PART FIVE

Examination Of Urine (Urinalysis)

Urinalysis provides information about the urinary system as well as other body systems. It is usually performed in-clinic rather than being sent to an outside laboratory, which is why it is important for the veterinary team to be able to effectively evaluate a complete Urinalysis.

The Urinalysis is designed to provide information needed to pursue a particular clinical problem or as part of a health screen, Therefore, it is usually included as part of the minimum database of diagnostic tests, along with a complete blood count and clinical biochemistry profile.

Indication for Urinalysis

1. To evaluate any animal with clinical signs related to the urinary tract (Acquiring a large amount of information about several body systems as a screening method)
2. To assess an animal with systemic illness
3. Evaluation of the kidneys, through detection of abnormal components in the urine that may be of renal origin such as cast and protein.
4. To monitor response to treatment.

Possible undesirable outcomes due to delayed Analysis

1. Bacterial contamination.
2. Altered pH..
3. Disrupted and/or dissolved casts .
4. Cellular detail loss especially WBCs and epithelial cells.
5. Chemical precipitation may be confused with crystals.

Macroscopic Examinations of the Urine

I. Physical examinations (Physical Properties of the urine)

Urine volume (quantity):

The amount of urine produced daily depends upon several physiological factors including ,water and other fluid intake, environmental condition, diet, size the activity of the animal, as well as the species of the animal.

1. Increased daily urine volume called polyuria.
2. Decreased daily urine output called oliguria.

Urine Color:

The normal color is pale yellow to yellow brown or light yellow depend on the concentration of urochrome.

Interpretation:

- Pale urine is seen in diabetes mellitus,diabetes insipidus, increased water intake.
- Dark urine due to the concentration of urochromes occurs in association with dehydration,fever, decreased blood pressure, and presence of bilirubin.
- Red to red-brown due to presence of hemoglobin and myoglobin.

Urine transparency:

The transparency (clarity or turbidity) of urine as observed in a test tube or urinometer cylinate should recorded as clear, flocculent or cloudy.

Interpretation :

- Normal urine is clear when freshly voided in all animals except in the horses which is normally thick and cloudy due to the presence of calcium carbonate crystals and mucus.
- Pathologically cloudy urine observed when leukocytes, erythrocytes, epithelial cells, bacteria,mucus, fat and crystal are present.

Urine Odor:

The odor of the urine is not diagnostic,although the urine of males of certain species (porcine, feline and caprine) has an especially strong odor.

Interpretation :

- The normal odor of urine is derived from the volatile organic acids present.
- Fruity odor may be detected in urine in associated with pregnancy disease, acetonemia and diabetes mellitus.
- In dogs and cats the most common abnormal odor of urine is an ammonia-like odor , urinary tract infection by urease-producing bacteria will result in hydrolysis of urea and the release of ammonia.

Urine Foam:

When shaken after collection, normal urine produces a white foam that is limited in quantity.

Interpretaion :

If there is proteinuria the amount of foam produced is in excess and slow to disappear.

Urine Specific gravity:

Specific gravity of urine is a measurement of the relative amount of solids in solution and is an indication of the degree of tubular reabsorption or concentration by the kidney.

There is a relationship between specific gravity and total solute concentration in urine, but specific gravity is dependent on molecular size and weight, as well as on the total number of solute molecules ,since total urine solute concentration is an important tool for clinical evaluation of renal functions and is most accurately determined by smometry

Method for determination:

1. Fill a cylinder to its three fourth its hight with urine.
2. Place the urinometer in the urine and rotate it to prevent its touching the sides of the bottom of the cylinder .
3. Read the specific gravity at the highest point at the bottom of the meniscus and record.

Interpretation :

1. Increased specific gravity physiologically occur as a consequence to decrease in water intake or in animal held in a high environmental temperature.
2. Several diseases caused increasing in specific gravity such as dehydration result from diarrhea, high fever, cystitis and diabetes mellitus,
4. Lower specific gravity occurs in advanced ureamia, diabetes insipidus and excessive fluid intake.

Urinometer

II. Chemical Examination (Chemical properties of the urine)

Urine pH or Acid-alkaline reaction :

Normal values of the pH reaction of urine from any species of animal must be carefully considered as the diet and state of metabolism. In general bovine, ovine and caprine have alkaline urine, while canine and feline have acid urine ,The hydrogen ion concentration can be determined by the use of litmus paper or hydrogen pH paper strips.

Interpretation:

1. Increased acidity of the urine may result from starvation, fever and metabolic or respiratory acidosis
2. Alkaline urine occur in cystitis, ingestion of salts such as sodium lactate, sodium bicarbonate, sodium citrate and nitrate, metabolic and respiratory alkalosis and when urine allowed to stand open to air at room temperature.

Protein in Urine:

Protein in urine can be estimated through ***Roberts' test*** as follows:

1. Pour 2ml of 20% sulphosalicylic acid solution into a test tube.
2. Carefully pour 6 drops of urine down the side of the tube from a dropping pipette to form a layer of urine above the acid.
3. Development of white ring at the junction indicate the presence of protein.

Interpretation :

Causes of Proteinuria:

- Tissue destruction and necrosis
- Fever & sever inflammatory processes
- Renal diseases(Increased glomerular filtration of protein, failure of tubular reabsorption of protein, tubular secretion of protein , protein leakage from damaged tubular cells, renal parenchymal inflammation).
- Diabetes mellitus

Glucose in urine :

Several methods are available for both qualitative and quantitative estimation of glucose in the urine. The simplest method is the use of ***Benedict reagent.***

a. Put 5 ml of Benedict reagent in a test tube.
b. Add 5 ml of urine.
c. Heat the mixture for 5 minutes in a boiling water bath.
d. Cool in air and read the result as follows:

- Clear blue -
- Greenish precipitate +
- Green-yellow precipitate ++
- Yellow-orange precipitate +++
- Red precipitate ++++

Interpretation :

a. Glucosuria is indicated in the case of diabetes mellitus.
b. Excessive administration of glucose-containing fluids
c. Renal tubular disorders
d. Stress or sever excitement
e. Glucosuria without hyperglycemia may occur in some dogs and cats with chronic disease, possibly due to altered proximal renal tubular function.
f. Chronic disease unrelated to the kidneys in some dogs and cats (associated with normoglycemia)

Ketone bodies in urine:

The ***Ross test*** has been widely utilized for the detection of ketone bodies in the urine.

