Clinical Analysis Course 2020-2021
Lecture: 1 - Fourth Stage – Biology Depart.

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The following is a detailed breakdown of the responsibilities of each unit:

- **Microbiology** receives almost any clinical specimen, including swabs, feces, urine, blood, sputum, cerebrospinal fluid, synovial fluid, as well as possible infected tissue.
- **Parasitology** is a microbiology unit that investigates parasites.
- **Virology** is concerned with identification of viruses in specimens such as blood, urine, and cerebrospinal fluid.
- **Hematology** works with whole blood to do full blood counts, and blood films as well as many other specialised tests.
- **Coagulation** requires citrated blood samples to analyze blood clotting times and coagulation factors.
- **Clinical Biochemistry** usually receives serum or plasma. They test the serum for chemicals present in blood. These include a wide array of substances, such as lipids, blood sugar, enzymes, and hormones.
- **Toxicology** mainly tests for pharmaceutical and recreational drugs. Urine and blood samples are submitted to this lab.
- **Immunology/Serology** uses the concept of antigen-antibody interaction as a diagnostic tool. Compatibility of transplanted organs is also determined.
- **Immunohaematology**, or Blood bank determines blood groups, and performs compatibility testing on donor blood and recipients.
- **Urinalysis tests urine** Urinalysis is one of the most commonly performed laboratory tests in clinical practice.
- **Histopathology processes** solid tissue removed from the body (biopsies) for evaluation at the microscopic level.
- **Cytopathology** examines smears of cells from all over the body (such as from the cervix) for evidence of inflammation, cancer, and other conditions.
- **Electron microscopy** prepares specimens and takes micrographs of very fine details.
- **Genetics** mainly performs DNA analysis.
- **Cytogenetics** involves using blood and other cells to get a karyotype. This can be helpful in prenatal diagnosis (e.g. Down's syndrome) as well as in cancer (some cancers have abnormal chromosomes).
- **Surgical pathology** examines organs, limbs, tumors, fetuses, and other tissues biopsied in surgery such as breast mastectomys.

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Urine analysis

INDICATIONS FOR URINALYSIS

1. Suspected renal diseases like glomerulonephritis
nephrotic syndrome, pyelonephritis, and renal failure
2. Detection of urinary tract infection
3. Detection and management of metabolic disorders like diabetes mellitus
4. Differential diagnosis of jaundice
5. Detection and management of plasma cell dyscrasias
6. Diagnosis of pregnancy.

Box 1.1: Collection of urine sample

- First morning, midstream: Preferred for routine urine examination.
- Random, midstream: Routine urine examination.
- First morning, midstream, clean catch: Bacteriological examination.
- Postprandial: Estimation of glucose, urobilinogen
- 24-hour: Quantitative estimation of proteins or hormones.
- Catheterised: Bacteriological examination in infants, bedridden patients, and in obstruction of urinary tract.
- Plastic bag (e.g. colostomy bag) tied around genitals: Infants; incontinent adults.

Box 1.2: Collection of urine for routine and culture examination

Collection for routine urinalysis

For routine examination of urine, a wide-mouthed glass bottle of 20-30 ml capacity, which is dry, chemically clean, leak-proof, and with a tight fitting stopper is used. About 15 ml of midstream sample is cleanly collected.

Collection for bacterial culture

- Use sterile container
- Collect midstream, clean catch sample
- Must be plated within 2 hours of collection
- If refrigerated, must be plated within 24 hours of collection
- No preservative should be added.

Changes which Occur in Standing Urine at Room Temperature

If urine is left standing at room temperature for long after collection, following changes occur:

• **Increase in pH** due to production of ammonia from urea by urease-producing bacteria.
• **Formation of crystals** due to precipitation of phosphates and calcium (making the urine turbid)
• **Loss of ketone bodies**, since they are volatile.
• **Decrease in glucose** due to glycolysis and utilization of glucose by cells and bacteria.
• **Oxidation of bilirubin to biliverdin** causing false negative test for bilirubin
• **Oxidation of urobilinogen to urobilin** causing false negative test for urobilinogen
• **Bacterial proliferation**
• **Disintegration of cellular elements**, especially in alkaline and hypotonic urine.

