perform biochemical tests for identification.

Report of the Result

The bacteria concentration before or without performing culture and identification of the bacteria can be reported as:

• Occasional bacteria / HPF
• Few bacteria / HPF
• Moderate bacteria / HPF
• Many bacteria / HPF
• Full of bacteria / HPF.

Elements in Urinary Sediment	Usual Distinguishing Color of Stained Elements		Comments
Squamous epithelial cells	Dark shade of orange-purple	Light purple or blue	
	Inclusions and Matrix		
Hyaline casts	Pale pink or pale purple		Very uniform color; slightly darker than mucous threads
Coarse granular inclusion casts	Dark purple granules in purple matrix		
Finely granular inclusion casts	Fine dark purple granules in pale pink or pale purple matrix		
Waxy casts	Pale pink or pale purple		Darker than hyaline casts, but of a pale even color; distinct broken ends
Fat inclusion casts	Fat globules unstained in a pink matrix		Rare; presence is confirmed if examination under polarized light indicates double refraction
Red cell inclusion casts	Pink to orange-red		Intact cells can be seen in matrix
Blood (hemoglobin) casts	Orange-red		No intact cells
Bacteria	Motile: do not stain Nonmotile: stain purple		Motile organisms are not impaired
Trichomonas vaginalis	Light blue-green		Motility is unimpaired in fresh specimens when recommended volumes of stain are used; immobile organisms also identifiable
Mucus	Pale pink or pale blue		
Background	Pale pink or pale purple		

5.5 Non-organized Elements (Urine Crystals)

Appear usually after the specimen (urine) collected and left withoutexamination. Mostly occur during metabolic abnormalities andexcessive consumption of certain foodstuffs. May be classified intoacidic, basic, and both acidic and basic based on:

• PH of urine in which they are usually seen.

• Solubility characters.

Identification of particular urine crystals from patient urine-sedimentmainly serves as

• Guide to diagnose most likely type of calculus present.

• Mode of therapy of calculus by adjusting of urine, and byavoiding the intake of certain calculus precursors.

• Occurrence of certain abnormal urine crystals, such ascystine,Leucine, and Tyrosine, indicate the patient is incertain metabolic disorders andsome drug crystals in the urine includesulfonamides,aspirin, and caffeine, used to follow the treatment condition.

The Usual Crystals Found in Urine	
Alkaline pH	Acid pH
Amorphous phosphates	Amorphous urates
Triple phosphates	Uric acid
Ammonium biurates	Calcium oxalates
Calcium phosphates	
Calcium carbonates	Cystine

Normal Crystals

- Uric acid Crystals
- Calcium Oxalate Crystals
- Hippuric Crystals
- Calcium Phosphate Crystals
- Triple Phosphate Crystals
- Calcium Carbonate Crystals
- Ammonium Biurate Crystals

Abnormal Crystals

- Bilirubin Crystals
- Cholesterol Crystals
- Cysteine Crystals
- Leucine Crystals
- Tyrosine Crystals
- Sulfa Crystals
- Indinavir Crystals

I. Acidic Urine Crystals

A. Amorphous Urates (Anhydrous uric acid)

- Normally present in urine in different quantity.

- Have pink to “brick red” color.

- From very small granules and seen in cluster.
- Dissolve in urine when the sample is gently heated.
- When urine is left in the refrigerator, it shows heavyprecipitation of urates.

B. Uric Acid Crystals

- Polymorphs (different in shape) i.e. square, prism,hexagonal, etc.
- Yellow to yellow brown in color.
- Size is 30-150 µm
- Small quantity found in normal urine, but increases inassociation with:
- Increased Purine metabolism in case of gout.
- Increased Nucleic Acid turn over, such as leukemia.

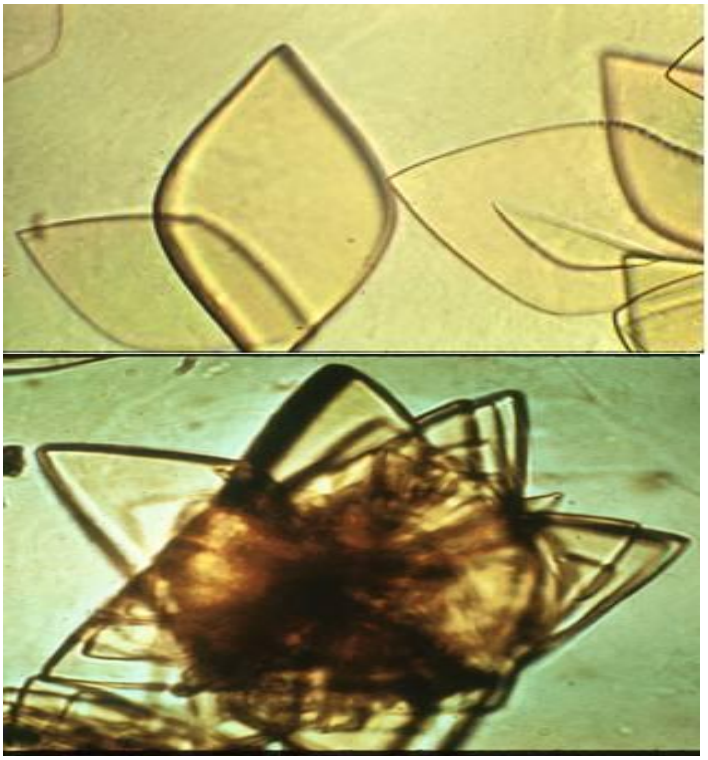


Figure 3.8.Uric Acid Crystals

G. Bilirubin

- Very rarely seen.
- Have reddish brown color.
- Seen in case of elevated Bilirubin.
- Have various tiny squarish, beads or amorphous needleshape.
- Size is 5 µm (half RBC).
- Chemical test for bile pigments positive.

I. Acidic, Neutral, or Basic Urine Crystals

- **Calcium Oxalate Crystal**

- Are colorless and refractive.

- Have octahedral, envelope, shape.

- Size 10-12 µm.

- Normally seen in small amount.

- After consumption of high calcium, or oxalate rich foods, such asmilk, tomatoes, asparagus, and orange, normally the crystals maybe seen.

- In dehydration condition, such as, in hot weather where there ishigh perspiration and only small amount of water is consumed perday Calcium oxalate crystals may be seen.

- Pathologically in large quantity may be seen in (severe chronicrenal disease, and urinary calculus).

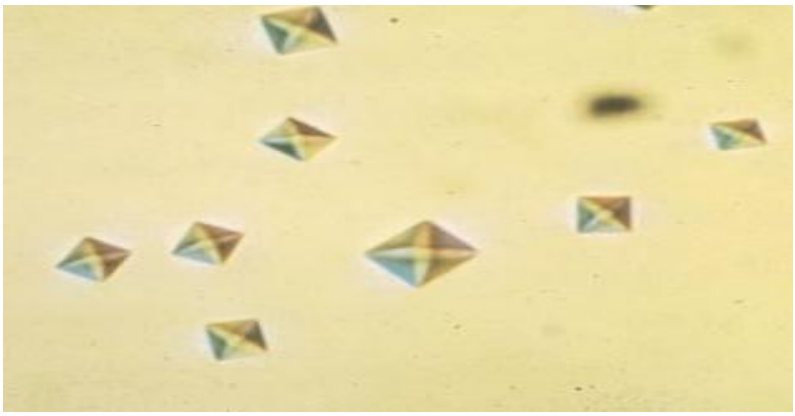


Figure 3.9. Calcium oxalate crystals

II. Alkaline, Neutral, or Slight Acidic Urine Crystals

- **Triple Phosphates**

- Colorless and refractive.

- Have “coffin lids” 3 to 4 to 6 – sided prism.

- Shape, or fern leaf or star shape.

- Size 13 0- 150µm.

- Seen in urine stasis (obstructive uropathy), or in urinary tract infections.

- Their presence is frequently indicative of bacterial infection by proteus mirabilis.

