Lab.N0-3

GENERAL URINE EXAMINATION

First: General Urine Examination (G.U.E):

It includes two basic tests:

A-Macroscopic examination

B-Microscopic examination

B-Microscopic examination of the urine:

A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a sediment (Deposit) is produced at the bottom of the tube.

The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is re-suspended in the remaining supernatant by flicking (shaking) the bottom of the tube several times. A drop of sediment is poured onto a glass slide and covered with cover slip. The sediment is first examined under low power (X10) to identify most crystals, casts, squamous cells, and other large objects. Next, examination is carried out at high power (X40) to identify crystals, cells, and bacteria. The following findings are observed.

1. Pus cells
2. RBCs
3. Epithelial cells
4. Crystals
5. Casts
6. Parasites
7. Others include: bacteria, monilia, mucous thread, ova spermatozoa.

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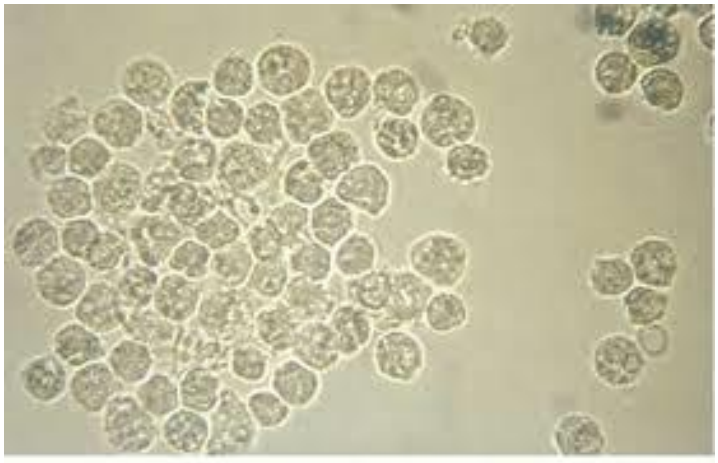
1. PUS CELLS:

Usually, they are WBC's are granulocytes have lobed nuclei and granular cytoplasm. It appear in urine in cases of inflammation of the urethra caused by a bacterial infection If two or more leukocytes per each high power field appear in non-contaminated urine, the specimen is probably abnormal while a number of more than 100/HPF pus cells is called pyuria,

Q/ Sometimes urine contains many P.C. but no bacteria is revealed in culture?

The reason behind is: ❶ The patient on antibiotics. ❷ The infection by fastidious bacteria i.e. *Neisseria gonorrhea*, T.B., or virus. ❸ Presence of renal stone produce P.C. however pus cells estimation depends on the following:

No. of pus cells / H.P.F.	**Result as written in the report**
No pus cells (0).	Nil.
0-1	Pus cells (0-1)/ HPF i.e. Rare
2-3	Pus cells (2-3)/ HPF i.e. Very Few
4-5	Pus cells (4-5)/ HPF i.e. Few
5-10	Pus cells (5-10)/ HPF i.e. (+).
10-20	Pus cells (10-20)/ HPF i.e. (++).
20-30	Pus cells (20-30)/ HPF i.e. (+++).
30-50	Pus cells (over 30)/ HPF i.e. (++++).
50 -100 (Full field)	Pus cells (over 50)/ HPF i.e. (++++).
>100 (loaded Full field)	Pus cells (over 100)/ HPF i.e. (++++).(Pyuria)



2. Red Blood Cells:

Hematuria is the presence of abnormal numbers of R.B.Cs in urine due to: ❶glomerular damage,❷ tumors, kidney trauma, ❸urinary tract stones,❹ upper and lower urinary tract infections, ❺ nephrotoxins, and ❻ physical stress. Red cells may also contaminate the urine from the vagina ❼ during menstrual period. Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals. However, if one or more red cells can be found in every high power field, and if contamination can be ruled out and neglected, so the specimen is probably abnormal.