1. Put 5 gm of provided reagent in a dry test tube.
2. Add 5 ml of urine to the test tube.
3. Add 1-2 ml concentrated. ammonium hydroxide to form a layer above the mixture.

Results : The reaction is reported as:

- Very slight purple color :Trace
- Slight purple color +
- Moderate purple color ++
- Dark purple color +++
- Black color ++++

Interpretation :

1. Diabetic ketoacidosis
2. Prolonged fasting or starvation
3. Glycogen storage disease specially in dogs and cats
4. Low carbohydrate diet
5. Persistent fever
6. Persistent hypoglycemia

Blood in urine:

Blood may be present in the urine in the form of non-districted erythrocytes (Hematuria) or the pigment hemoglobin (Hemoglobinuria).

Benizidine test:

1. Dissolve a small amount of benzidine base in 2 ml of glacial acetic acid.
2. Add 2 ml of urine and mix.
3. Add 1 ml of fresh hydrogen peroxide and mix.

Results:

- Appearance of a green or blue color within 5 minutes indicate the presence of blood.

Urine Bile salt:

This test is based on the fact that bile salt when present in the urine lower the surface tension.

Hay's test:

1. Place 10 ml of urine in a test tube.
2. Put a small amount of sulphur on the surface of the urine.

Results :

- If the bile salt are present, the sulphur will immediately start to sink through the urine.

Interpretation:

- Bile salts are present together with bile pigments in the urine in obstructive jaundice.

Urine bile pigments:

To detect the presence of bile pigments in the given sample of urine.

Harrison test:

- Take 3 ml of urine and add same quantity of 10% barium chloride in a test tube(Dissolve 10 gm. Barium chloride in 50 ml for dist. Water and make the volume up to 100 ml). mix and centrifuge. Discard the superannuate fluid and add 1 to 2 drops of Fouchet's reagent (Trichloracetic acid 25 gm, Dist.water 100 ml, 10% Ferric chloride solution 10 ml) to the residue in the test tube. A greenish blue color is obtained if bilirubin

Interpretaion:

Moderate to severe hepatocellular damage and obstruction of bile duct.

Urobilinogens in urine :

Ehrlicks' benzaldehyde test:

1. Put 5 ml of urine in a test tube.
2. Add 0.5 ml of Ehrlicks' reagent.
3. Leave at 5 minutes and read as follows:

Results :

- Pink color normal.
- Red color access of urobilinogen.

Interpretation:

- In haemolytic jaundice urobilinogens presence in high amounts.
- In obstructive jaundice complete absence of urine urobilinogen.

Microscopic Examination of the Urine

Microscopic examination of the urine is a clinically important component of the routine urinalysis,The portion of urine that have been used in a microscopic examination is the sediment.

1. Gently mix the urine then fill about 10 ml of urine on a conical centrifuge tube.
2. Centrifuge for 15 minutes at 1000 rpm.
3. Carefully pour away the supernatant liquid
4. and leave about 0.5 ml of material at the
5. bottom of the tube.
6. Pull 1-2 drops from the deposit by pasture pipette on a clean microscopic slide and apply cover slip.

7. Examine the slide under the power 10x. The differentiation could be made through high power (40x).
8. Addition of stain to urine sediment will facilitate identification of cells and structure.One drop of 0.5% New methylene blue stain preserved by addition of a drop, or two of formalin provides a most satisfasctory stain and permits easy identification of cells and other organized elements.

Note:

- The sediment should be made using fresh specimen. If there is delay (within 2 hours before examination) the sample should be preserved by refrigeration or adding formalin.

Component of Urinary Sediment:

Urinary sediment can generally be divided into organized and unorganized elements

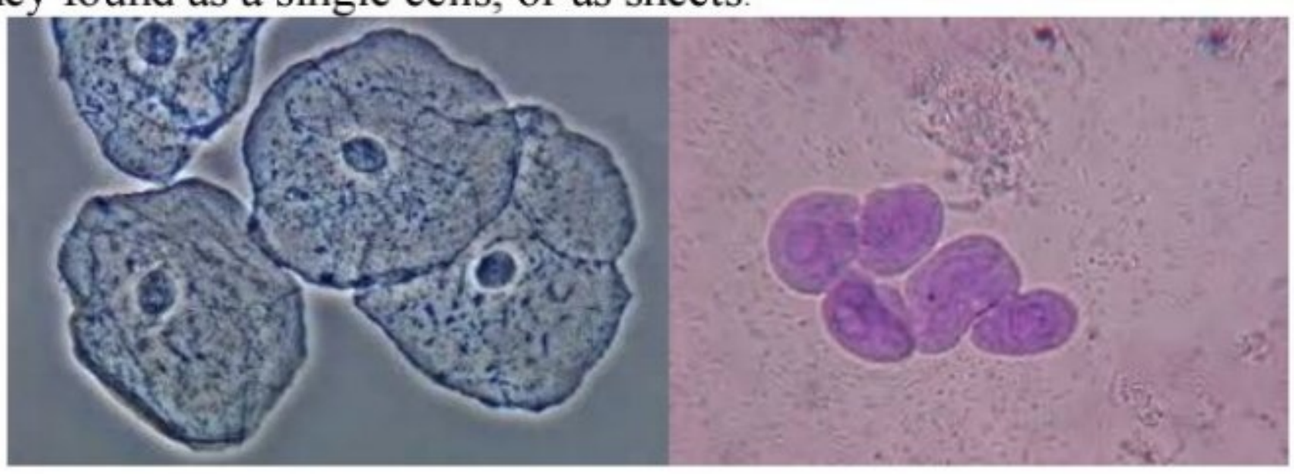
I. Organized Sediment:

Organized structures, which are primarily body cells and their derivatives, may be found in small numbers in all urine specimens, However, if they are present in any appreciable amount, they are usually associated with a pathological condition.