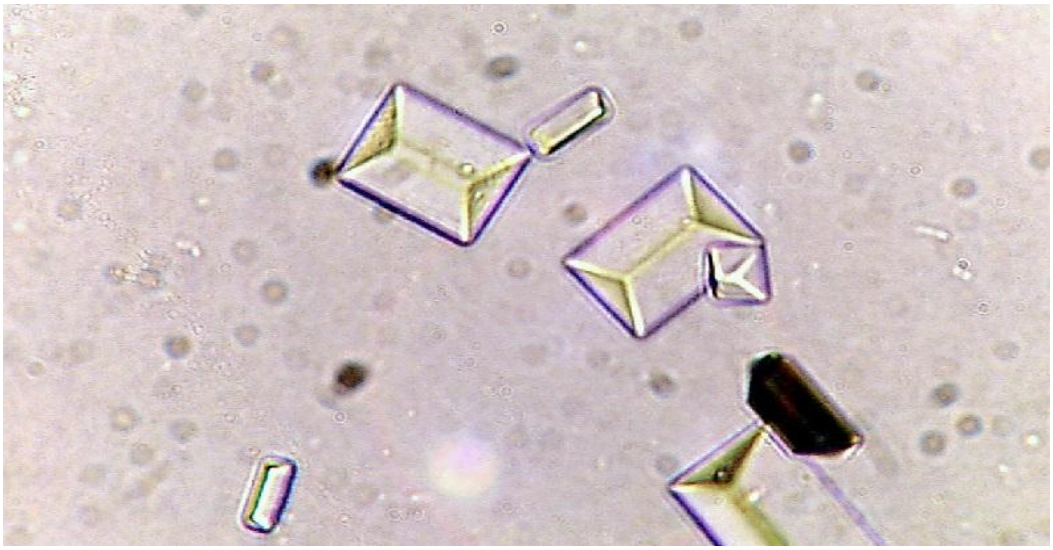


Fig.3.10. Triple phosphate crystals

III. Alkaline Urine Crystals

- ***Amorphous Phosphates***

- Normally seen in alkaline urine.

- Small, whitish granules usually seen scattered, &Soluble in 100g/1 acetic acid.

B. Calcium Carbonate

- Less commonly seen.

- Colorless.

- Have needle, spherical or dumbbells shape.

- Have very small crystals.

- If 100g/1, i.e.10% acetic acid is added, they dissolve, give offbubbles of gas.

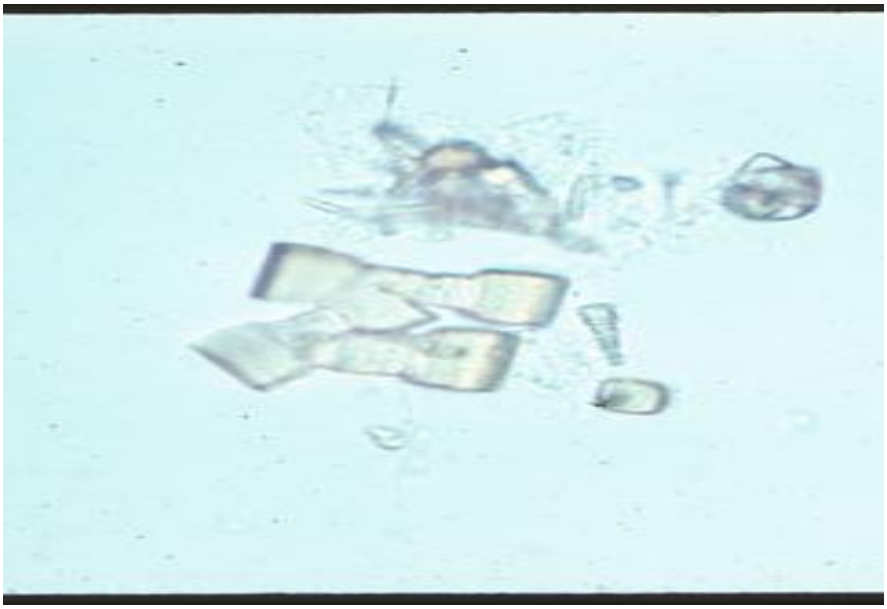


Fig. 3.11. Calcium Carbonate crystals

C. Calcium Phosphates

- Seen in small amount in normal individual urine, and when theyare in large amount, may indicate chronic cystitis, or prosthetichypertrophy.

. Have star or needle shape.

- Colorless.

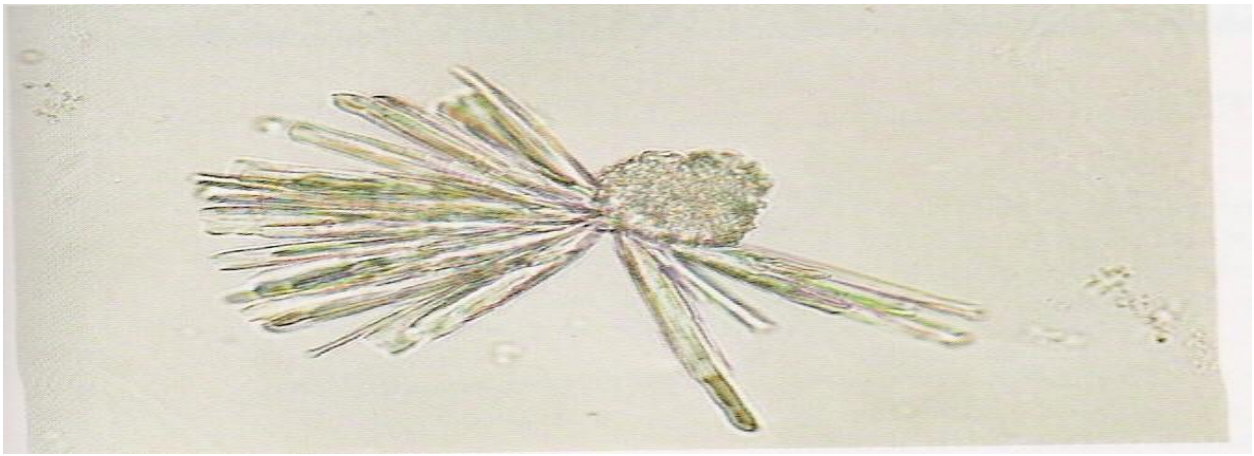


Fig. 3.12. Calcium Phosphates

MISCELLANEOUS

I. Spermatozoa

- Are small structures consisting of a head and tail, connected by ashort middle piece (neck).
- Easily recognized especially if they are motile.
- Frequently seen in the urine of males.
- They may see in the urine of females, when the urine collectedafter coitus usually not reported, unless the physician has specialinterest in it.

II Mucus Trades

- Formed by the precipitation of mucoproteins in cooled urine.
- Normally little mucus trades seen in normal individuals.
- Have fine, fiber like appearance.
- Wavy in shape and tapered at ends.
- If not examined carefully may confuse with hyaline casts.
- Their presence in large amount with WBCs may indicate UTI.

III. Other Contaminates and Artifact Structure

- Muscle fibers
- Vegetable cells - all are fairly seen and easily
- Cotton fibers (wool fibers) recognizable.
- Structure from slide or cover slide - high retractile andnon-uniform insize.

Fat droplets (other bubbles)

- Not evenly distributed.
- Oil droplets
- Pollen greens - are seasonal.
- Starch granules - incomplete digestion ofstarch

They can be confirmedby logos iodine.

**** To minimize the above mentioned contaminants and artifacts***

- Don't use dirty containers, slides and cover slides.
- Don't let urine specimen to open-air.
- Avoid contamination of urine with fats and oils.
- Avoid the drying of sediments.

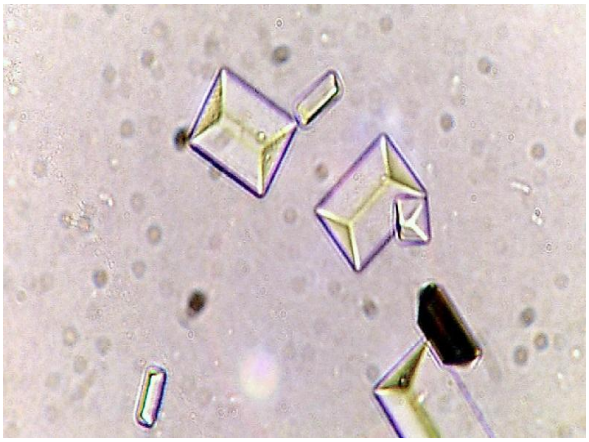
Self-Check 5	**Written Test**

Instruction 1: Say True or False

1. The number of casts preserved decrease as the pH of the urine decreases.
2. Presence of RBCs in the urine is always indicative of a renal disease.
3. Waxy casts are the end stage in the degeneration of cellular casts.
4. Pyuria refers to elevated numbers of leucocytes in the urine.
5. The presence of Bacteria in the Urine is determined using only Microscope.