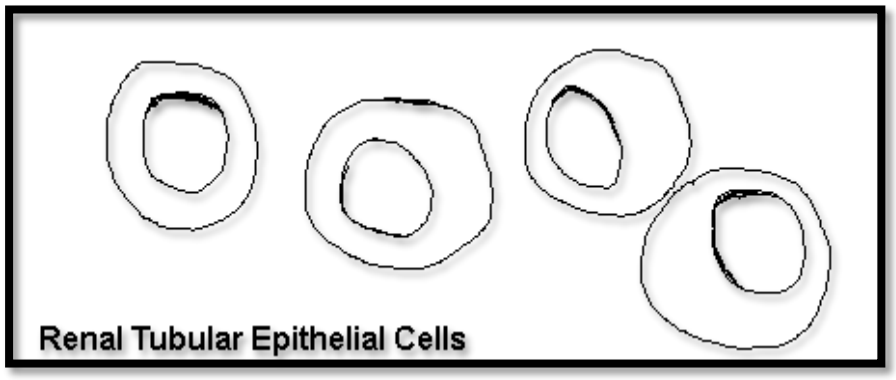
Shape of RBC's may appear in urine:
① Normal, ② swollen by dilute urine (in fact, only cell ghosts and free hemoglobin may remain), ③crenated by concentrated urine.

All swollen, partly hemolyzed and crenated RBC's are sometimes difficult to distinguish from WBC's in the urine.
RBC's are estimated in urine the same way of pus cells.

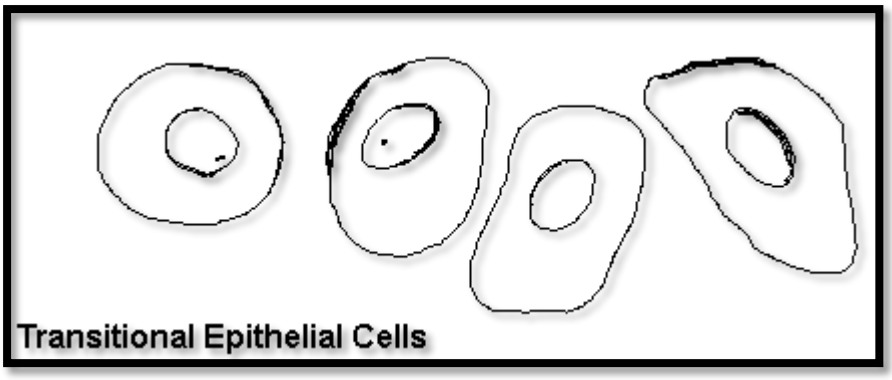
3. Epithelial cells:

Epithelial cells exist in lining of renal pelvis, ureters, urinary bladder, and upper urethra. These cells are large, and diamond- shaped. Usually found in urine in small numbers, but large numbers or sheets of these cells in urine occur in many conditions, found in female's more than males. There are types of epithelial cells according to its origin:

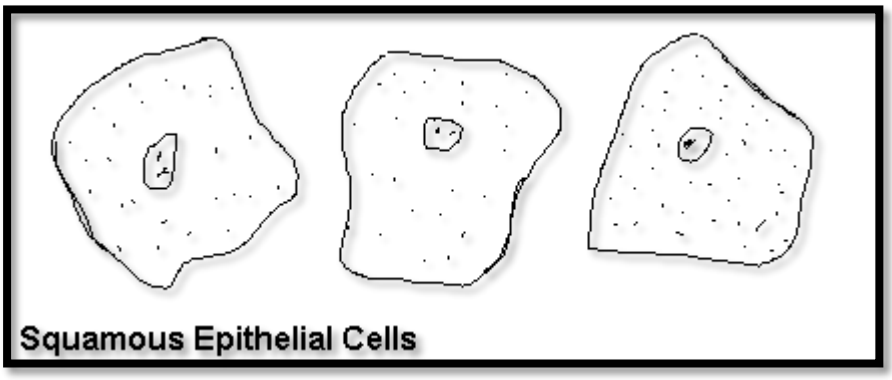
- Renal tubular epithelial cells, usually larger than Pus cells, contain a large round or oval nucleus and normally in small numbers. Increase in nephrotic syndrome and tubular degeneration.



- Transitional epithelial cells from the renal pelvis, ureter, or bladder. Have more regular cell borders, larger nuclei, and smaller overall size than squamous epithelium. Renal tubular epithelial and transitional epithelium, and their nucleus occupies more of the total cell volume.



- Squamous epithelial cells from the skin surface or from the outer urethra can appear in urine. Their significance is that they represent possible contamination of the specimen with skin flora.