(1) Epithelial cells:

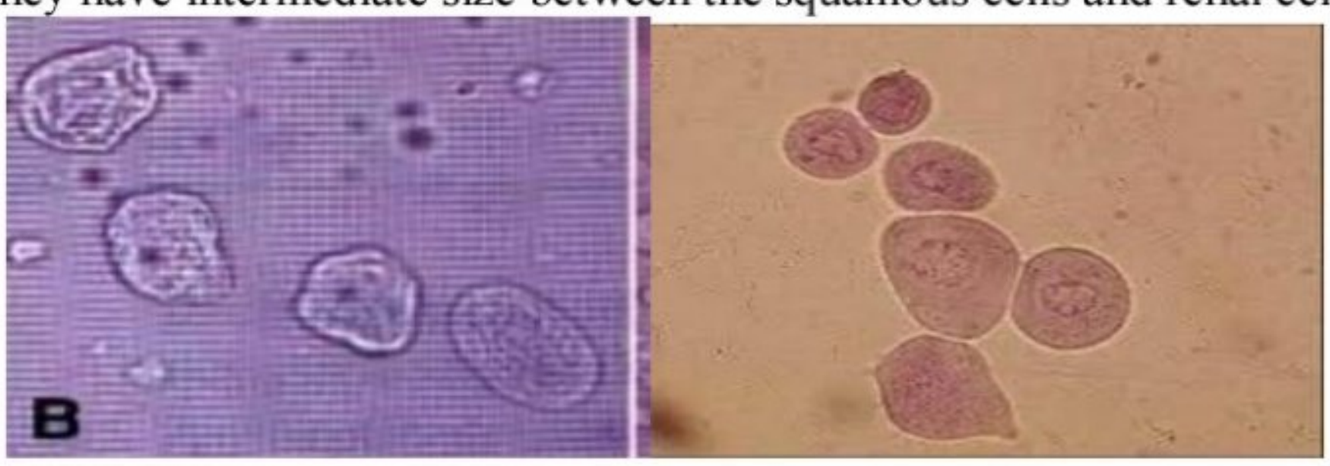
a- Squamous epithelial cells:

1. They are the largest cells that appear in the urine sediment, derived from superficial layer of the urethra and vagina.
2. They have an irregular outline, and contain a small round nucleus.
3. They found as a single cells, or as sheets.



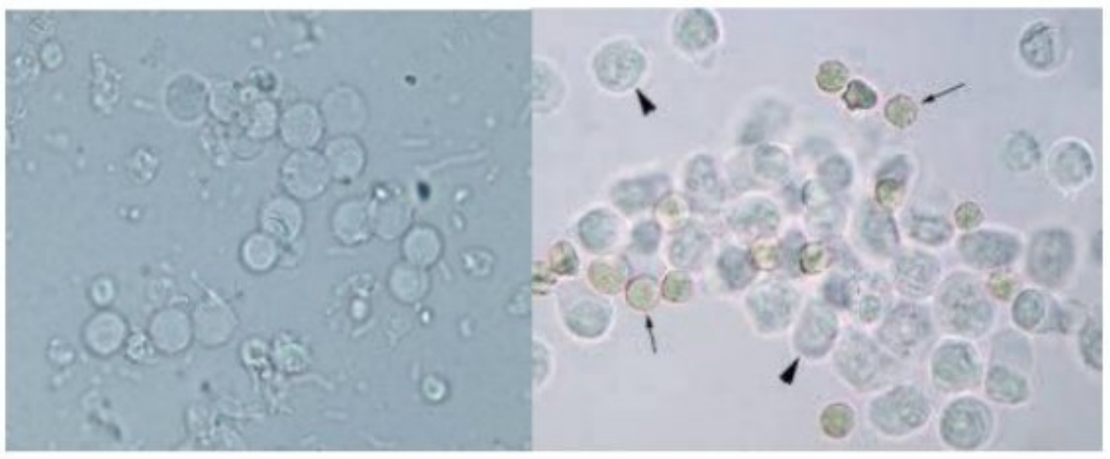
b- Transitional epithelial cells:

1. They have various forms including round,oval and spindle shape.
2. They have intermediate size between the squamous cells and renal cells.



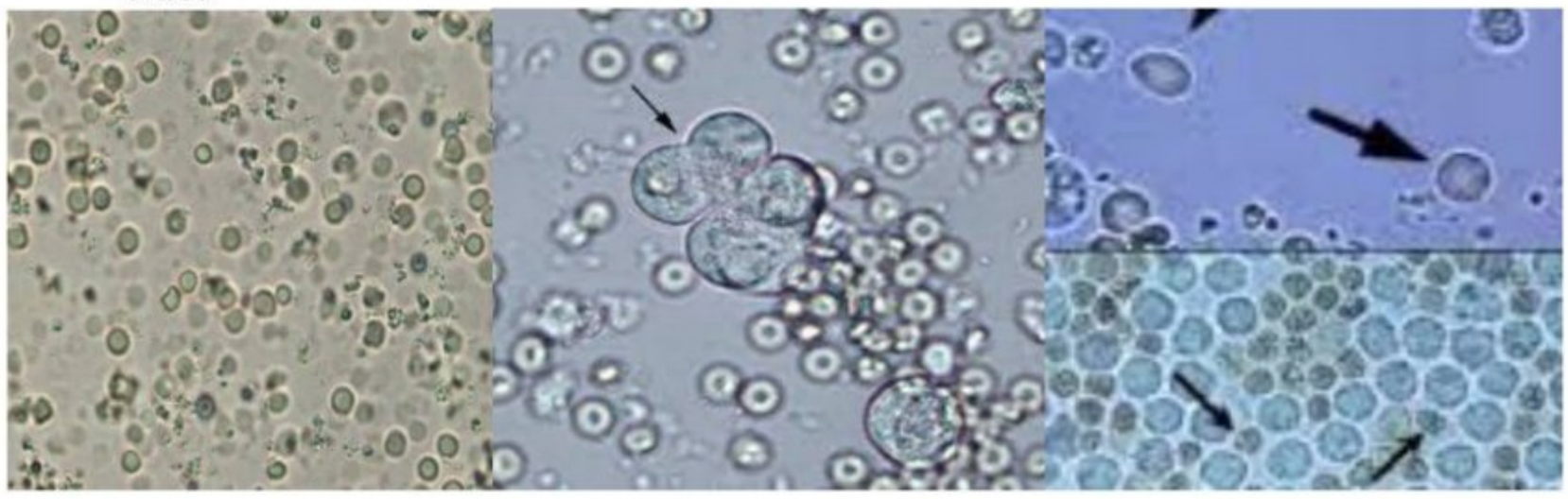
(2) Leukocytes or pus cells:

1. They are granulated cells, smaller than epithelial cells, contain nucleus.
2. Normal urine contains 1-2 white cells/HPF,but 5-9/HPF indicates urethritis, cystitis,nephritis, vaginitis and metritis.



(3) Erythrocytes:

1. They are yellow to orange cells, smaller than leukocytes, contain no nuclei.
2. In normal urine 1-2 RBC in each high power field, but presence of larger numbers of red cells more than 5-9/HPF denotes hemorrhage in genito-urinary tract.



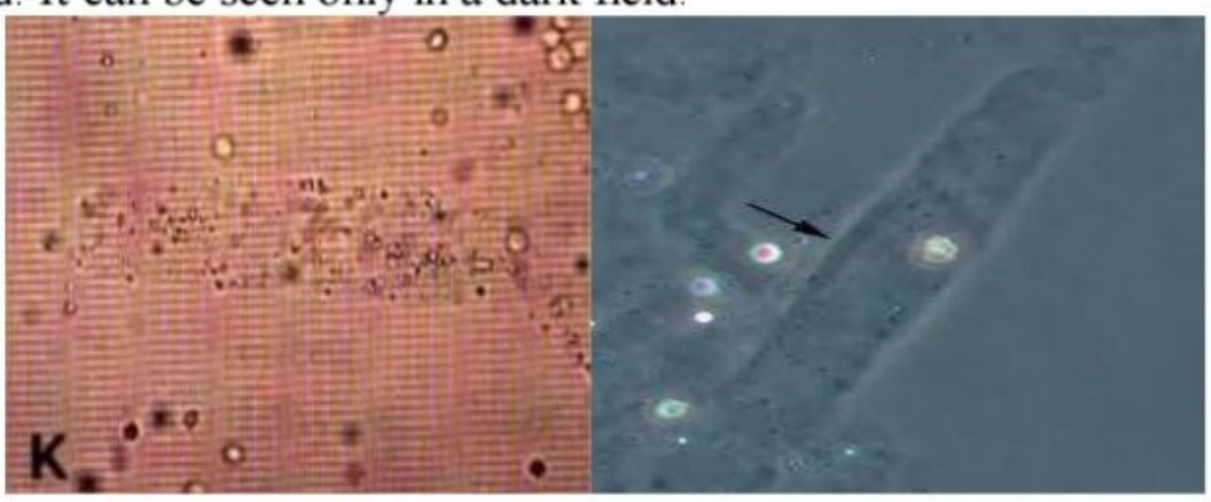
(3) Casts:

Casts are cylindrical structures comprised of varying combinations of cells and matrix mucoprotein, they are principally formed in the lumen of the distal tubules ascending loop of Henle and collecting tubules of the kidneys.

There are several types of casts:

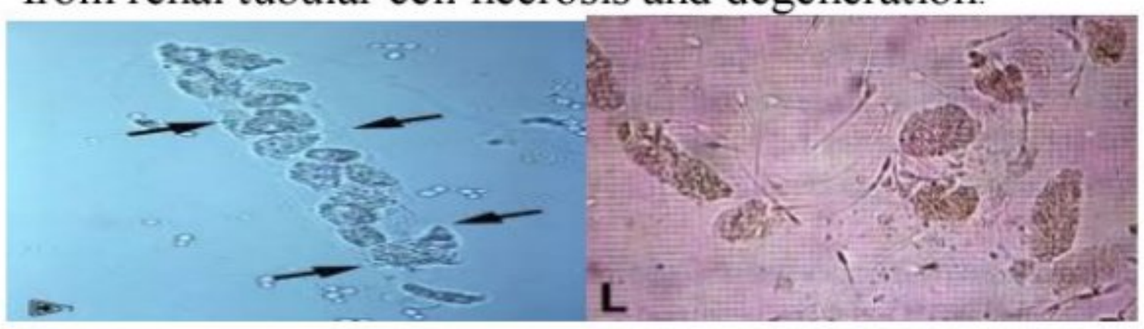
a- *Hyaline cast*.

- It is composed of protein and mucoprotein(They are pure protein precipitates of and small amounts of albumin). It is hemogenous, semitransparent colorless, cylindrical structure and having rounded end. It can be seen only in a dark field.



b- *Granular cast.*

- Granules in casts are thought to represent particulate matter arising from renal tubular cell necrosis and degeneration.

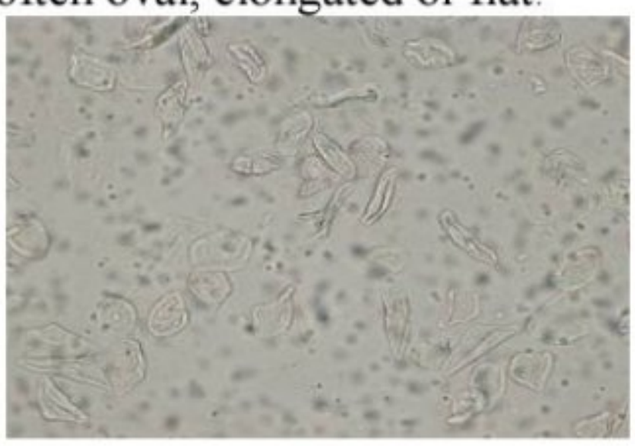


c- ***Waxy cast***.