• **Physical examination**
• Volume, Odor, Color, Specific gravity, Appearance, pH

• **Volume** of only the 24-hr specimen of urine needs to be measured and reported. The average 24-hr urinary output in adults is 600-2000 ml. The volume varies according to fluid intake, diet, and climate. Abnormalities of urinary volume are as follows:
• **Polyuria** means urinary volume > 2000 ml/24 hours. This is seen in diabetes mellitus (osmotic diuresis), diabetes insipidus (failure of secretion of antidiuretic

hormone), chronic renal failure (loss of concentrating ability of kidneys) or diuretic therapy.

• **Oliguria** means urinary volume < 400 ml/24 hours. Causes include febrile states, acute glomerulonephritis (decreased glomerular filtration), congestive cardiac failure or dehydration (decreased renal bloodflow).

• **Anuria** means urinary output < 100 ml/24 hours or complete cessation of urine output. It occurs in acute tubular necrosis (e.g. in shock, hemolytic transfusion reaction), **acute glomerulonephritis**, and **complete urinary tract obstruction**.

- **Color**: Normal urine color in a fresh state is pale yellow or amber and is due to the presence of various pigments collectively called urochrome. Depending on the state of hydration urine may normally be colorless (over hydration) or dark yellow (dehydration). Some of the abnormal colors with associated conditions are listed in Table 1.2

Table 1.2: Different colors of urine

Colors	*Conditions*
Colorless	Dilute urine (diabetes mellitus, diabetes insipidus, overhydration)
Red	Hematuria, Hemoglobinuria, Porphyria, Myoglobinuria
Dark brown or black	Alkaptonuria, Melanoma
Brown	Hemoglobinuria
Yellow	Concentrated urine
Yellow-green or green	Biliverdin
Deep yellow with yellow foam	Bilirubin
Orange or orange-brown	Urobilinogen Porphobilinogen
Milky-white	Chyluria
Red or orange fluorescence with UV light	Porphyria

- **Appearance:** Normal, freshly voided urine is clear in appearance. Causes of cloudy or turbid urine are listed in Table 1.3 Foamy urine occurs in the presence of excess proteins or bilirubin

Table 1.3: Causes of cloudy or turbid urine

Cause	*Appearance*	*Diagnosis*
1. Amorphous phosphates	White and cloudy on standing in alkaline urine	Disappear on addition of a drop of dilute acetic acid
2. Amorphous urates	Pink and cloudy in acid urine	Dissolve on warming
3. Pus cells	Varying grades of turbidity	Microscopy
4. Bacteria	Uniformly cloudy; do not settle at the bottom following centrifugation	Microscopy, Nitrite test

- **Odor:** Freshly voided urine has a **typical aromatic odor** due to volatile organic acids. After standing, urine develops ammoniacal odor (formation of ammonia occurs when urea is decomposed by bacteria). Some abnormal odors with associated conditions are:

• Fruity: Ketoacidosis, starvation
• Mousy or musty: Phenylketonuria
• Fishy: Urinary tract infection with *Proteus*, tyrosinaemia.
• Ammoniacal: Urinary tract infection with *Escherichia coli*, old standing urine.
• Foul: Urinary tract infection
• Sulfurous: Cystinuria

- **The Specific gravity**

This is also called as relative mass density. It depends on amount of solutes in solution. It is basically a comparison of density of urine against the density of distilled water at a particular temperature. Specific gravity of distilled water is 1.000. **Normal SG of urine is 1.003 to 1.030** and depends on the state of hydration. SG of normal urine is mainly related to urea and sodium. SG increases as solute concentration increases and decreases when temperature rises (since volume expands with rise in temperature).

SG of urine is a measure of concentrating ability of kidneys and is determined to get information about this tubular function. SG, however, is affected by proteinuria and glycosuria.