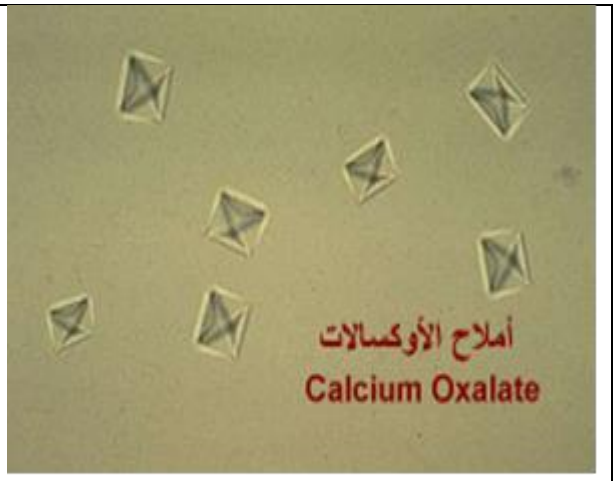
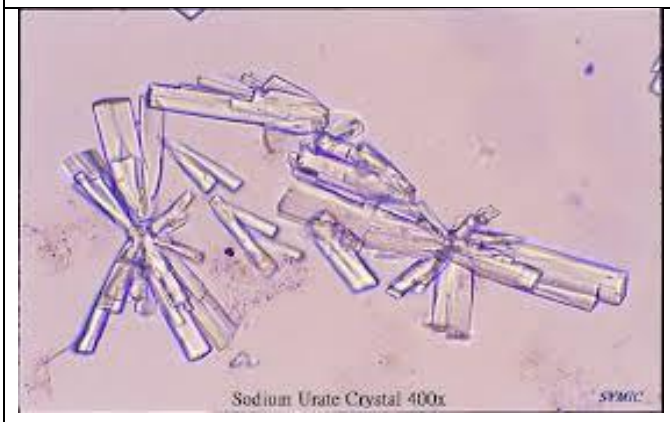
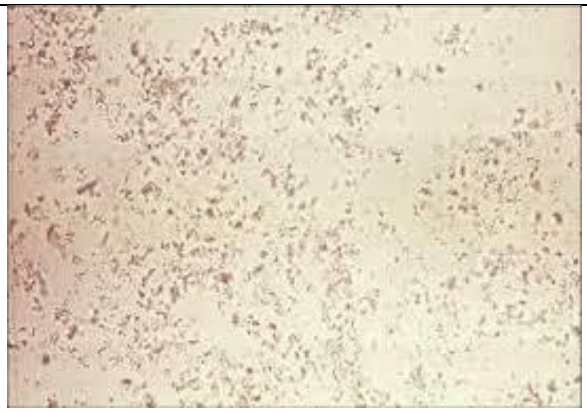
4. Crystals in urine:

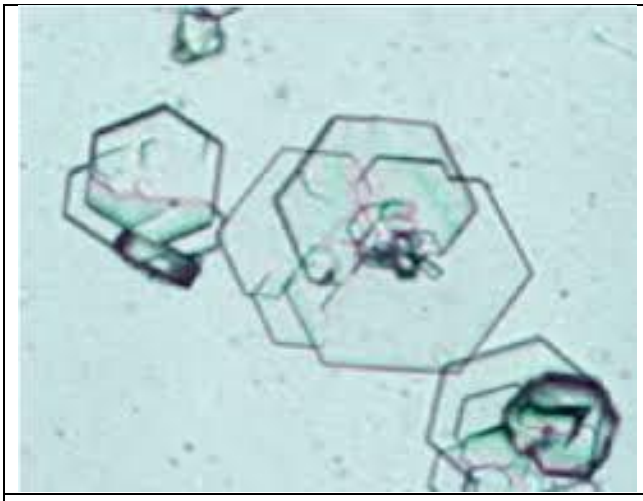
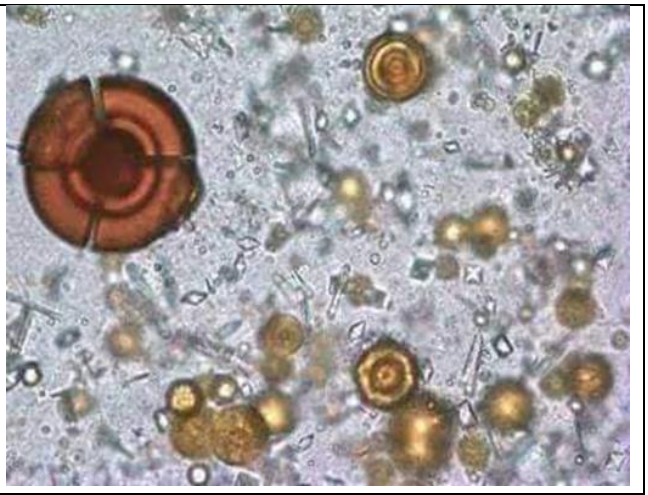
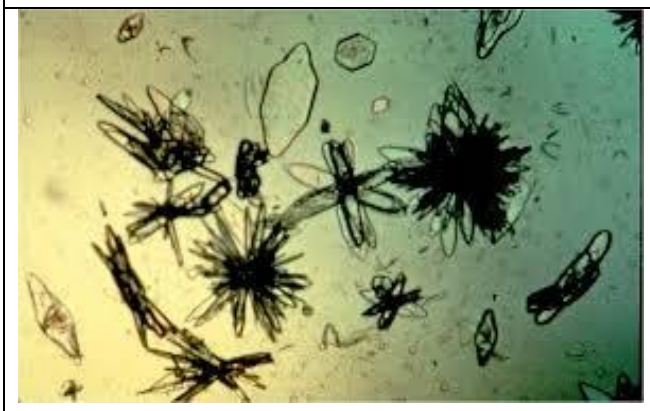
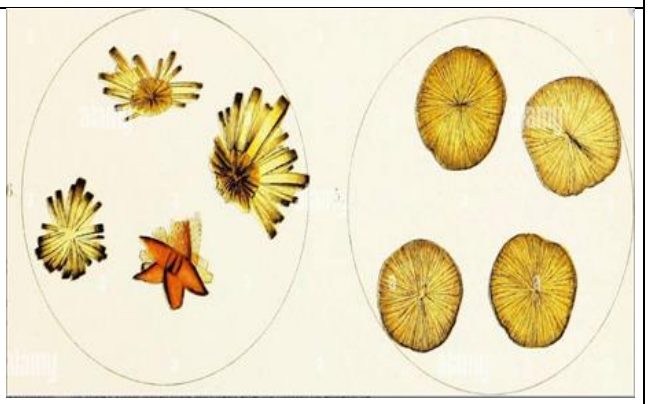
Crystals are structures with a definite geometric shape due to orderly 3-dimensional arrangement of its atoms and molecules. Amorphous material (or crystals) has no definite shape and is commonly seen in the form of granular aggregates or clumps. Before reporting presence of any abnormal crystals in urine, it is necessary to confirm them by chemical tests which dived them into two types:

A- Crystls in Acidic urine.

B- Crystls in Alkaline urine.

A- Crystls in Acidic urine:

	
1. Uric acid crystals: These are variable in shape (diamond, rosette, plates), and yellow or red-brown in color (due to urinary pigment). They are soluble in alkali, and insoluble in acid. Increased in case of gout and leukemia.	**2. Calcium oxalate crystals:** These are colorless, refractile, and envelope-shaped. Sometimes dumbbell-shaped or peanut-like forms are seen.
	
3. Sodium urate: Sodium urate crystals may appear as small, brown needle-like crystals. Sodium urate crystals are the sodium salt of uric acid crystals.	4. Amorphous urate: These are urate salts of potassium, magnesium, or calcium in acid urine. They are usually yellow, fine granules in compact masses.

	
5. Cysteine crystals: These are colorless, clear, hexagonal (having 6 sides). They often occur in layers. They are seen in cysteinuria, an inborn error of metabolism. Cysteine crystals are often associated with formation of cysteine stones.	**6. Leucine crystals:** These are refractile, yellow or brown, spheres with radial or concentric striations. They are soluble in alkali. They are usually found in urine along with tyrosine in severe liver disease (cirrhosis).
	
7. Tyrosine crystals: They appear as clusters of fine, delicate, colorless or yellow needles and are seen in liver disease and tyrosinemia (an inborn error of metabolism). They dissolve in alkali.	**8. Bilirubin crystals:** These are small (5 μ), brown crystals of variable shape (square, bead-like, or fine needles). They are seen in severe obstructive liver disease.