Waxy casts are thought to represent the final stage of granular cast , They are easy to see because of their high refractive index and homogeneous, translucent appearance, and appears more opaque than the hyaline cast, grayish or colorless. It is indicates a chronic lesion of the tubules.

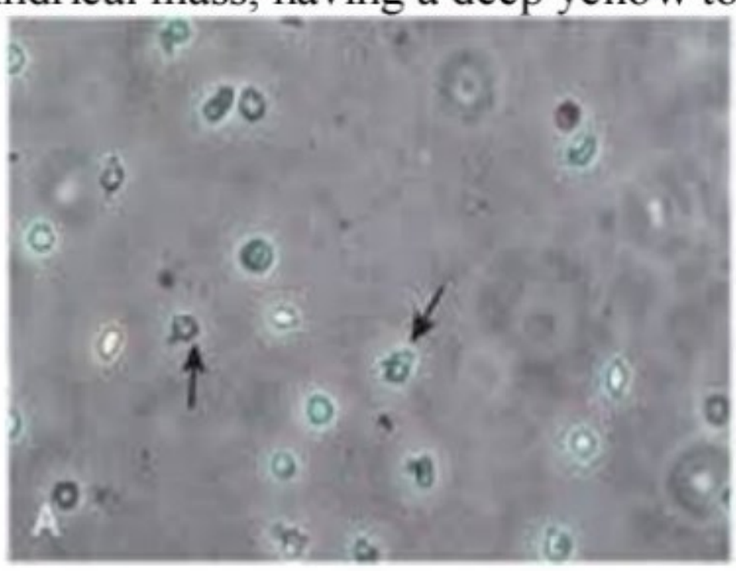
d- ***Epithelial cast.***

It is formed from the desquamated cells derived from the renal tubules. Cells within these cast vary in size and often oval, elongated or flat.



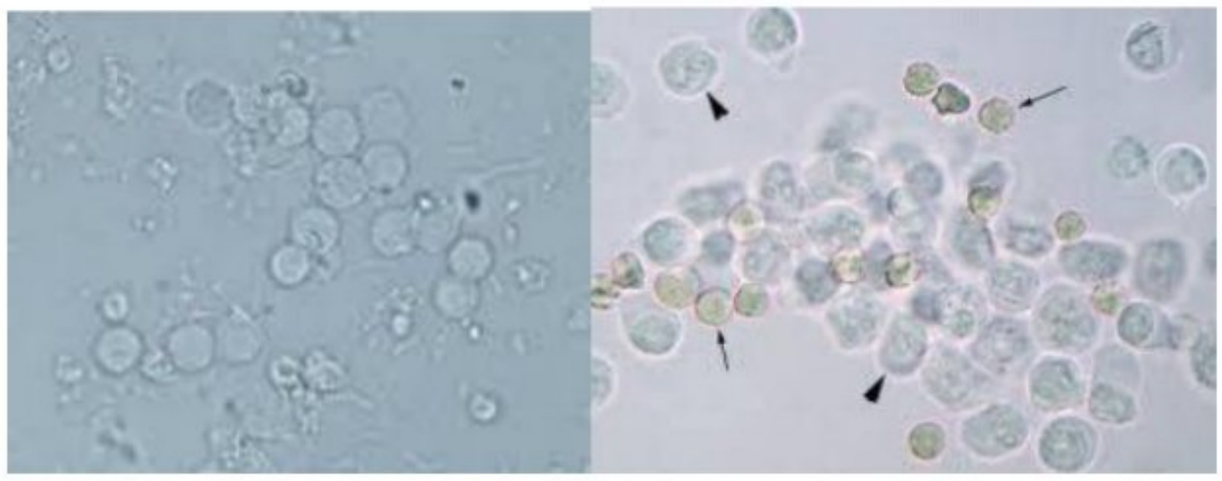
e- ***Erythrocytic cast.***

It is a hemogenous, cylindrical mass, having a deep yellow to orange color.



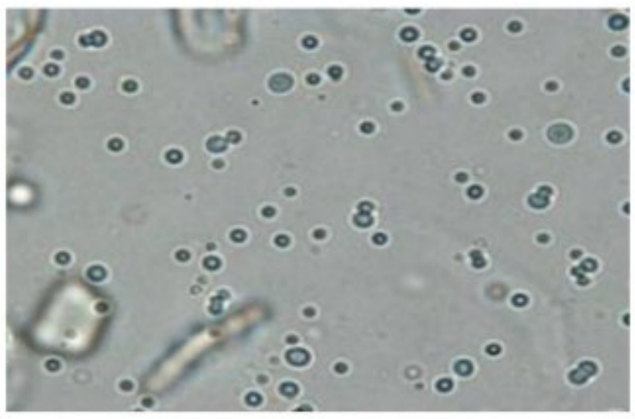
f-*Leukocytic cast*.

It is characterized by the presence of mucous pus cells adherent to or within a hyaline matrix.



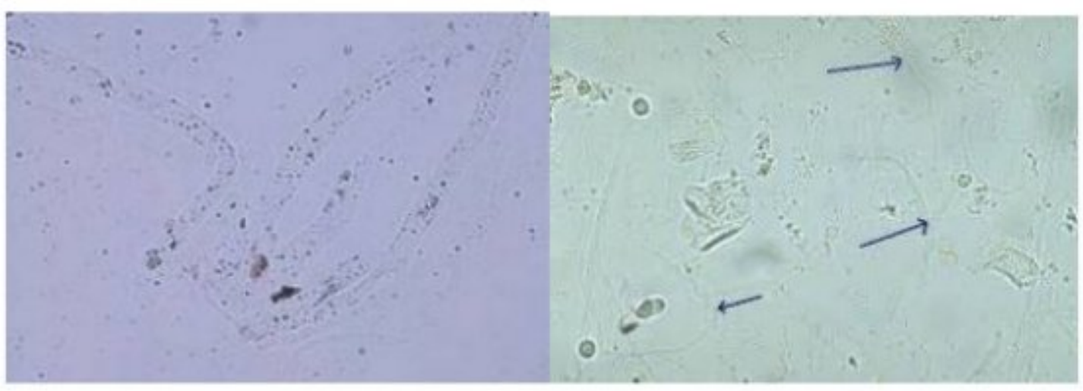
g- ***Fatty cast.***

Contain small droplets that appear as refractile bodies, usually colorless but can be stained with suddan III.