Causes of increase in SG of urine are diabetes mellitus (glycosuria), nephrotic syndrome (proteinuria), fever, and dehydration *Causes of decrease in SG of urine are* diabetes insipidus (SG consistently between 1.002-1.003), chronic renal failure (low and fixed SG at 1.010 due to loss of concentrating ability of tubules) and compulsive water drinking.

- **Microscopic examination**

1-Red blood cells (R.B.Cs)

The presence of abnormal number of intact red blood cells in urine is called as **hematuria**. It implies presence of a **bleeding lesion in the urinary tract**. Bleeding in urine may be noted macroscopically or with naked eye (gross hematuria). If bleeding is noted only by microscopic examination or by chemical tests, then it is called as occult, microscopic or hidden hematuria. Or **Hemoglobin** :Presence of free hemoglobin in urine is called as **hemoglobinuria**.

The Causes of Hematuria and hemoglobinuria

1. Diseases of urinary tract :Glomerular diseases: Glomerulonephritis, Berger's disease, lupus nephritis

2. Hematological conditions: Coagulation disorders, sickle cell disease Presence of red cell casts and proteinuria along with hematuria suggests glomerular cause of hematuria.

2. Hemoglobinuria: Hemoglobin will appear in urine when **haptoglobin** (to which hemoglobin binds in plasma) is completely saturated with hemoglobin.

Intravascular hemolysis occurs in infections (severe falciparum malaria, clostridial infection, *E. coli* septicemia), trauma to red cells (march hemoglobinuria, extensive burns, **prosthetic heart valves**), **glucose-6-phosphate dehydrogenase deficiency** following exposure to oxidant drugs, immune hemolysis (mismatched blood transfusion.

Tests for Detection of Blood in Urine

1. Microscopic examination of urinary sediment:

Definition of microscopic hematuria is presence **of 3 or more number of red blood** cells per high power field on microscopic examination of urinary sediment.

2. Chemical tests: These detect both intracellular and extracellular hemoglobin (i.e. intact and lysed red cells) as well as myoglobin. **Heme** proteins in hemoglobin act as **peroxidase**, which reduces hydrogen peroxide to water. This process needs a hydrogen donor (**benzidine, orthotoluidine**, or **guaiac**). Oxidation of hydrogen donor leads to development of a color (Fig. 1.19).

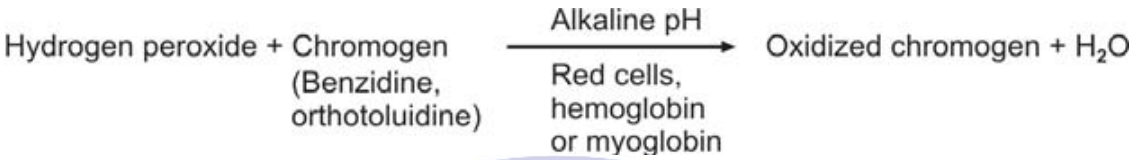

Fig. 1.19: Principle of chemical test for red cells, hemoglobin, or myoglobin in urine