Instruction 2:- Choose the best possible answer for each of the following questions

1. The crystal found in the diagram below is--------?



A. Triple Phosphate C. Amorphous Phosphate

B. Calcium oxalate D. Cysteine crystal

2. Which one is true about crystal in the Q-1 above?

A. Found in acidic urine only C. It is iatrogenic' crystals

B. Composed of magnesium, ammonium and phosphate D. All of the above

3. The normal yellow color of urine is produced by:

A. Bilirubin B. Urochrome C. Urobilinogen D. Hemoglobin

4. Ms. Darmi brought the urine specimen which is Yellow brown or "beer brown" in color. This may indicate?

A. presence of hemoglobin C.presence of bilirubin

B. Indicates hematuria D. Presence of protein

5. Which of the following elements are commonly confused with RBCs?

A. Yeast cells B. leukocytes C. bubbles D. All

6. In a microscopic examination of clear urine that produces a pink precipitate after refrigeration will show triple phosphate crystals.

A. True B. False C. Unknown

7. A yellow-brown specimen that produces a yellow foam when shaken can be suspected of containing:

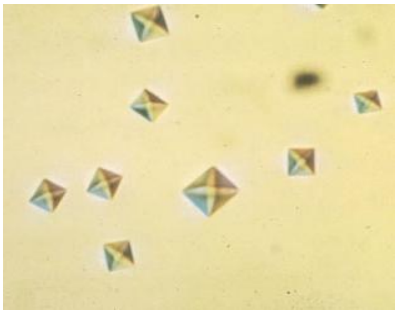
A. Bilirubin B. Carrots C. Hemoglobin D. Rhubarb

8. The fungus Candida species (Candida albicans) are common in patients with

A. Diabetic mellitus C. Immunosuppressive therapy

B. Vaginites D. All of the above

9. What is the name of this crystal?



A. Calcium phosphate C. Amorphous urate

B. Calcium oxalate D. Uric acid crystal

10. Which one is not true about crystal in Q-9 above?

A. Found in alkaline urine C. Indicate Presence of bilirubin in the urine

B. May found in monohydrate& dehydrate form D. All are true

Answer sheet

Score—————————
Rate—————————
Remark—————————

Name————————————ID.No————————Date————————

4.1. Performing urine examination

Procedure for Microscopic examination of Urine specimens

1. Collect all necessary materials used for the collection, centrifugation and examination of urine specimens
2. Preparation of patient
3. Explain the purpose of the test by using simple language. Do not use medical terms or try to explain details of the procedure.
4. Advise the patient how to collect the specimen. The first morning urine or mid-stream urine specimen is more preferable, because it is more concentrated.
5. If the patient is female, advice her to wash her genital organ before giving the specimen. This is because bacteria that are normally found on the genital tract may contaminate the sample and affect the result.
6. Advise the patient to collect at least 15 ml of urine in to the clean, sterilize and dry urine cup that is supplied from the laboratory.
7. The collected urine sample should arrive at a diagnostic laboratory as soon as possible.
8. Centrifugation of the urine specimen
9. Mix the urine specimen
10. Transfer about 10 ml of urine in the centrifuge tube.
11. Balance tubes in the centrifuge.
12. Centrifuge the specimen at a medium speed (from 1500 –2000 rpm) for 3-5 minutes
13. Discard the supernatant by quick inversion of the tube
14. Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers
15. Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean, sterilized and dry slide. If Pasteur pipette is not available, gently incline the tube and place drop of sediment into the clean, sterilized and dry slide.
16. Apply cover slide on the urine sediment that is on the slide.This will make specimen to be spread on the slide on one cell thickness.
17. Put the slide on the stage of microscope and tie it by clips on the stage.
18. Lower the condenser, close the diaphragm and look under10x objective of the microscope. Casts tend to concentrate near the edge of cover slide.

19. Then after looking through at least 20 fields of the low power objective, change the objective in to 40x objective. Do not forget to raise the condenser and opening of the diaphragm when you change the objective in to the high power (40x).Under high power objective also you should have to look for a minimum of 10-15 fields).
20. Then report what you get under10 x (low power) and 40 x(high power) on the laboratory request form of the patient.

- *For determination of cellular elements, casts, etc, the number of elements seen under at least 10 fields should be counted and the average of this number is used for report value. Other elements such as parasites are usually reported as well.*

LAP TEST	**PRACTICAL DEMONSTRATION**

Name————————————ID.No————————Date————————

Time started————————Time ended————————

Instruction:- Demonstrate the following tasks(1hr)

1. Perform microscopic examination of urine according to the SOPs?
2. Identify urine crystals microscopically?

6.1. Performing Body Fluid Analysis

Objectives: At the end of this chapter the trainees beable to:

- ✓ Describe the overview of body fluids
- ✓ Describe body fluid analysis methods.
- ✓ Perform semen analysis.
- ✓ Perform cerebrospinal fluid analysis.

6.1. Cerebrospinalfluid(CSF)

Fluid in the space called sub-arachnoids' space between the arachnoids mater and pia mater

Protects the underlying tissues of the central nervous system (CNS)

- Serve as mechanical buffer to
- Prevent trauma,
- Regulate the volume of intracranial pressure
- Circulate nutrients
- Remove metabolic waste products from the CNS
- Act as lubricant

Has composition similar to plasma except that it has less protein, less glucose and more chloride ion

  - Itisoneofthevertebratesbodyfluidcontainedinthecavitythatsurroundsthebrainandthe spinal cord.
  - It supplies nutrients to the tissues of the central nervous system
  - It helps to protect the brain and spinal cord from injury.
  - ThevolumeoftheCSFinadultsis100–150ml;inchildrenthevolumeislessandvaries according to the body length.

- Maximum volume of CSF
  - Adults 150 mL
  - Neonates 60 mL
- Rate of formation in adult is 450-750 mL per day or 20 ml per hour

- reabsorbed at the same rate to maintain constant volume

- Collection by lumbar puncture done by experienced medical personnel
- About 1-2ml of CSF is collected for examination
    - lumbar puncture is made from the space between the 4th and 5thlumbar vertebrae under sterile conditions.
- Collected in three sequentially labeled tubes
    - Tube 1 Chemical and immunologic tests
    - Tube 2 Microbiology
    - Tube 3 Hematology (gross examination, total WBC & Diff)
        - This is the least likely to contain cells introduced by the puncture procedure

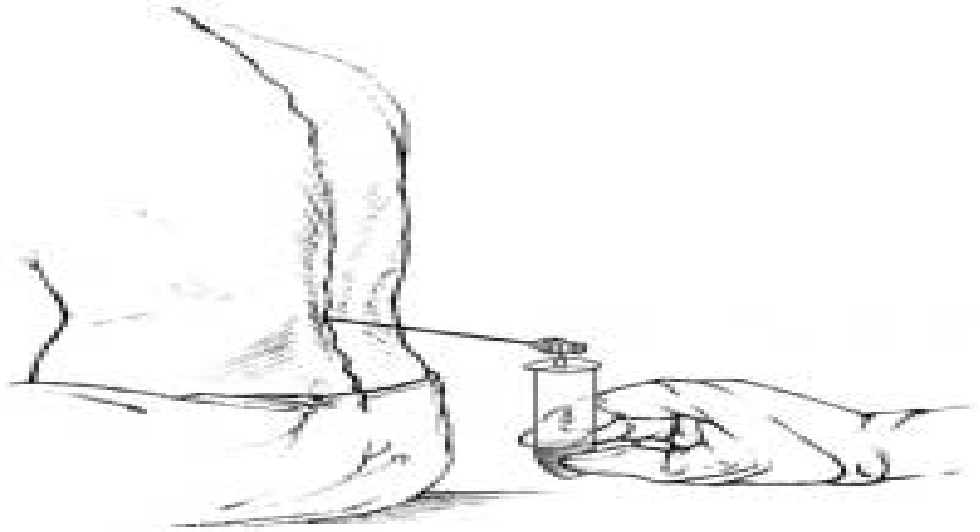


Fig. 3.13. location of CSF collection

Lab analysis

Clinical Significance

- Diagnosis of meningitis of bacterial, fungal, mycobacterial and amoebic origin or differential diagnosis of other infectious diseases
- subarachnoid hemorrhage or intracerebral hemorrhage