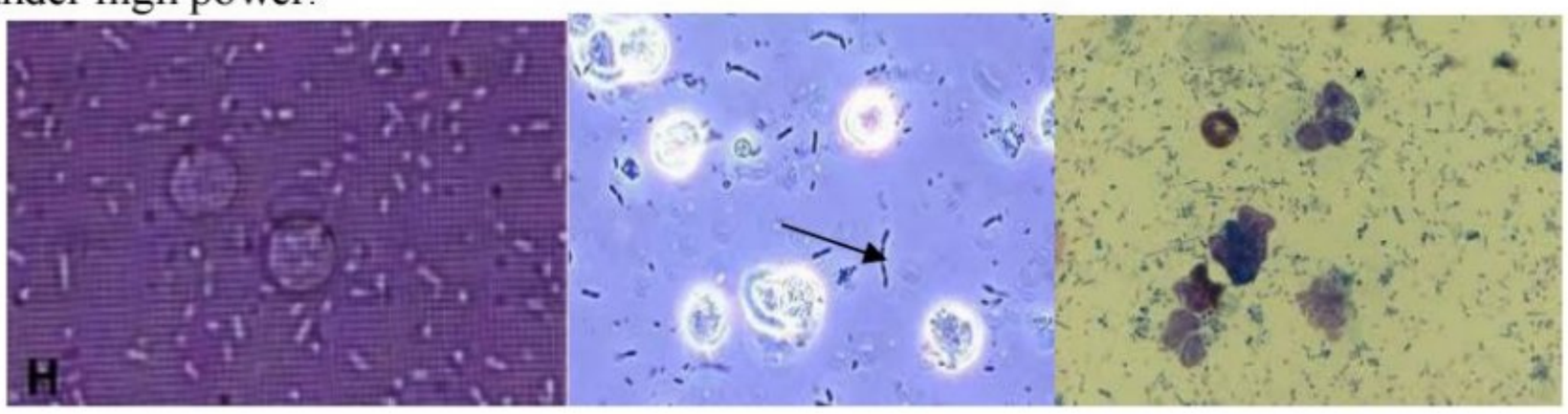
(4) Mucous strands:

They are derived from the mucous glands of the urinary tract appear as long, translucent shreds, They are normal in horses.



(5) Bacteria:

Normal bladder urine is sterile, The distal urethra and genital tract harbor bacteria and voided or catheterized urine samples may be contaminated with bacteria from the distal urethra or genital tract,They are seen as small objects displaying true motility under high power.

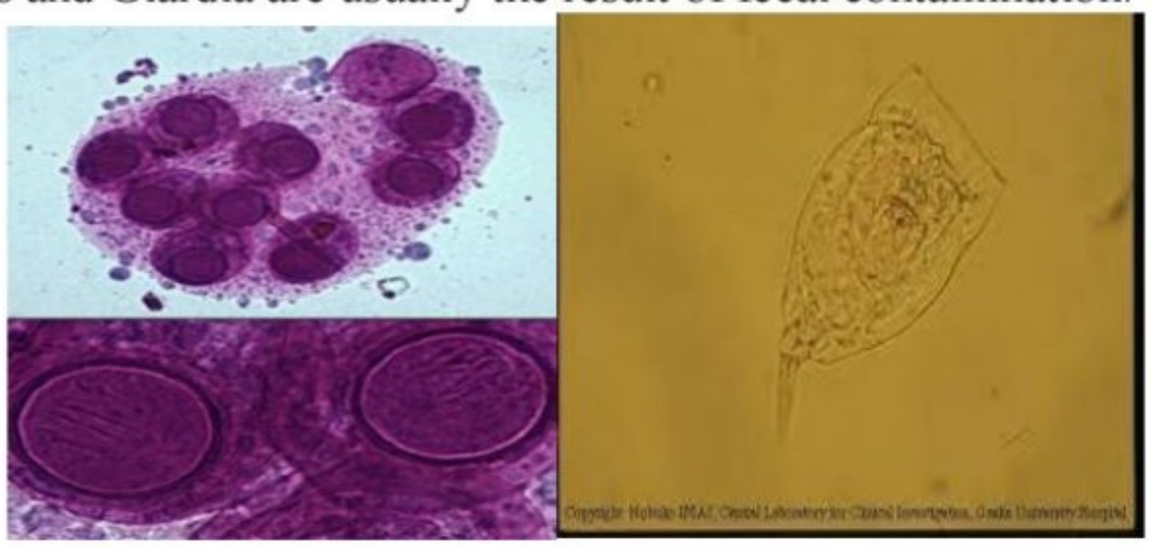


(6) Yeast:

Yeast and fungal hyphae in the sediment usually are contaminants since fungal urinary tract infection occurs and usually is seen in urinary tract obstruction or with prolonged use of antimicrobial agents or immunosuppressive therapy, it appear as Unnucleated round and oval bodies, larger than bacteria but smaller than leukocytes.

