CHAPTER 3

Collection, Preservation and Examination Of Urine Specimen

Objectives

- At the end of this chapter the students will be able to :
- Identify factors affecting the quality of a specimen.
- List the basic procedures and prequations of urine collection.
- Describe types of urine specimens.
- Identify the commonly used preservatives and differentiate the advantages and disadvantages of their use.
- carry out physical examination of urine such as odour, volume, color, transparency, foam, specific gravity of urine and interpretation of the test result.

Out line

- ✓ Collection of urine specimen
- ✓ Preservation of urine specimen
- ✓ Types of Examination in Routine Urinalysis

Collection of Urine Specimen

- In order to make Urinalysis reliable the urine must be properly collected.
- Improper collection may invalidate the results of the laboratory procedures, no matter how carefully and skillfully the tests are performed.
- Sample should be delivered as soon as possible to the lab after collection
- If delay is necessary the appropriate preservatives based on test types should be added.
- instruct the patient to bring the mid-stream urine
- Avoid samples that is contaminated with blood in case of manusteral women
- The container should be dried and clean

Urine Containers

- types of containers used for collection of urine
- Urine bottles
- Disposable plastic containers
- Poystrine containers
- Waxy coated paper
- Plastic caps
- URIN-TEK tube
- Note : The container should be wide mouth, clean ,dry, leak proof

Urine Containers cont’d…

- The container should be labeled with :
  - The test order
  - The patient’s name
  - Time and date of collection
  - The preservative added
  - NOTE :Specially samples that comes from ward should contain all the above information in addition to the above the labeling on the sample container must matched with the request paper

Types of Specimen

- ***First Morning Specimen :*** *obtained during the first urination of the day*
  - Most concentrated
  - Bladder incubated

  preferable for:
    - Nitrite
    - Protein
    - Microscopic examination

Types of Specimen cont'd…

- ***Random Specimen*** **-** a spciemen obtained at any time during examination**.**
  - Most convenient
  - Most common

  Preferable for
    - Chemical Screen
    - Microscopic examination

Types of Specimen cont'd…

- **Postprandial** : a specimen obtained 2 hours after meal**.**
  - Good for glucose determination

- **24- Hour specimen -** obtained within 24 hours**.**

  Necessary for quantitative tests, especially for quantitative determination of protein.

Types of Specimen cont'd…

Collection of 24 hour Urine Specimen

Procedure

1. Instruct patient to completely empty his bladder and discard his urine exactly at the beginning of the 24 hour time collection (let say at 6:00 a.m.).
2. Collect all urine voided during the following 24 hours, including that voided exactly at the end of the 24 hour period in a container (at 6:00 a.m.) of the following (second) day.
3. Provide the patient with appropriate type and amount of preservative.
4. The container should be labeled

Types of Specimen cont'd…

- **Clean Catch Urine can be collected by two ways:**

A) Mid- stream Specimen - a specimen obtained from the middle part of the first urine.

- commonly used for routine urinalysis.
- important for bacteriological urine culture.

B) Catheterization is the process of passing a tube through the urethra to the bladder for the withdrawal of urine (it may introduce urinary tract infection).

Types of Specimen cont'd…

The best method of 'clean catch' urine collected as follows:

1. The genital area should be cleaned with soap and water and rinsed well. This is to keep off bacteria on the skin from contaminating the urine specimen.
2. The patient should urinate a small amount and this is discarded.
3. The urine that comes next, the mid-stream specimen, should be collected into a sterile container of 30 to 50 ml.
4. After obtaining the specimen the patient continues to urinate and this is discarded.

Types of Specimen cont'd…

- **Sources of Errors**
  - Bacteriologically or chemically contaminated specimen such as detergent remnants in the container.
  - Wrong type/amount of preservative.
  - Partial loss of specimen or inclusion of two-morning specimen in the 24 hr collection.
  - Inadequate mixing of specimen before examination.
  - Careless measuring of the 24 hr volume.
  - Wrong type of urine specimen, Such as using random sample for urine culture
  - Inappropriate storage condition .***

Preservation of Urine Specimen

- Urine specimen after voided in a container, should be examined immediately, or within maximum delay of 30 minutes.
- If not the specimen should be properly preserved to prevent those changes that occur in old urine sample.