Principle of the test

- CSF specimen examined visually and microscopically and total number of cells can be counted and identified

Specimen: the third tube in the sequentially collected tubes*

- must be counted within 1 hour of collection (cells disintegrate rapidly). If delay is unavoidable store 2-8^{o}C.
- All specimens should be handled as biologically hazardous

6.2. Semen analysis

- Used in the evaluation of reproductive dysfunction (infertility) in the male
- Used to select donors for therapeutic insemination
- Is a cost-effective and relatively simple procedure.
- Consists of microscopic and macroscopic components

Tests for semen

- Macroscopic

-Physical (volume, viscosity, liquefaction)

-chemical l(eg. ph)

- Microscopic

-stained preparation

- wet-mount

- When investigating infertility, the basic analysis of

semen (seminal fluid) usually includes:

- Measurement of volume
- Measurement of pH
- Examination of a wet preparation to estimate the percentage of motile spermatozoa and viable forms and to look for cells and bacteria.
- Sperm count
- Examination of a stained preparation to estimate the percentage of spermatozoa with normal morphology.

Caution: Handle semen with care because it may

- contain infectious pathogens, e.g. HIV, hepatitis
- viruses, herpes viruses.

Macroscopic Examination

Measure the volume

- Normal semen is thick and viscous when ejaculated.
- It becomes liquefied usually within 60 minutes due to a fibrinolysin in the fluid.
- Failure to liquefy may indicate inadequate prostate secretion.
- When liquefied, measure the volume of fluid in millilitres using a small graduated cylinder.
- *Normal specimens*: Usually 2 ml or more

Measure the pH

- Using a narrow range pH paper, e.g. pH 6.4–8.0, spread a drop of liquefied semen on the paper.
- After 30 seconds, record the pH.
- *pH of normal semen*: Should be pH 7.2 – 7.8
- When the pH is over 7.8 this may be due to infection.
- When the pH is below 7.0 and the semen is found to contain no sperm, this may indicate dysgenesis (failure to develop) of the vas deferens, seminal vesicles, orepididymis.

Microscopic Examination

- be performed to obtain estimates of sperm concentration, motility, and agglutination.
- polygonal cells of the urethral tract and ‘round cells' such as spermatogenic cells and leukocytes can also be observed when sperm are counted in a hemocytometer.
- Motility (normal range 50% or above) is expressed as the percentage of sperm that move.
    - **Estimate the percentage of motile and viable spermatozoa**

Motility

- – Place 1 drop of*well-mixed* liquefied semen on a slide and cover with cover glass.

- ❖ – Focus the specimen using the 10_ objective.
- ❖ – Ensure the spermatozoa are evenly distributed
- ❖ – if not, re-mix the semen and examine anew preparation.
- ❖ – Using the 40_ objective, examine several fields
- ■ to assess motility, i.e. whether excellent (rapid and progressive) or weak (slow and non progressive).
- ■ Count a *Normal motility*: Over 50% of spermatozoa are motile within 60 minutes of ejaculation.

Reporting of results

- ■ Motility (normal range 50% or above) is expressed as the percentage of sperm that move.
- ■ Sperm moving rapidly in a straight line with little yaw and lateral movement are Grade 4
- ■ if they move more slowly, Grade 3.
- ■ Grade 2 sperm move even more slowly and with substantial yaw.
- ■ Grade 1 sperm have no forward progression.
- ■ Zero progression denotes absence of any motility
- ■ If motility is less than 50%, a viability stain of eosin Y with nigrosin as a counterstain is done.
- ■ dead sperm will stain red, whereas live sperm will exclude the dye and appear unstained.
- ■ In samples with no visible sperm, such as post-vasectomy semen, the entire sample should be centrifuged, and the pellet examined for intact or damaged sperm fragments.
- ■ The spermatozoa remain motile for several hours.
- ■ Perform gram stain smear:
  - ■ When more than 60% of spermatozoa are non motile,
  - ■ when more than a few leucocytes and

- > 6 red blood cell/ HPF
- Look for the type of bacteria that exist in the semen

Viability

procedure

- Mix one drop of semen with 1 drop of 0.5% eosin solution on a slide.
- After 2 minutes examine microscopically.
- Use the 40X objective to count the percentage of viable and non-viable spermatozoa.
- Viable spermatozoa remain unstained,
- non-viable spermatozoa stain red.
- *Normal viability*: 75% or more of spermatozoa should be viable (unstained).

Self-check 6	**Written examination**

Instruction1:-Say true or false for each of the following questions

1. Semen analysis used in the evaluation of reproductive dysfunction (infertility) in the male
2. CSF is used to select donors for therapeutic insemination
3. Lumbar puncture is made from the space between the 4^{th} and 5^{th} lumbar vertebrae under sterile conditions.
4. CSF should be collected in three sequentially labeled tubes among them Tube is used for Chemical and immunologic tests.
5. Tube 2 is used for Hematology (gross examination, total WBC & Diff) whereas Tube 3 is for Microbiology tests.
   1.

Score—————
Remark—————

6.1. Procedures for Collection and transportation of semen

1. Give the person a clean, dry, leak-proof container,

and request him to collect a specimen of semen at home following 3 days of sexual abstinence. Condom is used to collect the fluid, this must be well-washed to remove the powder which coats the rubber. It must be dried completely before being used.

2. Label the container (name ,date and time of collection, period of abstinence
3. Deliver the specimen to the laboratory within 1 hour
4. Fluid should be kept as near as possible to body temperature.
5. This is best achieved by placing the container inside a plastic bag and transporting it in the person's armpit . .

a. **Procedure for Estimating the percentage of motility of spermatozoa**

1. Place 1 drop of*well-mixed* liquefied semen on a slide and cover with cover glass.
2. Focus the specimen using the 10_ objective.
3. Ensure the spermatozoa are evenly distributed
4. if not, re-mix the semen and examine anew preparation.
5. Using the 40_ objective, examine several fields

b. **Procedure for Estimating the percentage of viability of spermatozoa**

1. Mix one drop of semen with 1 drop of 0.5% eosin solution on a slide.
2. After 2 minutes examine microscopically.
3. Use the 40X objective to count the percentage of viable and non-viable spermatozoa.
4. Viable spermatozoa remain unstained,
5. non-viable spermatozoa stain red.
6. *Normal viability*: 75% or more of spermatozoa should be viable (unstained).

Lap test	Practical demonstration

Name————————————ID.No————————Date————————

Time started————————Time ended————————

Instruction:- Demonstrate the following tasks(1hr)

Project 1:- Performing semen analysis

Task1:-Perform Collection and transportation of semen

Task2:- Estimate the percentage of motility of spermatozoa

Task3:- Estimate the percentage of viability of spermatozoa

Quality control in urinalysis.

- Quality assurance is a set of activates starting from specimen collection to issuing test results that ensure test results are accurate and precise as possible.
- It is the sum of all the activates of the laboratory that ensures test results are of good quality.
- Quality assurance includes
- inside and outside the laboratory performance standards
- good laboratory practice and management skills that are required by achieving and maintaining a quality service and that provide for continuing improvement
  - Part of quality assurance, which primarily concern the control of errors in the performance of tests and verification of test results.
  - must be practical, achievable, affordable, and above all continuous
  - The purpose of quality control procedure is to monitor analytical processes, analytical error and to correct result of analysis.