B- Crystls in Alkaline urine:

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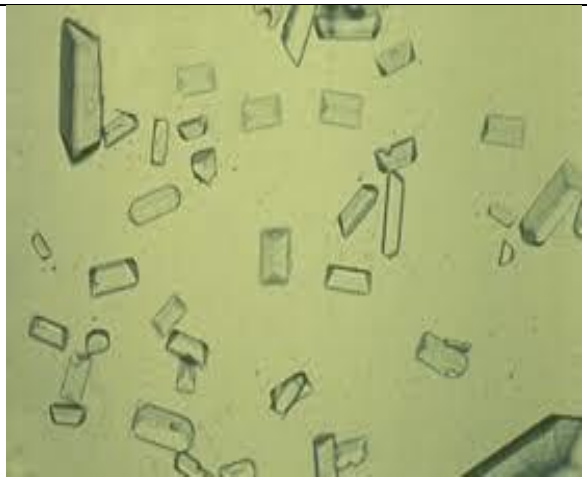
<td>  </td>
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<td>1. Calcium carbonate crystals: These are small, colorless, and grouped in pairs. They are soluble in acetic acid and give off bubbles of gas when they dissolve.</td>
<td>2. Triple phosphates (ammonium magnesium phosphate): They are colorless, shiny, 3-6 sided prisms with oblique surfaces at the ends (“coffinlids توابيت”) appearance.</td>
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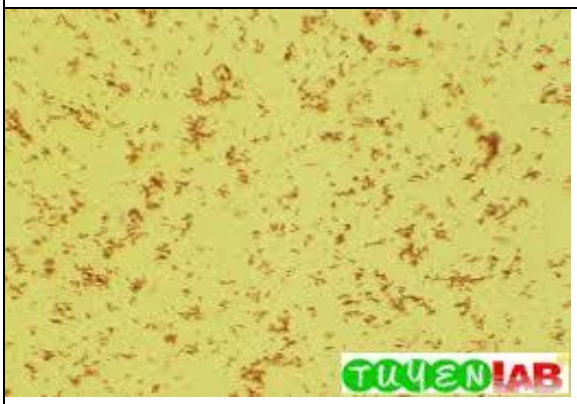
<td> 
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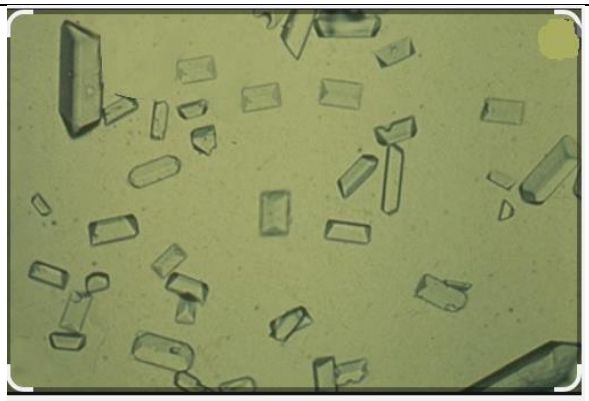
<td>  </td>
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<td>3. Amorphous phosphates: These occur as colorless small granules, often dispersed.
All phosphates are soluble in dilute acetic acid.</td>
<td>4. Cholesterol crystals: These are colorless, refractile, flat rectangular plates with notched (missing) corners, and appear stacked in a stair-step arrangement. They are seen in lipiduria, hypercholesterolemia. They can be positively identified by polarizing microscope.</td>
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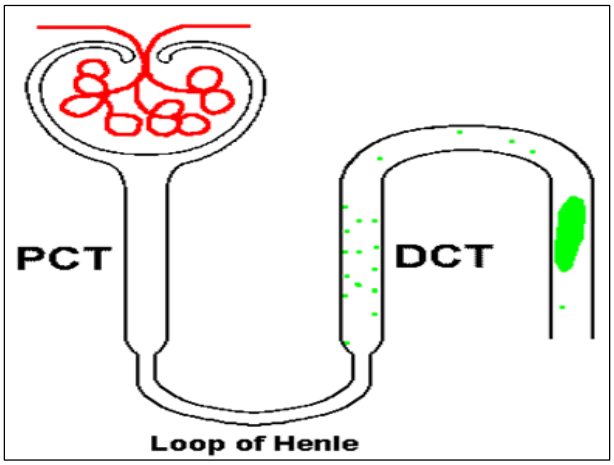
5. CASTS:

Urinary casts are cylindrical, cigar-shaped microscopic structures that form in distal renal tubules (DCT) and collecting ducts. They take the shape and diameter of the renal tubules (molds or 'casts'). They have parallel sides and rounded ends. Casts are basically composed of a precipitate of a protein that is secreted by tubules (Tamm-Horsfall protein). Since casts form only in renal tubules their presence is indicative of disease of the renal parenchyma. Although there are several types of casts, all urine casts are basically hyaline; various types of casts are formed when different elements get deposited on the hyaline material. Casts are best seen under low power objective (×10) with condenser lowered down to reduce the illumination.