Urine preservation cont'd…

- **Changes that occur in old urine specimen**
  - Bacteria will grow, and change the pH into alkaline.
  - This environment will convert urea into ammonia;
  - In Acidic urine glucose will be converted into acids and alcohol
  - Concentration of glucose will decrease, because of utilization of the glucose by the bacteria
  - Acetones will evaporated as a result Ketones concentration will decrease

Urine preservation cont'd…

- Bilirubin will converted into biliverdin by light
- Urobilinogen change into urobilin, and concentration will also decrease by light
- Nitrate concentration either increased if bacteria reduce nitrites, or decrease if it is converted to ammonia, which is volatile; and urine pH will become alkaline
- Turbidity will increase, because of bacterial growth, crystal formation, and precipitation of amorphous substances
- Red blood cells, leucocytes, and casts will layzed

Urine preservation cont’d…

Methods used

A. Physical methods
B. Chemical methods

Urine preservation cont'd…

A. Physical method of urine preservation

- **Refrigeration**
  - used for short period of time
  - serves for 6 to 8 hours

Limitation:

  - turbidity due to amorphous may affect microscopical exam.

- **freezing**
  - Good for bilirubin and urobilinogen

Limitation:

  - increase sp.gravity
  - destroy formed elements

Urine preservation cont'd…

B. Chemical Methods

- Different chemical are available to preserve urine for long period of time
- The choice of the chemical, depend on the required preserved substance in the urine sample.

Urine preservation cont'd…

Chemical Preservatives

- **Toluene**_
  - It preserves acetone, protein and other reducing substances like glucose; it is added into the urine sample by making thin layer over the urine
  - The disadvantage is risk of fire accidents
- **Thymole**
  - It preserves most urine constituents; it is added into the urine by making small crystal of 5mm diameter for a 100ml of urine.
  - But it causes false positive results for protein tests

Urine preservation cont'd…

- **Chloroform**
  - It preserves urine aldosterole level
  - Has higher specific gravity than urine it settles at the bottom of the urine sample thus needs frequent mixing
- **Formaldehyde**
  - It preserves most of the formed elements; [one drop per 30 ml]
  - The disadvantage of this chemical preservative is that it interferes with urine glucose determination
- **Boric acid**
  - It preserves most chemicals and formed elements
  - But the problem is that it precipitate uric acid

- **Exercises**:

Answer the following questions:

1. What type of specimen would be appropriate for both routine urinalysis and bacteriological culture?
2. List important points that should be labeled on the 24 hr urine collecting bottle.
3. Discuss about source of errors in the collection of urine.
4. What kind of changes take place in old urine specimen?
5. Discus about physical and chemical preservatives of urine with their respective advantage and disadvantages.
6. Mention limitation of chemical tests
7. Asume, you are instructed to bring your urine sample for student demonstration? Are you voluntary?, if you say yes, show the important procedures during collection ,if No reason out it

Types of Urinalysis Examination

1. Physical Examination of Urine
2. Chemical Analysis of Urine
3. Microscopic Examination of Urine

Physical Examination of Urine

-Volume

-Color

-Transparency

-odor

-Foam

-Specific gravity

Introduction

- Physical examination of urine :
  - the first part of routine urinalysis.
  - It is simplest procedure of all urine examination
  - But this simplicity doesn’t mean that any one can do it without any background knowledge and experiences.
  - Despite its simplicity, It provides hint for the subsequent chemical and microscopic urinalysis
  - It includes measurement of the volume of urine, examination of color and appearance, detecting the foam, odour, and measuring the specific gravity.

Volume

- Not measured as part of a routine urinalysis
- But volume of urine excreted in 24 hours aids for clinical diagnosis.
- The 24-hour urine voided by a healthy
  - adult range 600-2000ml
  - Children (6 to 12 years) about 1000ml
  - infants about 600ml.