Two types of quality control programs

A) Internal quality control

- Is carried out in the laboratory, an intra-lab program.
- Encompasses all measurements made, technical skills performed within an individual laboratory.
- use control samples, like pooled serum
- The purpose of quality control program is to insure tests are performed reliably and reported correctly.
- Effective quality control systems detect errors at an early stage, before they lead to incorrect test results.

- **B) External quality control.**

1. External quality control is observation of variance in results when the same material is analyzed in different laboratories

External quality control is observation of variance in results when the same material is analyzed in different laboratories

Quality control steps:

- Pre analytical steps
- Analytical steps
- Post analytical steps

1. Pre analytical Quality control in urinalysis

- Read and understand requested paper
- guide the patient to bring an appropriate urine sample
- Labeling the urine container after collecting the sample
- Cheek the material we are going to use whether they are properly cleaned or not
- Ask the patient whether the urine sample left ,more than two hours, after it is voided.
- Do not accept contaminated requested paper
- Cheek the slide, the microscope, & all needed material before taking the next procedure.
- If the urine comes from far place ask or read the preservative applied
- Concentrate and find out an abnormality related to chemical & physical appearance.
- Proper sample preparation is also most important.
- Reduce possible source of errors
- Do not open the centrifuge while it is not stopped
- Proper balance of urine in the centrifuge

2. analytical quality control in urinalysis

- small urine sample how to be rejected
- Follow exactly standard operation procedure (sop)
- Check and read reagent strip chemical test according to the instruction of the manual of the manufacturer, at the right time
- Write the physical appearances properly
- use the needed amount of urine for centrifugation
- When discarding the supernatant, it has to be quick and vertical upside down in order not to lose the sediment
- Examine as quickly as possible

3. post analytical quality control in urinalysis

- Proper written result
- Correct calculation
- Result interpretation

Self-Check 7	Written Test

Exercises4: Say True or False

1. External quality control is observation of variance in results when the same material is analyzed in different laboratories
2. Quality assurance is a set of activates starting from specimen collection to issuing test results that ensure test results are accurate and precise as possible.
3. Quality control is the sum of all the activates of the laboratory that ensures test results are of good quality.
4. Quality assurance includes inside and outside the laboratory performance standards
5. Proper sample preparation is part of post-analytical quality assurance.

Answer sheet

Name————————————————ID.No————————

1. ————
2. ————
3. ————
4. ————
5. ————

Score————————————

Remark————————————————————————

3.1. Verifying Laboratory results before releasing for clinician/client

In this topic, a review is given of all elements involved in the validation of clinical laboratory results. Validation will include:

1. Method validation,
2. Instrument validation,
3. Preanalytical validation procedures,
4. Analytical validation procedures, and
5. Postanalytical validation procedures.

Within the scope of this sub-topic, all of these different elements are discussed in detail. The management of all these steps is the only way to guarantee a correct result, if this is used either for patient treatment or in clinical evaluation studies.

All the types of validation is expressed in the diagram in page 64 below

Checklist for validation of test results

A validation of patient results should be performed using this checklist. Only when a complete validation is performed the report may be authorized to be sent to the requester.

Patient ID:-——————————

Pre-analytical phase

- ✓ □Patient was correctly identified
- ✓ □Patient was properly prepared for sample collection
- ✓ □The person collecting the samples was correctly identified
- ✓ □Sample was labeled correctly and clear
- ✓ □The request form matches the specimen
- ✓ □The request form contains correct and clear contact details of the requester
- ✓ □The date and time of collection is indicated on the request form
- ✓ □The specimen was transported appropriately to the laboratory
- ✓ □The specimen was received in acceptable condition

- ✓ The log book entry matches the specimen label

Analytical phase

- ✓ Reagents and test kits used were within expiry date
- ✓ Quality controlsassociated with the result wereacceptable
- ✓ There were no flags on the analyzer's results that need investigation
- ✓ If diluted, the final results were calculated correctly with the correct dilution factor
- ✓ Results are within the biological reference intervals
- ✓ Panic (critical) values are confirmed
- ✓ The results make clinical sense
- ✓ Confirmatory testing or established testing algorithms werecompleted
- ✓ If applicable: previous patient results are available to assist with interpretation of current sample's result

Post Analytical phase

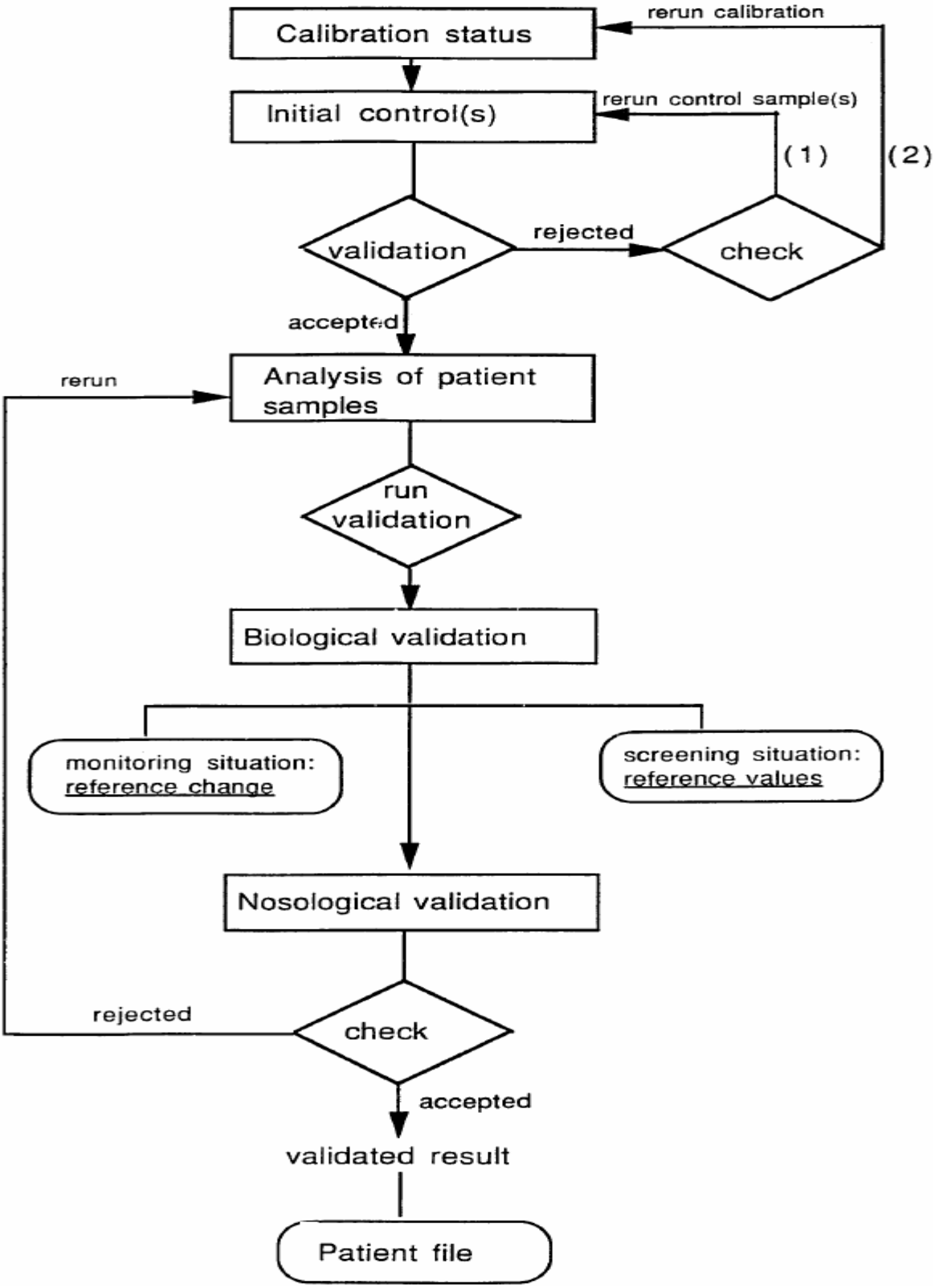
- ✓ The report shows an appropriate result including test and result matchfor each test requested
- ✓ Proper concentration units for resultsareused
- ✓ The decimal place is correct(if resultshavedecimals)
- ✓ The personsperforming the tests areidentified
- ✓ All results and documentation are legible
- ✓ In case of results within critical intervals the need for immediate notification is indicated on the report and an immediate notification form is used to verify correct reception of the result report by the requester
- ✓ If applicable, the report contains interpretative information to assists the clinician
- ✓ The release of the results is dated and timed

Remarks:————————————————————————
_____

Authorizer's name, signature and date for completion of validation and correctness of results:

Date:—————— Name:—————— Signature:——————

validation of results



References

LG52. Maintain laboratory records

Welcome to the module "Performing Urine and Body Fluid analysis". This learner's guide was prepared to help you achieve the required competence in "**Medical laboratory services Level-III** this will be the source of information for you to acquire knowledge and skills in this particular occupation with minimum supervision or help from your trainer.