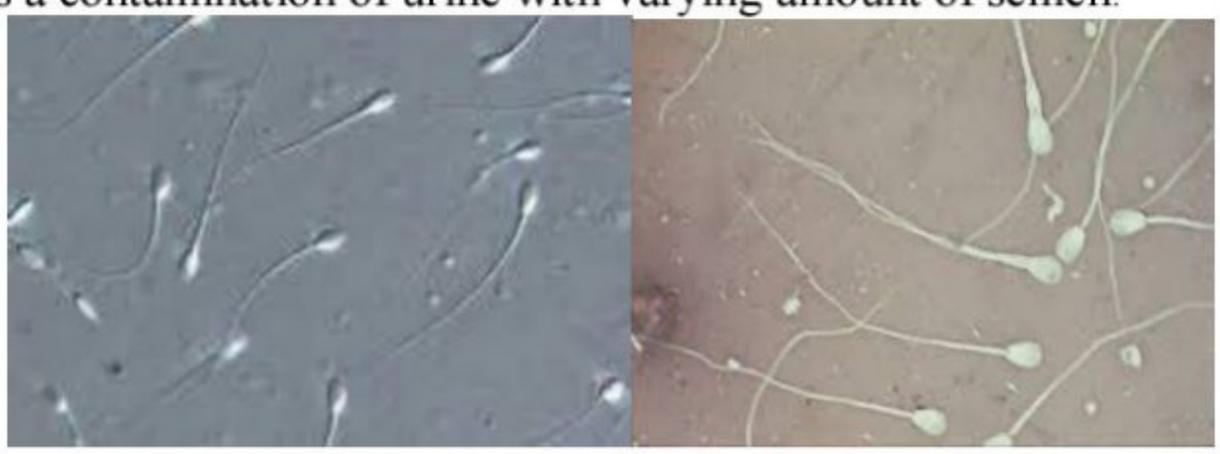
(7) Protozoa:

Trichomonads and Giardia are usually the result of fecal contamination.



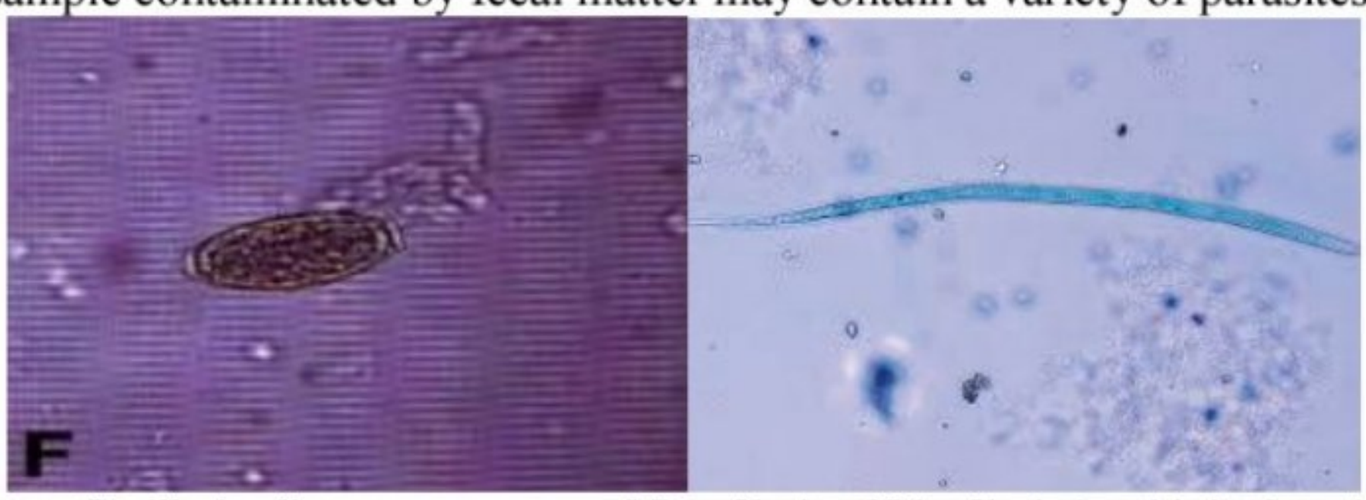
(8) Spermatozoa:

It appears as a contamination of urine with varying amount of semen.



(9) Parasites ova:

Urine sample contaminated by fecal matter may contain a variety of parasites ova.

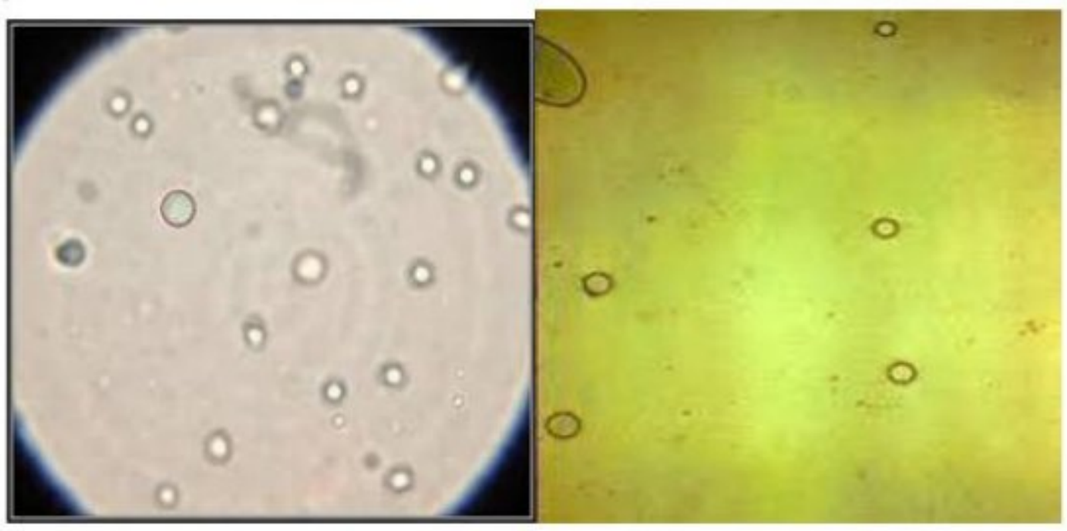


Capillaria plica ovum *Microfilaria of Dirofilaria immitis in the urine*

II. Unorganized Sediment:

(1) Fat droplets:

They are appear as round, highly retractile bodies of various size.Positive identification of fat droplets can be made by the addition of suddan III to the urinary sediment.