Casts formed in distal convoluted tubule (DCT) at the green color in this diagram.

Casts are the only elements in the urinary sediment that are specifically of renal origin.

Casts are of two main types:

1. **Noncellular:** ① Hyaline, ② Granular, ③ Waxy, ④ Fatty casts.
2. **Cellular:** ① R.B.C. cast, ② W.B.C cast, ③Renal tubular epithelial cell.

1. Noncellular casts:

① **Hyaline casts:** These are the most common type of casts in urine and are homogenous, colorless, and transparent. They are cylindrical with parallel sides, and rounded ends. Presence of occasional (1or2) hyaline cast is considered as normal. Their presence in increased numbers ("cylinduria") is abnormal. They occur transiently after muscle exercise in healthy persons and during fever. Increased numbers are found in conditions causing glomerular proteinuria.

② **Granular casts:** Presence of degenerated cellular debris in a cast makes it granular in appearance. These are cylindrical structures with coarse or fine granules (which represent degenerated renal tubular epithelial cells). They are

seen after strenuous muscle exercise and in fever, acute glomerulonephritis, and pyelonephritis.

③ **Waxy cast:** These are the most easily recognized of all casts. They form when hyaline casts remain in renal tubules for long time (prolonged stasis). They have homogenous, smooth glassy appearance, cracked or serrated margins and irregular broken-off ends. The ends are straight and sharp and not rounded as in other casts. They are light yellow in color. They are most commonly seen in End-stage renal failure.

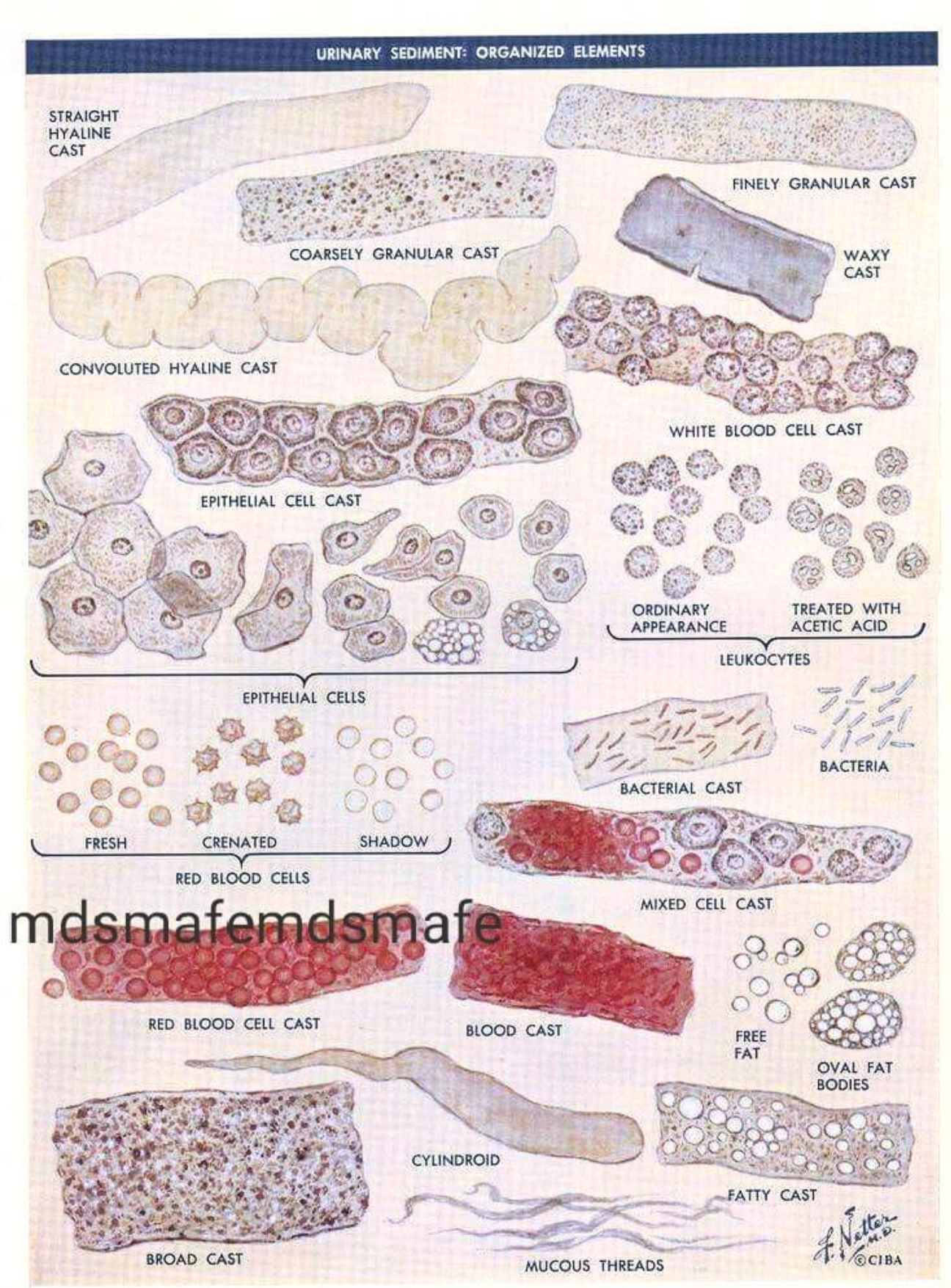
④ **Fatty casts**: These are cylindrical structures filled with fat globules (triglycerides and cholesterol esters). They are seen in nephrotic syndrome.