Summary of Learning Outcomes

After completing this learning guide, you should be able to:

LO4. Maintain laboratory records

1. Entering of data on report forms or into computer systems
2. Maintaining log of Instruments
3. Recording of received urine
4. Maintaining Security and confidentiality
5. Maintaining Laboratory data and records

Learning-instructions

1. Read the contents of this Learning Guide. It is divided into sections that cover all the skills and knowledge that you need.
2. Read the information written in the "Information Sheet #1, #2, and # 3".

3. Accomplish the “Self-check #1on page 15 &16, #2 on page 20, and #3 on page 23
4. If you earned a satisfactory evaluation on self-check proceed to next learning Guide. However, if your rating is unsatisfactory, see your teacher for further instructions.
5. Read the “Operation Sheet” and try to understand the procedures discussed.
6. Practice the steps or procedures as illustrated in the operation sheet. Go to your teacher if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedures

This learning guide is developed to provide you the necessary information regarding the

Following content coverage and topics –

- Entering of data on report forms or into computer systems
- Maintaining log of Instruments
- Recording of received urine
- Maintaining Security and confidentiality
- Maintaining Laboratory data and records

Learning Activities

5. Read the information written in the “Information Sheets”.
6. If you earned a satisfactory evaluation proceed to next module. However, if your rating is unsatisfactory, see your teacher for further instructions.
7. Read the “Operation Sheet” and try to understand the procedures discussed.
8. Practice the steps or procedures as illustrated in the operation sheet. Go to your teacher if you need clarification or you want answers to your questions or you need assistance in understanding a particular step or procedure

LO4. Maintain laboratory records

4.1.Record keeping/information transcription

Increasingly, service providers are expected to keep records of interventions with clients. Whilethis can seem time-consuming and difficult, good record keeping is:

 Key to an effective service.

 help in monitoring and improvement of your service delivery.

 Help you in obtaining funding - they are a way of demonstrating the work you do and the successes you have.

Minimum Standards of records

 The provider has policies and procedures for handling information about clients, including confidentiality and data protection

 Record keeping systems are maintained and regularly monitored.

 Staffs are trained in the operation of recording systems and understand the scope of their authority to access information.

 Staffs understand and work in line with the requirements of the Data Protection Act.

 Clients are aware of their rights to access information and are enabled to exercise these rights.

 There are policies and procedures for sharing information with external agencies and clients are made aware of this on admission.

 Records are written in a clear, concise and impartial manner and are dated and signed by the author

 Statistical data is made available to inform development of local homelessness strategy.

☐ Most health service providers keep records in order to provide better support to clients.

5.1.1. Types of records

Service providers keep a large quantity of information relating to individual clients, often of a sensitive nature, contained in all or any of the following records:

- Referral and admission forms.
- Key working notes, agreements, needs assessments, and plans
- Resettlement agreements and plans
- Needs assessments
- Risk assessments and management plans
- Minutes of meetings with clients
- Records of warnings, exclusions and bans

- Correspondence on behalf of or about clients.

These records are usually combined to form a ‘client file’.

Some services have revolutionized the system of the client or client file by allowing people to look after their own file.

In day centers this system is probably best administered where the worker takes copies for a central 'staff' file, but this is with the consent and sign off of the client. This system is felt to be empowering to the clients, and encourage real partnership working on key work/support plans.

- Other records need to be kept of daily operations in:
- Log book (day book)
- Diary
- Hand-over records
- Medication records
- Accident book (health and safety)
- Incident reporting file.

4.2. Characteristics of records

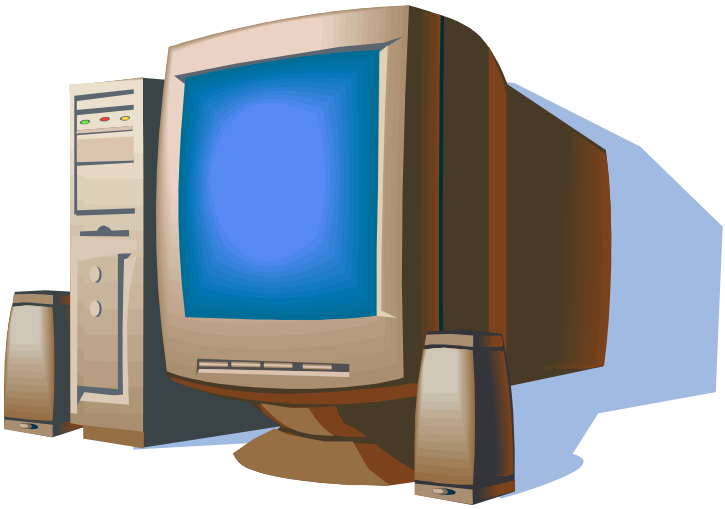
1 Recordkeeping should be Compliant

2 Recordkeeping should be Reliable

Recordkeeping systems, procedures and practices should work reliably to ensure that records are credible and authoritative.

3 Recordkeeping should be Systematic

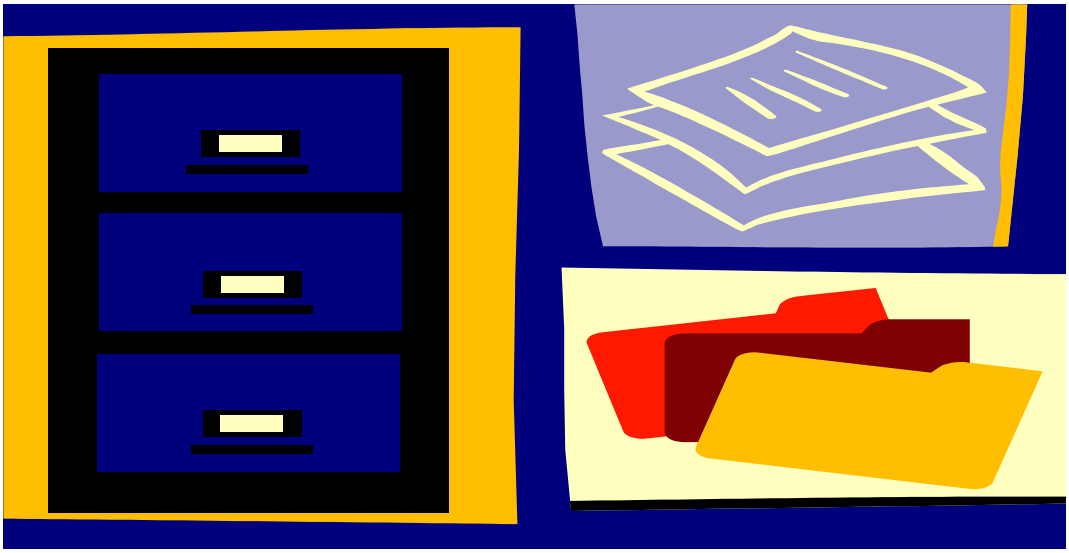
Records should be made, maintained and managed systematically.



4 Recordkeeping should be Managed

Recordkeeping must be managed through an identifiable records management program.

Recordkeeping systems must have accurately documented policies, assigned responsibilities, and formal methodologies for their management. This applies equally to dedicated recordkeeping systems and to business application systems functioning as recordkeeping systems.