Cont…

- Factors that affect the urine output
  - fluid intake
  - Diet
  - physiological and environmental factors of the b

Cont…

- **Procedures :for measurement of urine volume**
  - collect 24-hour urine specimen in a container of clean and dry, brown colored.
  - Add appropriate preservative for the desired urine chemical test
  - Label on the container that indicates name of patient, collection time and date, type of chemical test ordered and preservative used.
  - Pour the collected urine into a graduated cylinder of suitable size (500ml or 2000ml)
  - measure the volume and report in terms of ml in 24hrs

Cont…

- **Clinical Significance**
- Abnormal urine volume
  - suggests several renal diseases but it is confirmed by confirmatory tests
  - Helps for determination of protein
  - Indicates some clinical features like abnormal execration of urine/ day

Cont…

- Measurement of urine volume used for:
  - Quantitative tests
  - GFR
  - Clearance tests
  - Evaluation of fluid balance and kidney function

Cont…

- Anuria urine formation<100ml per 24 hours
  - Complete urinary tract obstruction
  - Hemolytic transfusion reaction
- Polyuria urine volume is greater than normal >2000ml per 24 hours for prolonged period.
  - Diabetic mellitus
  - Tubular necrosis [improper function of urine tubules]
  - Intravenous fluid intake

Cont…

- **Oliguria :**urine volume is less normal <400ml per 24 hours for prolonged period
  - Dehydration or poor blood supply to the kidneys
  - Mechanical obstruction of the urinary system [e.g. due to renal calculi or tumors]
  - Excessive salt intake.
- **Diuresis:** temporal increment of urine due to excessive fluid intake

color

- Urine color is recorded within 30 minute after collection
- Pale to colorless urine may indicate
  - Large fluid intake
  - Diabetic mellitus
  - Alcohol consumption
- Dark yellow or brown red urine may indicate
  - Concentrated urine
  - Decreased fluid intake
  - Fever
  - Dehydration

Cont…

- Yellow brown or “beer brown” color may indicate the presence of bilirubin.
- Clear red color may indicate presence of hemoglobin
- Cloudy red or smoky red color indicates hematuria.

odour

- Normally freshly voided urine from healthy individuals has faint aromatic odor
- The test is conducted by smelling of urine and the result is not used for reporting but for the purpose of comparison with the subsequent chemical test result.
- If the urine specimen is old, it will have ammonical or pungent odor, which results from the break down and conversion of urea in the urine into ammonia by the action of bacteria.
- Sweet fruity odor of urine results from the presence of ketone bodies due to diabetes mellitus, prolonged vomiting, starvation or strenuous exercise.

foam

- Normally fresh urine produces small amount of white foam, But during certain abnormal conditions, it may be changed.
  - Yellowish foam indicates the presence of bilirubin in the urine
  - must be confirmed by chemical test
  - When high concentrations of protein are present in the urine, a large amount of white foam may be seen

Transparency

- Freshly voided urine specimen is normally clear and transparent
- Excretion of turbid urine is not always un abnormal
  - Refrigeration of urine results in the turbidity of the urine.
- Occasionally turbidity of urine may result from
  - White blood cells [pus cells]
  - Kidney stones
  - Yeast cells
  - High number of bacteria cells
  - High number of mucus treads

  N.B All the above physical findings must be confirmed by chemical and/or microscopic examination

- **Note:**
  - If the urine is so cloudy, microscopical examinations should be done for the suspended solutes in the sample.
  - All urine specimens should be assessed for color and transparency in similar containers to ensure consistent results.

Specific gravity (Sp.g)

- **(Sp.g):**
  - ratio of the weight of a fixed volume of solution to that of the same volume of water at a specified temperature usually
- Indicator of the kidneys ability to concentrate or dilute urine.
- Loss of concentrating or diluting capacity is an indication of renal dysfunction.
- Reflect degree of hydration or dehydration.
- A concentrated urine may have a specific gravity of **1.025 –1.030**
- A diluted urine can have a specific gravity of **1.001-1.010.**

Chemical analysis of urine

Introduction

- Chemical analysis of urine deals with examinations of chemical reactions.
- Chemical test are done for a quick screening of urine for certain substances which have a diagnostic significance.
- In this category of tests, abnormal or pathological dissolved constituents of urine (chemicals) performed in routine urinalysis

Introduction Cont…

- Chemical analysis of urine is important in the detection of many diseases.
- Urine contains normal chemical compositions. But in abnormal (pathological) conditions its composition varies in kind and quantities.
- So the chemical changes of urine can indicate disease at the early stage.