2. Cellular casts:

① **Red cell casts:** These are cylindrical structures with red cells. They may appear brown in color due to hemoglobin pigmentation. These have greater diagnostic importance than any other cast. If present, they help to differentiate hematuria due to glomerular disease from hematuria due to other causes. RBC casts usually denote تدل على glomerular pathology e.g. acute glomerulonephritis.

② **White cell casts:** These are cylindrical structures with white blood cells. Leucocytes usually enter into tubules from the interstitium and therefore presence of leucocyte casts indicates tubulointerstitial disease like pyelonephritis.

③ **Renal tubular epithelial cell casts:** These are composed of renal tubular epithelial cells that have been sloughed off. They are seen in acute tubular necrosis, viral renal disease, heavy metal poisoning, and acute allograft rejection. Even an occasional renal tubular cast is a significant finding.



6. **Others include:**
   Parasites, Bacteria, Monilia (Candida), mucous thread, ova and spermatozoa.

Bacteria: in urine can be detected by microscopic examination, Significant bacteriuria exists when there are >105 bacterial colony forming units/ml of urine in a cleancatch midstream sample, >104 colony forming units/ml of urine in catheterized sample, and >103 colonyforming units/ml of urine in a suprapubic aspiration sample.

Parasites:

Trichomonas vaginalis

These are motile organisms with pear shape, undulating membrane on one side, and four flagellae. They cause vaginitis in females and are thus contaminants in urine. They are easily detected in fresh urine due to their motility.

Yeast Cells (*Candida*)

These are round or oval structures of approximately the same size as red blood cells. In contrast to red cells, they show budding, are oval and more refractile, and are not soluble in 2% acetic acid.
Presence of *Candida* in urine may suggest immunocompromised state, vaginal candidiasis, or diabetes mellitus. Usually pyuria is present if there is infection by *Candida*. *Candida* may also be a contaminant in the sample and therefore urine sample must be examined in a fresh state.

Eggs (Ova) of *Schistosoma haematobium* the incidence of urinary Bilharziasis Fork Terminal ovum of Schistosoma could be seen in bloody urine in most cases.

Spermatozoa

They may sometimes be seen in urine of men.