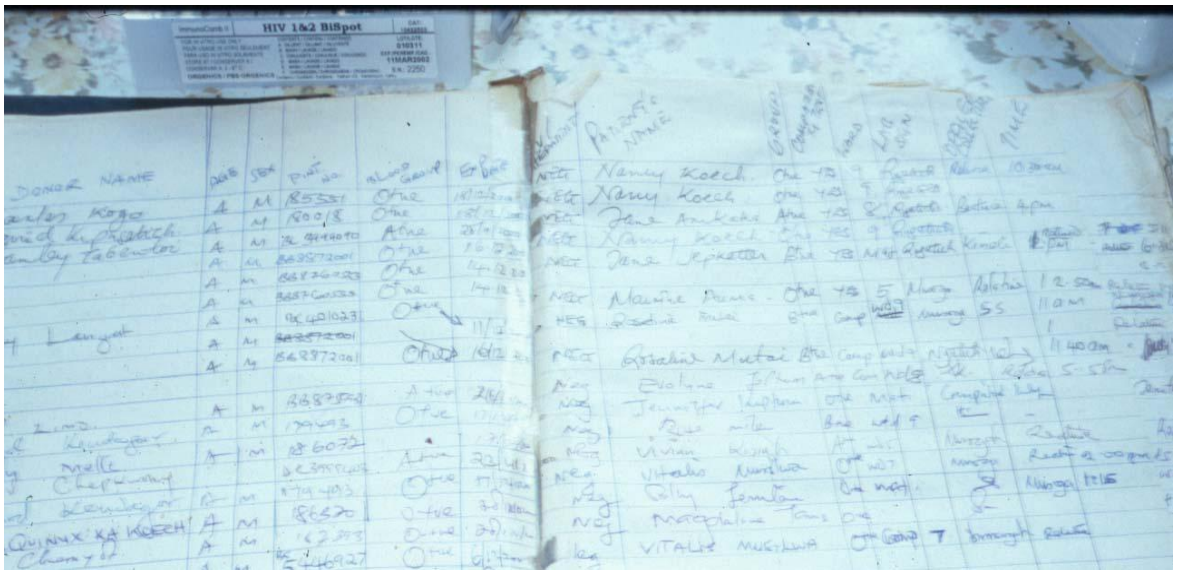


5 Recordkeeping should be Audited

Recordkeeping systems, procedures and practices should be audited to ensure compliance with regulatory requirements.

Recordkeeping practices, systems and procedures of public sector bodies operate within a regulatory regime. This regime may consist of standards and requirements to ensure the creation, management and disposal of full and accurate records. It is essential that the recordkeeping practices, systems and procedures are audited on a regular basis. The audits will:

- Identify areas of non-compliance within existing regulatory requirements
- Identify problem areas for public sector bodies, thus allowing for internal corrective actions
- Improve the quality and reliability of public records.



6 Recordkeeping should be Routine

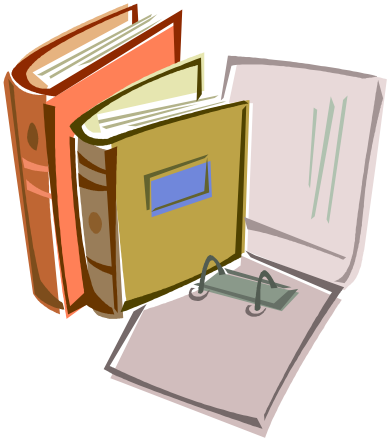
Recordkeeping systems should be used when transacting business.

7 Records should be made

Records should be made to document and facilitate the transaction of business and captured into recordkeeping systems.

8 Records should be retained

Records should be retained for as long as they are needed.



9 Records should be Complete

A record should contain not only the content, but also the structural and contextual information necessary to document a transaction. It should be possible to understand a record in the context of the organizational processes that produced it and of other, linked records.

A record comprises content, structure and context. The elements that make up the structural and contextual parts of the record are known as recordkeeping metadata.

10 Records should be Comprehensive

Records should document the whole of the business of a public sector bodies.

Records should be made of all facets of the public sector body's operations. Recordkeeping should not be selective, so that some parts of the business have no records at all. Recordkeeping should take place in all technological environments in which the organization carries out its business.

11 Records should be Adequate

Records should be adequate for the purposes for which they are kept.

Records are kept to support future business activity and to meet accountability requirements. A record must be adequate to the extent necessary to:

- facilitate action by employees (including agents and contractors) at any level and by their successors
- make possible a proper scrutiny of the conduct of business by anyone authorized to undertake such scrutiny, and
- Protect the financial, legal and other rights of the organization, its clients and any other people affected by its actions and decisions.

12 Records should be Accurate

Records should correctly reflect what was communicated, decided or done.

Recordkeeping procedures and practices must be designed to ensure that a record correctly reflects what occurred. Business processes and systems should be designed to make it easy, or even automatic, to make accurate records of transactions.

Falsifying information in a record is illegal.

13 Records should be Authentic

Records should be what they purport to be.

It must be possible to prove that records are what they purport to be and that their purported creators, including the senders of communications, indeed created them. The recordkeeping system must operate so that the records derived from it are credible and authoritative. It should be possible to show that the recordkeeping system was operating normally at the time the records were captured by the system.

14 Records should be Useable

Records should be identifiable, retrievable, accessible and available when needed.

To be able to be used, records must be maintained in such a way that they can be quickly and easily identified and retrieved when they are required. Availability is different, however, from accessibility. Records are not available unless retrieval systems are adequate, but access to records may be tightly restricted (for example, for security or privacy reasons). It is not necessary that access to records be unrestricted to comply with this principle.

15 Records should be Inviolate

Records should be securely maintained to prevent unauthorized access, destruction, alteration or removal.

Records should be kept using facilities, materials and methods which promote their survival undamaged for as long as they are needed. Records should be protected from tampering, unauthorized alteration, and from accidental or intended damage or destruction. The protection can include the physical security of premises, the selection of appropriate materials and systems, and procedures which hinder loss or unauthorized alteration.

Patient confidentiality

Confidentiality is the right of an individual to have personal, identifiable medical information kept private.

Patient confidentiality means that personal and medical information given to a health care provider will not be disclosed to others unless the individual has given specific permission for such release.

Because the disclosure of personal information could cause professional or personal problems, patients rely on physicians to keep their medical information private. It is rare for medical records to remain completely sealed, however. The most benign breach of confidentiality takes place when clinicians share medical information as case studies. When this data is published in professional journals the identity of the patient is never divulged, and all identifying data is either eliminated or changed. If this confidentiality is breached in any way, patients may have the right to sue.

The greatest threat to medical privacy, however, occurs because most medical bills are paid by some form of health insurance, either private or public. This makes it difficult, if not impossible, to keep information truly confidential.

LO5. Maintain a safe work environment

Common hazards in health laboratories

The following are important hazards that require assessment and management in health laboratories:

- Naked flames
- Microbial hazards
- Chemical hazards
- Equipment hazards
- Explosions
- Infestation by ants,
- Glassware hazards

- Unreliable water supply
- Sharps hazards

Common causes of accidents in health laboratories

Hazards	
Types of laboratory hazards	**Injury from chemicals** – When chemicals with irritating fumes are used in a laboratory with Inadequate ventilation. – When hazardous chemicals are stored on high shelves or on the floor Under benches. **Injury from equipment:** – When electrical equipment has faulty earthling or insufficient ventilation. – when unsafe adaptors or extension leads are used because there are Insufficient electric wall points. – when the laboratory has no preventive maintenance schedules and equipment is not inspected regularly for defective insulation, corrosion, And loose connections.
Naked flames	**Injury from fire caused by lighted Bunsen burners, spirit burners, tapers,** **matches, alcohol swabs, ring burners, stoves:** – When a lighted burner is placed in sunlight, making the flame difficult to see

	– When a Bunsen burner, ring burner, match, or taper is lit too close to a Flammable chemical. – When a lighted taper is carried across the laboratory close to where a flammable stain or reagent is being used or stored
Chemical hazards	**Toxic or harmful chemicals causing serious ill health, injury, or irritation:** – When toxic or harmful chemicals are swallowed by being mouth- Pipetting. – When fumes from irritant chemicals are inhaled in poorly ventilated areas of the laboratory – When no protective goggles or gloves are worn and harmful chemicals enter the eye or come in contact with the skin **Flammable chemicals causing fire:** – When flammable chemicals are used or stored near a naked flame – When a lighted ‘swab’ is used to heat stain in the Ziehl-Neelsen method and ignites nearby flammable chemicals – When the neck of a bottle containing a flammable chemical is accidentally flamed – When a flammable chemical is spilled near a flame **Corrosive chemicals causing serious injury and burns:**