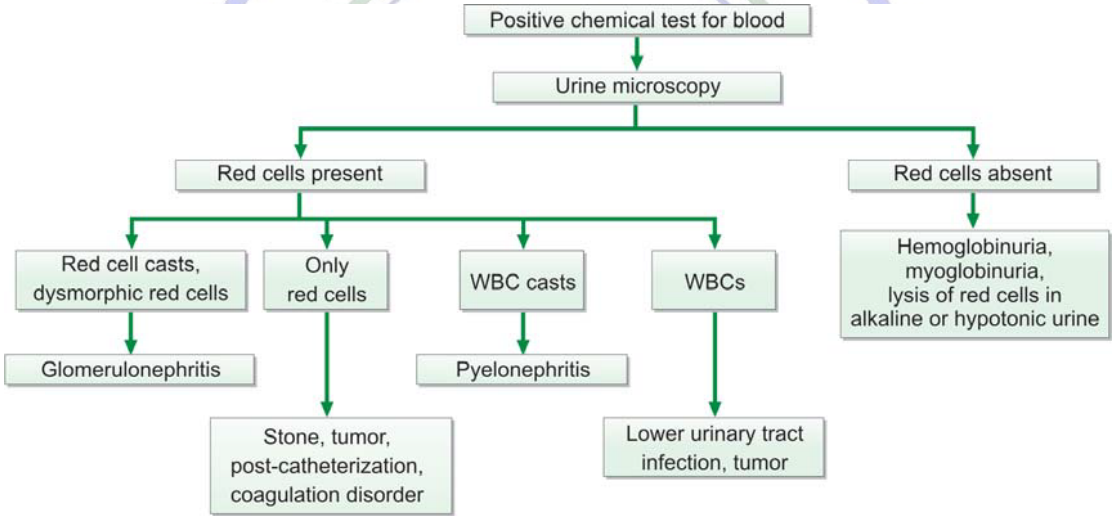

Fig. 1.20: Evaluation of positive chemical test for blood in urine

2- White Blood Cells (Pus Cells)

White blood cells are spherical, **10-15 µ in size**, **granular** in appearance in which **nuclei** may be visible. Degenerated white cells are distorted, smaller, and have fewer granules. Clumps of numerous white cells are seen in infections. Presence of many white cells in urine is called as **pyuria**. In hypotonic urine white cells are swollen and the granules are highly refractile and show Brownian movement; such cells are called as **glitter cells**; large numbers are indicative of injury to urinary tract.

Normally 0-2 white cells may be seen per high power field. **Pus cells greater than 10/HPF or presence of clumps is suggestive of urinary tract infection**.

Increased numbers of white cells occur in fever, pyelonephritis, lower urinary tract infection, tubulointerstitial nephritis, and renal transplant rejection.
In urinary tract infection, following are usually seen in combination:
• Clumps of pus cells or pus cells >10/HPF
• Bacteria
• Albuminuria
• Positive nitrite test
Simultaneous presence of white cells and white cell casts indicates presence of renal infection (**pyelonephritis**). Eosinophils (>1% of urinary **leucocytes**) are a characteristic feature of acute interstitial nephritis due to drug reaction (better appreciated with a **Wright's stain**).

3-Renal Tubular Epithelial Cells

Presence of renal tubular epithelial cells is a significant finding. Increased numbers are found in conditions causing tubular damage like acute **tubular necrosis**, **pyelonephritis**, viral infection of kidney, allograft rejection, and salicylate or **heavy metal poisoning**.

Squamous Epithelial Cells

Squamous epithelial cells line the lower **urethra and vagina**. They are best seen under low power objective (×10). Presence of large numbers of squamous cells in urine **indicates contamination of urine with vaginal fluid.**
These are large cells, rectangular in shape, flat with abundant cytoplasm and a small, central nucleus.

Transitional Epithelial Cells

Transitional cells line **renal pelvis, ureters**, **urinary bladder**, and **upper urethra**. These cells are large

4- Casts

Urinary casts are cylindrical, cigar-shaped microscopic structures that form in distal renal tubules and collecting ducts. They take the shape and diameter of the lumina (molds or 'casts') of the renal tubules. They have parallel sides and rounded ends. Their length and width may be variable. 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

• **The types of the casts:**

1- Hyaline casts: colourless, homogenous and transperance.
The cause of this type:
A-Heart failure **B**-Renal defect in Diabetic patients,
C-Chronic renal failure **D-**Glumerulonephritis and pyelonephritis**.**

2- Granular casts: This kind divided in to: fine granular cast (consist of fine granules in all part of the cast), and coarse granular cast (consist of fats, analysied cells or protein clumping which are seen as dark granules). The causes of these casts are:
A-nephron syndrom **B-** poisoning with iron
C- Glumerulonephritis and pyelonephritis.

3- RBCs casts: It is consisting of haemoglobin produced by haemolysis of RBCs. The colour of this casts yellow to orang. The causes: **A-** acute glumerulonephritis **B-** semi acute bacteria infection.