Introduction Cont…

- Urine composition depends greatly on how much and what specific waste material is to be excreted.
- Urea, creatinine, uric acid, ammonium salts, chlorides, sulphates and phosphates of sodium, potassium, calcium and magnesiumn are the normal composition of urine.
- Glucose, protein, ketone bodies, bilirubin , bile salts etc. are the abnormal constituents of urine. Normaly these substances do not appear in the urine in detectable amount . So their appearance in the urine shows the pathological condition.

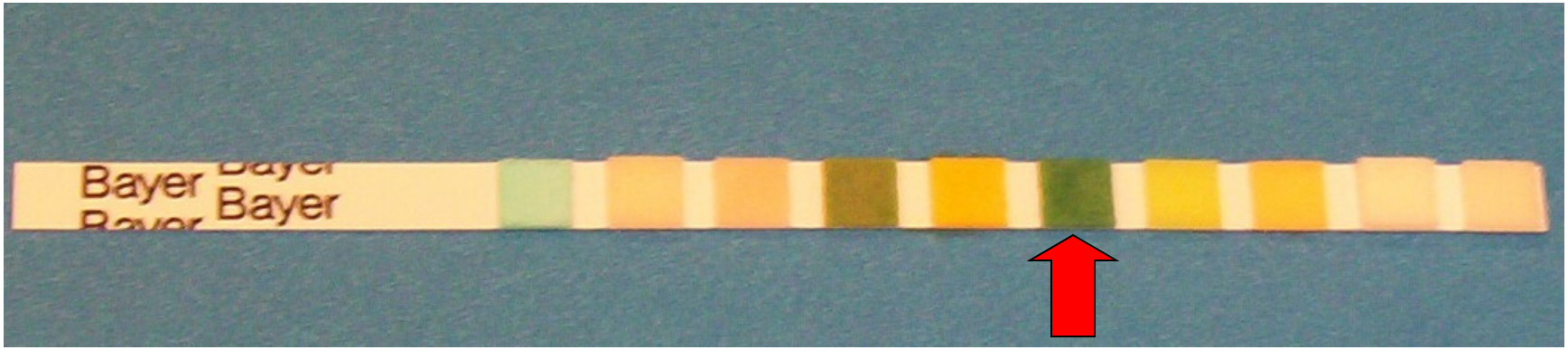
Chemical analysis

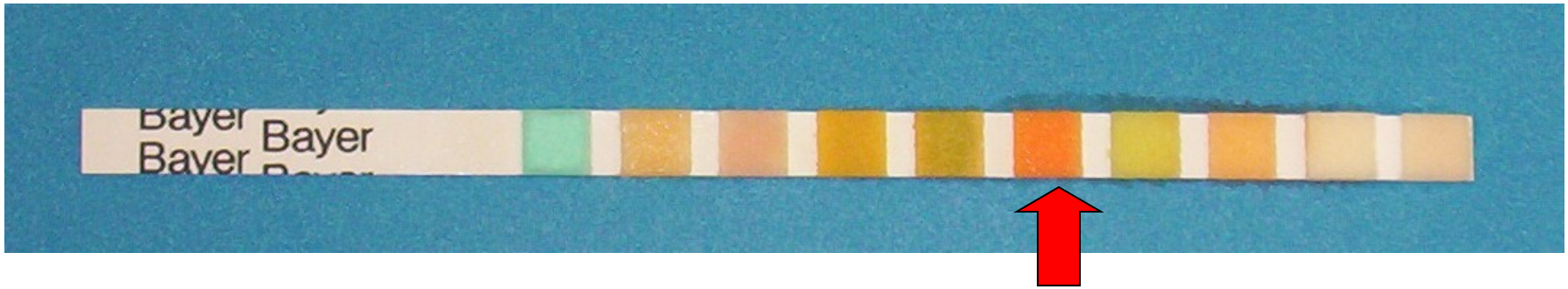
- Chemical analysis of urine can be classified as:
- Screening test
- Qualitative test
- Quantitative

Chemical analysis

- Screening test : tells the presence or absence of the abnormal components of urine
- Qualitative test: tells semi-quantitatively the amount of solutes that exist at the detectable level in pathological or physiological conditions
- Quantitative test: tells the exact amount of abnormal constituents if they are detectable