	– When corrosive reagents are ingested by being mouth-pipetted – When strong acids are accidentally knocked from shelves or spilled – When intense heat is produced during the dilution or dissolving of a strong acid or alkali or when water is added to a concentrated acid – When a corrosive chemical comes into contact with the skin, or the eyes are splashed when opening and pouring a corrosive chemical
Equipment hazards	**Electric shock:** – When equipment is not reliably earthed or electrical circuits are faulty – When touching live wires in attempting to repair equipment or replace components, e.g. lamp, without first disconnecting the equipment from the mains – When handling electrical equipment with wet hands or standing on a wet floor **Fire:** – When cables and electrical equipment overheat due to overloading of conductors – When there is overheating caused by the overuse of adaptors – When insulation is inadequate or becomes damaged – When thermostats fail and there is no temperature cut-out

	device to prevent overheating – When electrical sparking or arching causes flammable material to ignite – When preventive maintenance is not carried out to check for corrosion, wear, and loose connections. **Injury from moving parts:** – When an open hand-centrifuge is used in a part of the laboratory where it can easily injure a person. – When a person opens a centrifuge lid and tries to stop the motor manually (where the equipment does not have a safety device to prevent this) – When a centrifuge is not balanced, resulting in the buckets and trunnions spinning off the rotor, particularly when there is corrosion

General factors that contribute to the occurrence of accidents

- Inexperience and insufficient training and supervision of staff and lack of health and safety awareness by senior laboratory officers
- Untidy working, allowing the bench to become cluttered and not using racks to avoid spillages
- Too heavy a workload for the size of laboratory and number of staff

- Rushing to finish work ‘on time’
- Loss of concentration due to a noisy working environment, constant interruptions, and excessive heat particularly in small poorly ventilated outreach laboratories
- Fatigue due to frequent emergency work during night hours.

Many of these factors can be remedied by:

- On-going health and safety training in the workplace
- Good laboratory practice and common sense
- Changing the work attitudes of laboratory staff
- Increasing health and safety awareness in the laboratory by frequent discussions on safety issues and displaying appropriate safety symbols and notices
- Monitoring and improving the working conditions of district laboratory personnel as part oftotal quality management

Safe working environment

- Rules concerning access to the laboratory and displaying of safety signs and notices for staff, patients, and visitors to the laboratory
- Procedures to follow to maintain local laboratory security
- How to keep the laboratory clean
- How to separate and dispose of general waste, broken glass and other ‘sharps’, contaminated materials, and different specimens
- Decontamination procedures
- Washing of reusable specimen containers, needles, syringes, lancets, slides, cover glasses, pipettes
- Disinfectants and their use in the laboratory
- Sterilization procedures
- Ventilation of the laboratory
- How to check the laboratory for structural damage and wear that may lead to accidents or make the premise less secure
- Maintenance schedules and routine cleaning of equipment

- Inspecting electrical equipment for damage to insulation and loose connections in plugs
- Rules for the storage and labeling of chemicals and reagents and how to keep an inventory of chemicals
- Regulations covering the safe packing and transport of specimens
- Procedure for the reporting of faults

Safe working practices

- Personal hygiene measures and wearing of safe footwear
- Regulations concerning the wearing, storing, decontamination and laundering of protective clothing
- Preventing laboratory acquired infection including regulations to avoid the accidental:
  - –Ingestion of pathogens
  - – Inhaling of pathogens
  - – Inoculation of pathogens
- What to do when there is a spillage of a specimen or liquid culture
- Safety rules concerning the handling and storage of chemicals and reagents that are flammable, oxidizing, toxic, harmful, irritant, and corrosive, and how to manage chemical spillages
- What to do when there is a glass breakage
- How to pipette and dispense safely
- Safe operation of manual, electrical, and battery operated laboratory equipment
- Working tidily, use of racks, and rules to prevent the floor and benches from becoming cluttered and exits obstructed
- Use of protective gloves, goggles, face shield dust mask, eyewash bottle
- How to control noise levels and other causes of loss of concentration

Safe laboratory working environment

The safety of the working environment must take into consideration:

- Type of work being performed, i.e. specimens which the laboratory handles and pathogens which may be encountered
- Working practices including the procedures and equipment used
- Number of staff and workload
- Laboratory's location, climatic conditions, and security of premise

The following are important points in making the workplace safe:

- ✓ Laboratory premise that is structurally sound and in good repair with a reliable water supply and a safe plumbing and waste disposal system. Drainage from sinks must be closed and connected to a septic tank or to a deep pit. *Note:* If there is a shortage of piped water, provision must be made for the storage of water, e.g. collection of rain water in storage tanks. It is not safe for a laboratory to function without an adequate water supply
- ✓ Adequate floor and bench space and storage areas. The overall size of the laboratory must be appropriate for the workload, staff numbers, storage and equipment requirements
- ✓ Well constructed floor with a surface that is nonslip, impermeable to liquids, and resistant to those chemicals used in the laboratory. It should be bevelled to the wall and the entire floor should be accessible for washing. The floor must not be waxed or covered with matting. Floor drains are recommended
- ✓ Walls that are smooth, free from cracks, impermeable to liquids, and painted with washable light colored paint
- ✓ When practical, a door at each end of the laboratory so that laboratory staff will not be trapped should a fire break out. Doors should open outwards and exit routes must never be obstructed. Where fitted, internal doors should be self closing and contain upper viewing panes. External doors must be fitted with secure locks
- ✓ Adequate ventilation supplied by wall vents and windows that can be opened. The windows should not face the prevailing winds to avoid excessive dust entering the laboratory in the dry season and the wind interfering with work activities. Windows

should be fitted with sun blinds and insect proof screens, and when indicated secure window bars

- ✓ Sectioning of the laboratory into separate rooms or working areas. The area where blood samples are collected from patients must be away from the testing area of the laboratory. Seating should be provided for patients outside the laboratory. The specimen reception area must be equipped with a table or hatchway which has a surface that is impervious, washable, and resistant to disinfectants. There should also be a First Aid area in the laboratory containing a First Aid box, eyewash bottle and fire blanket
- ✓ Bench surfaces that are without cracks, impervious, washable, and resistant to the disinfectants and chemicals used in the laboratory. Benches, shelving, and cupboards need to be well constructed and kept free of insect and rodent infestation. Benches should be kept as clear as possible to provide maximum working area and facilitate cleaning
- ✓ Suitable storage facilities, including a ventilated locked store for the storage of chemicals and expensive equipment
- ✓ Where required, a gas supply that is piped into the laboratory with the gas cylinder stored in an outside weatherproof, well-ventilated locked store
- ✓ A staff room that is separate from the working area where refreshments can be taken and personal food and other belongings stored safely. Near to the staff room there should be a separate room with toilet and hand-washing facilities. There should be separate toilet facilities for patients.
- ✓ A hand basin with running water preferably sited near the door. Whenever possible, taps should be operated by wrist levers or foot pedals. Bars of soap should be provided, not soap dispensers. Ideally paper towels should be used. If this is not possible small cloth hand towels that are laundered daily should be provided
- ✓ Provision of protective safety cabinets and fume cupboards as required and when feasible

- ✓ Safe electricity supply with sufficient wall electric points to avoid the use of adaptors and extension leads
- ✓ Fire extinguishers sited at accessible points. These need to be of the dry chemical type. Several buckets of sand and a fire blanket are also required
- ✓ As good illumination as possible. Low energy tube lights are recommended. Window screens must be fitted to protect from direct sunlight and glare but these should not make the working areas too dark
- ✓ Provision of *separate* labeled containers for the decontamination of infected material, discarding of needles, syringes, lancets, glassware for cleaning, broken glass, and general laboratory waste. A warning symbol such as a red triangle can be used to mark containers in which infectedmaterial is placed.

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