4-leukocytes casts: It was consisting of pus cells aggregation the cause are: **A-** pyelonephritis **B-** Interstitial nephritis

5- Tubular epithelial casts: this is a similar of leukocytes casts but seen as two rows of the cells. The cause is: **A-** Tubular Necrosis **B-** viral infection C- poisoning with heavy metales.

6- Waxy casts: It is yellow and homogenous.

The cause is: **A-** Renal failure **B-** Marked tubular atrophy.

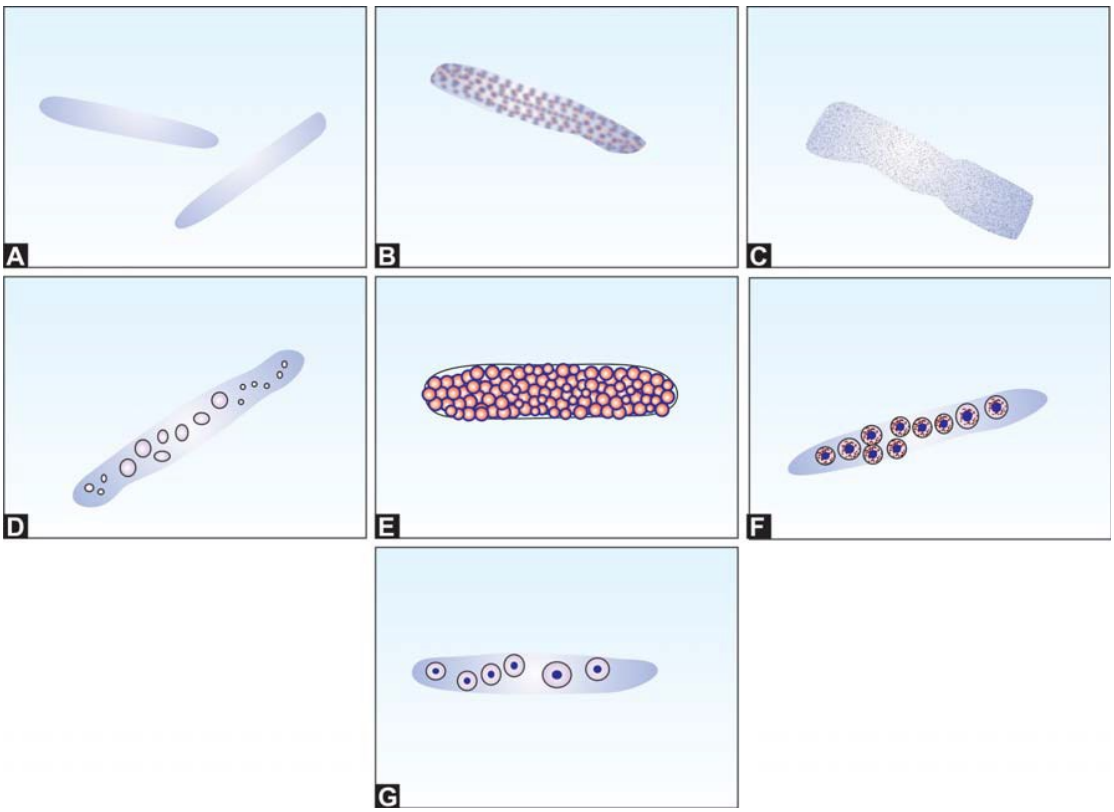

Fig. 1.27: Urinary casts: (A) Hyaline cast, (B) Granular cast, (C) Waxy cast, (D) Fatty cast, (E) Red cell cast, (F) White cell cast, and (G) Epithelial cast

5-Oval Fat Bodies

These are degenerated renal tubular epithelial cells filled with highly refractile lipid **(cholesterol) droplets**. Under polarized light, they show a characteristic "Maltese cross" pattern. They can be stained with a fat stain such as **Sudan III or Oil Red O**. **They are seen in nephrotic syndrome in which there is lipiduria**.

***6*-Spermatozoa**

They may sometimes be seen in urine of men. *Telescoped urinary sediment*: This refers to urinary sediment consisting of **red blood cells, white blood cells, oval fat**

bodies. It occurs in lupus nephritis, **malignant hypertension**, rapidly proliferative **glomerulonephritis, and diabetic glomerulosclerosis**.