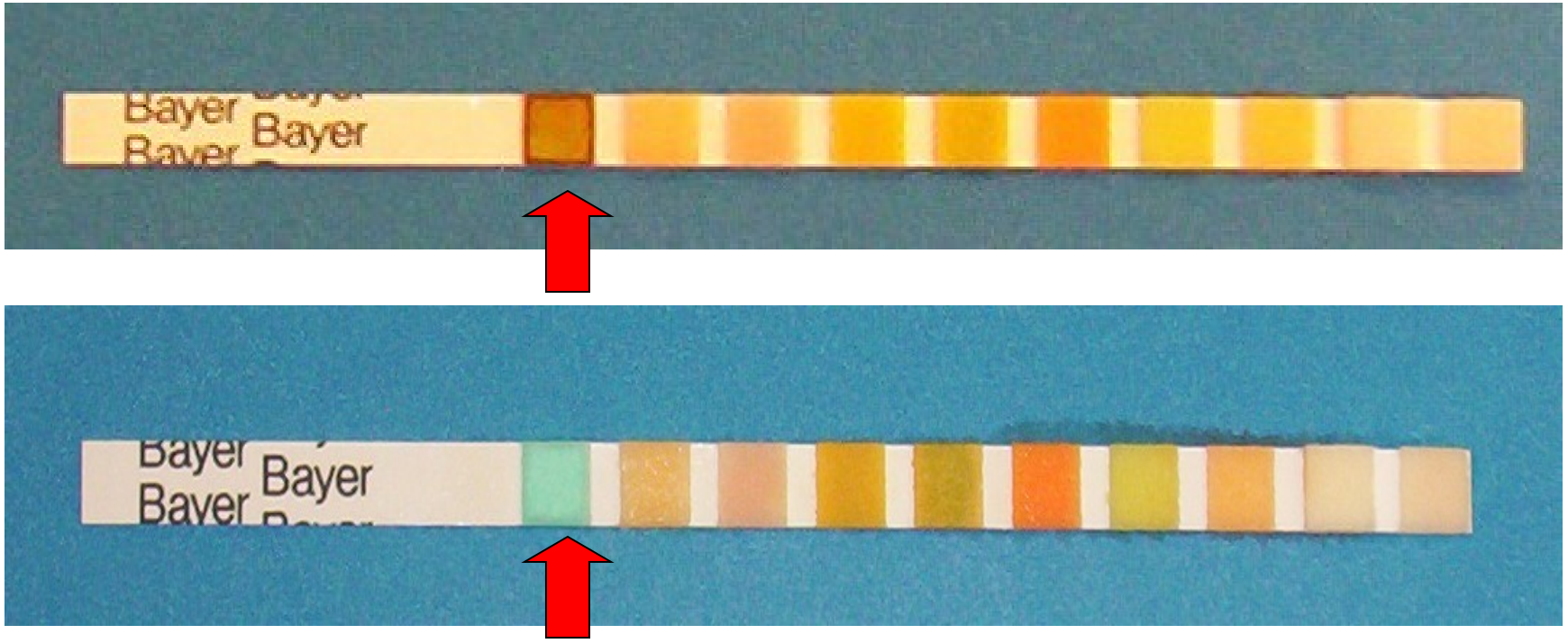
Elevated pH (alkaline)