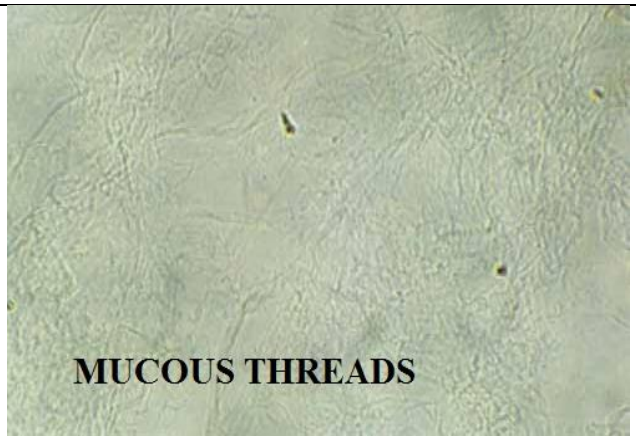
MUCOUS THREADS

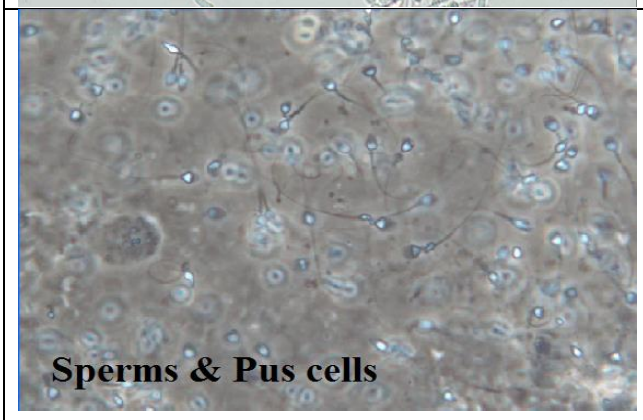
Mucous threads are long, thin, wavy threads structures which may show faint longitudinal striations. Mucous threads are present in normal urine in small numbers, but they may be very abundant in the presence of inflammation or irritation of the urinary tract.

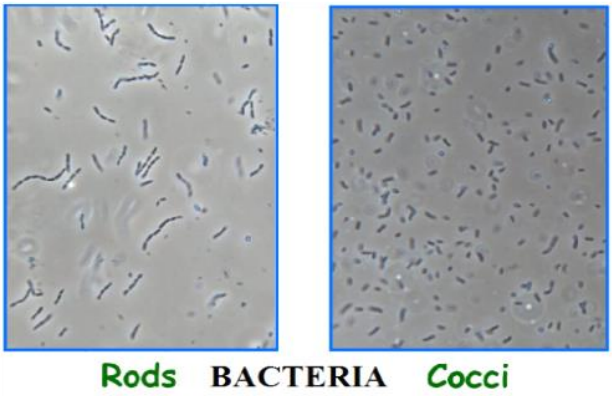

بويضات البلهارسيا البولية


بويضات ديدان الأكسيورس
Enterobius vermicularis Egg


Candida albicans. (Monelia).
budding & Hyphae


MUCOUS THREADS


Sperms & Pus cells


Rods
BACTERIA
Cocci

Second: Urine for Culture and Sensitivity test (urine for c/s)

Urine cultures are performed to detect organisms that are the causative agents of urinary tract infections which is one of the most common infections. Normally the urinary tract is sterile above the urethra. , a colony count of more than 105/ml is strongly suggestive of urinary tract infection and should be cultured. Positive culture is followed by sensitivity test. Most infections are due to Gram-negative enteric bacteria, particularly ***Escherichia coli.***

Urine samples for culture characterized by the following

1. The urine is collected in sterile container.
2. Method used in urine collection is clean catch or (Mid-stream urine).
3. Urine should be cultured directly after collection.
4. That patient is not taking antibiotics at least 3 days before test.

(N.B. sometimes urine culture is done with antibiotic intake to ensure its activity)

Culture media

One drop of urine is taken by using loop ring of (0.3 cm diameter) and cultured on selected media by streaking method and incubated for (24 to 48) hours at 37 °C.

1-**Blood agar media**: It is enrichment media it promote growth of gram positive and gram negative bacteria and is used to detect hemolysis of blood phenomenon and the color of the colonies.

2-**Mackonky agar**: It is differential media used to:

A. Differentiate between gram positive and negative bacteria it allows the growth of gram negative bacteria only.

B. Differentiate between lactose fermenter and non-fermenter of gram negative bacteria which appears in a pink color colonies when lactose fermented and pale yellow colonies in non-lactose fermented.

Culture Identification tests:

1. Macroscopic tests: Done by observation the form of colonies, color, viscosity pigments produced and the ability to blood hemolysis.

2. Microscopic test is done by Gram stain to determine the type of bacteria.

3. Identification: By using biotyping and serotyping and sometimes using phage typing, to identify bacterial type isolated from urine which most likely include *E.coli* .*Klebseilla spp, Pseudomon*as, *Proteus* and *Enterobacter*, Which is considered the most common causative of UTI.

4. Sensitivity test:

Also called Antibiogram, it is carried out by cultivating bacterial isolates on Muller Hinton agar to determine its sensitivity to selected antibiotics and use the most proper one in U.T.I. treatment through a list of antibiotics like Penicillin, Nitrofuantoin, Erythromycin, Streptomycin, Ciprofloxacin, Methoprim, and Tetracycline.