7-Organisms

Organisms detectable in urine. Bacteria in urine can be detected by microscopic examination, reagent strip tests for significant **bacteriuria** (nitrite test, leucocyte esterase test), **and culture.** Method of collection for bacteriologic examination is **given earlier in Box 1.2.**

8- Crystals: are refractile structures with a definite geometric shape due to orderly 3-dimensional arrangement of its atoms and molecules. Amorphous material (or deposit) has no definite shape and is commonly seen in the form of granular aggregates or clumps.

Crystals in urine (Fig. 1.28) can be divided into two main types:

(1) Normal (seen in normal urinary sediment)

(2) Abnormal (seen in diseased states).

Normal Crystals

Crystals present in acid urine

a. *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 numbers are found in **gout and leukemia**. Flat hexagonal uric acid crystals may be mistaken for **cysteine crystals** that also form in acid urine.

b. *Calcium oxalate crystals*: These are colorless, refractile, and envelope-shaped. Sometimes dumbbell-shaped or peanut-like forms are seen. They are **soluble in dilute hydrochloric acid**. Ingestion of certain foods like **tomatoes, spinach, cabbage, asparagus, and rhubarb causes** increase in their numbers. Their increased number in fresh urine (oxaluria) may also suggest oxalate stones. A large number are seen in ethylene glycol poisoning.

c. *Amorphous urates*: These are urate salts **of potassium, magnesium, or calcium in acid urine**. They are usually **yellow**, fine granules in compact masses. They are **soluble in alkali or saline at 60°C.**

***Crystals present in alkaline urine*:**

a. 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.

b. Phosphates: Phosphates may occur as crystals (triple phosphates, calcium hydrogen phosphate), or as amorphous deposits.

• **Phosphate crystals**: Triple phosphates (ammonium magnesium phosphate): They are colorless, shiny, 3-6 sided prisms with oblique surfaces at the ends (“coffinlids”), or may have a feathery fern-like appearance.

• **Amorphous phosphates**: These occur as colorless small granules, often dispersed. All phosphates are soluble in dilute **acetic acid.**

c. Ammonium urate crystals: These occur as cactus-like (covered with spines) and called as '**thornapple' crystals**. They are yellow-brown and soluble in acetic acid at 60°C.

Abnormal Crystals

They are rare, but result from a pathological process. These occur in acid pH, often in large amounts. Abnormal crystals should not be reported on microscopy alone; additional chemical tests are done for confirmation.

1. Cysteine crystals: These are colorless, clear, hexagonal (having 6 sides), very refractile plates in acid urine. They often occur in layers. They are **soluble in 30% hydrochloric acid**. They are seen in cysteinuria, an **inborn error of metabolism**. Cysteine crystals are often associated with formation of **cysteine stones.**

2. Cholesterol crystals: These are colorless, refractile, flat rectangular plates with notched (missing) corners, and appear stacked in a stair-step arrangement. They are soluble in **ether, chloroform, or alcohol**. They are seen in **lipiduria** e.g. nephrotic syndrome and hypercholesterolemia. They can be positively identified by polarizing microscope.

3. Bilirubin crystals: These are small (5 µ), brown crystals of variable shape (square, bead-like, or fine needles). Their presence can be confirmed by doing reagent strip or **chemical test for bilirubin**. These crystals are soluble in strong acid or alkali. They are seen in **severe obstructive liver disease.**

4. 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**).

5. 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.**

6. Sulfonamide crystals: They are variably shaped crystals, but usually appear as **sheaves of needles**. They occur following **sulfonamide therapy**. They are **soluble in acetone**.