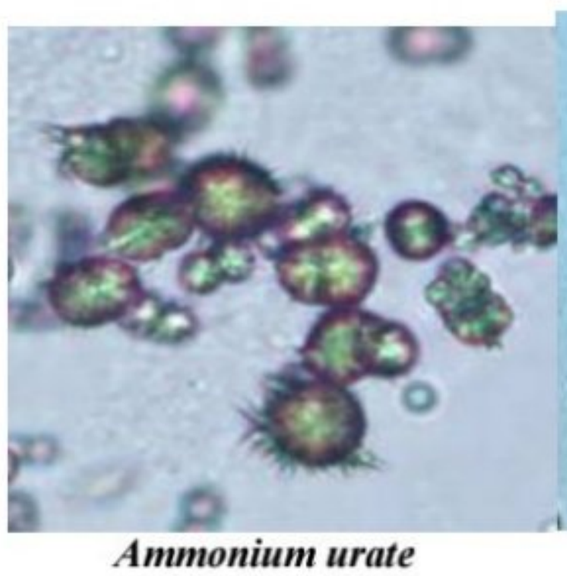
(2) Crystal:

The observation of crystals in urine sediment depends on a number of factors, the extent of urinary saturation with crystal precursors, urine pH, solubility and concentration of the crystalloid and colloids, total urine solute concentration, time between collection and analysis, and refrigeration before analysis.

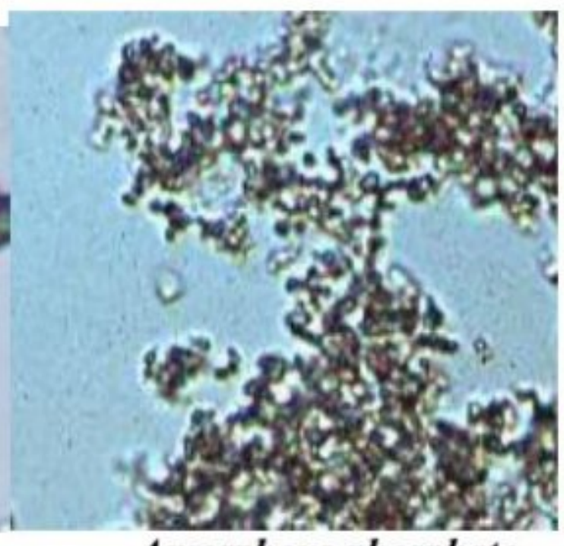
In general…

Alkaline urine will contain triple and amorphous phosphate, calcium carbonate,especially in the horse, and on rare occasions, ammonium urate crystals.

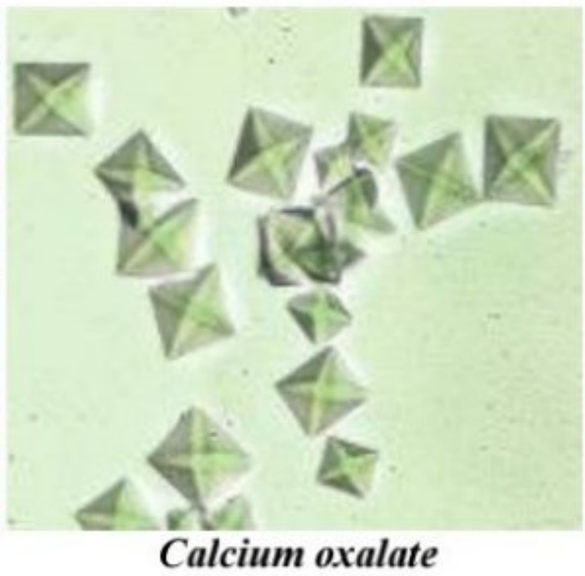
Acidic urine contains amorphous urate and uric acid, calcium oxalate and hippuric acid may be present, but are less common.



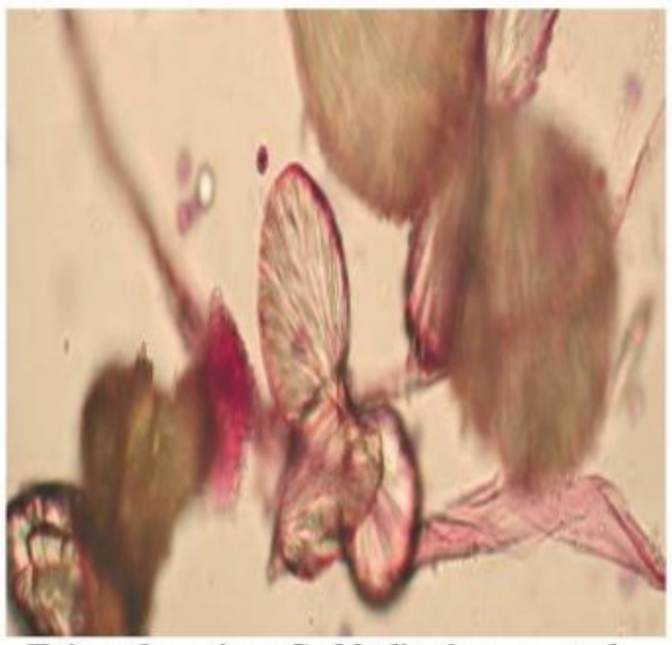
Ammonium urate



Amorphous phosphate



Calcium oxalate



Trimethoprime Sulfadiazine crystal

Urine Culture:

Normal urine is sterile, but it may become contaminated with members of the skin microbiots near the end of it's passage through the urethra.Urine itself is a good culture medium. The followings should be considered during urine collection for culture:

1. Urine specimen should be collected in the morning.
2. Urine specimen should be collected by the cytocentesis or catheterization.
3. All specimens should be processed by the laboratory within 2 hours of collection, or be kept refrigerated at 4˚c.The examination procedure include the following steps:
    - Examine gram-stained smear.
    - Culture the urine on suitable media such as MacConky agar, blood agar, incubate at 37˚c for 24 hours under aerobic condition.
    - Susceptibility tests on clinically significant bacterial isolates.