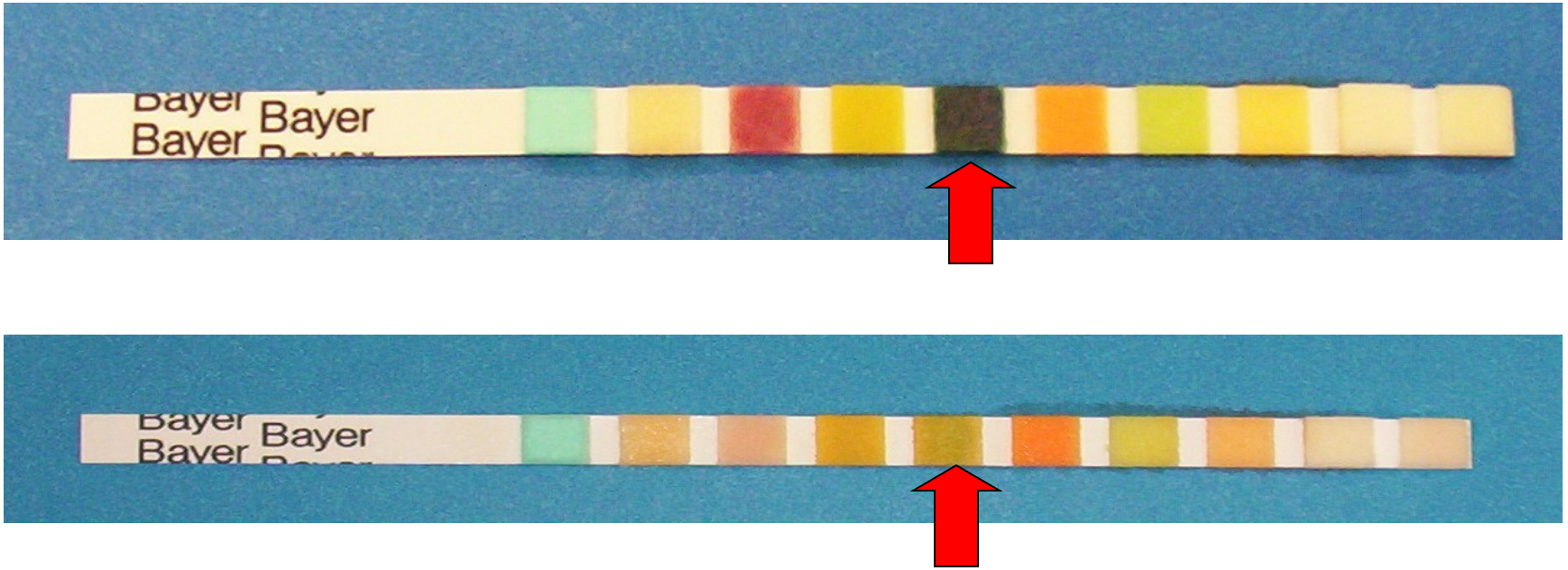
(Normal for comparison)

Positive Glucose



(Normal for comparison)

Positive urine Ketones



(Normal for comparison)

Microscopic Examination Of Urine

Introduction

- In examining urinary sediment it is necessary to learn the distinguishing characteristics of elements which have significance .
- If the urine sample is properly collected, the test is carefully performed, and the person performing the test is knowledgeable, skillful valuable information can be obtained from microscopic examination of urine specimen.

Procedure for Microscopic Examination

Assemble all necessary materials used for the collection, centrifugation and examination.

- Conical centrifuge tubes, or regular test tubes up to 15 ml.
- centrifuge.
- Pasture pipette with .
- Slides and cover slides 20 x 20 mm.
- microscope

Procedure for Microscopic Examination cont'd…

1. Mix the urine specimen
2. Transfer about 10 ml of urine into a labelled centrifuge tube.
3. Centrifuge the specimen at a medium speed (from 1500 – 2000 rpm) for 3-5 minutes
4. Discard the supernatant by quick inversion of the tube
5. Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers
6. Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean and dry slide.

Procedure for Microscopic Examination

- Apply cover slide on the urine sediment that is on the slide.
- Put on the microscope and look under 10x objective of the microscope.
- Then after looking through the low power objective, change the objective in to 40x objective .
- Then report what you get under low power and high power objective on the laboratory request form of the patient.

Organized (Formed) elements

- RBCs/HPF
- WBCs/HPF
- Epithelial cells / LPF
- Casts / LPF
- Parasites/LPF
- Bacteria / HPF

- Yeast Cells /`LPF
- Mucus trade/LPF
- Spermatozoa
- Miscellaneous substances

Non-organized (Non-living Material)

- ***Slightly acidic urine***
  - Triple phosphates
  - Amorphous phosphate
  - Calcium carbonate
  - Calcium phosphate
- ***Acidic, Neutral, or slightly alkaline Urine crystal***
  - Calcium Oxalate crystals
- ***Alkaline, Neutral, or Slightly acidic urine***
  - Triple phosphates
  - *Alkaline Urine Crystals*
  - Amorphous phosphate
  - Calcium carbonate
  - Calcium phosphate