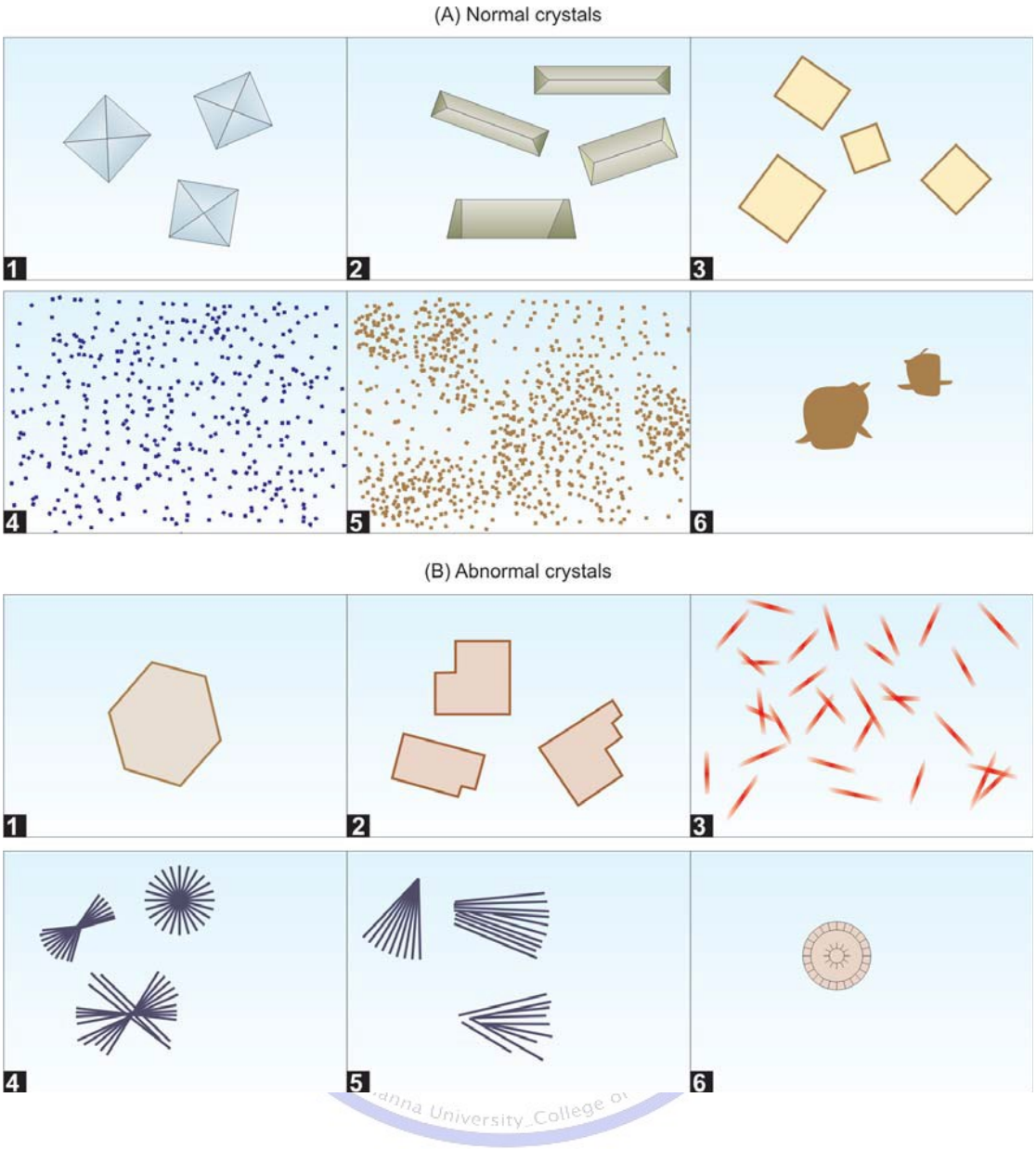

Fig. 1.28: Crystals in urine. (A) Normal crystals: (1) Calcium oxalate, (2) Triple phosphates, (3) Uric acid, (4) Amorphous phosphates, (5) Amorphous urates, (6) Ammonium urate.
(B) Abnormal crystals: (1) Cysteine, (2) Cholesterol, (3) Bilirubin, (4) Tyrosine, (5) Sulfonamide, and (6) Leucine