جامعة آشور

كلية الصيدلة

Sample Collection, Processing and Handling

Clinical Chemistry Practical | Fifth Stage



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Learning outcomes



- ✓ **Outline the main types of biological and medical specimens.**
- ✓ **Explain whole blood, serum, plasma, and their clinical use.**
- ✓ **Describe capillary, arterial, and venous blood collection techniques.**
- ✓ **List key venipuncture equipment and major procedural precautions.**
- ✓ **Recognize timing requirements, hemolysis causes, anticoagulants, transport media, and processing principles.**

Types of biological samples

Blood: whole blood, serum, plasma.

Urine and feces are common routine laboratory specimens.

Other body fluids include saliva, spinal fluid, synovial fluid, pleural fluid, pericardial fluid, and peritoneal fluid.

Choice of specimen depends on the clinical question and required analysis.



Common clinical specimen containers

Types of medical specimens

Group	Examples / source
Skin & wound	Pus, ulcers, wounds, hair, nail
Eyes / nose / mouth	Swabs or secretions from local sites
Respiratory	Upper and lower respiratory tract specimens
Urogenital	Semen, urethral, vaginal or cervical specimens
Body fluids	Effusions, CSF, synovial, pleural, peritoneal
Routine	Urine, feces, blood



Different specimens require different handling

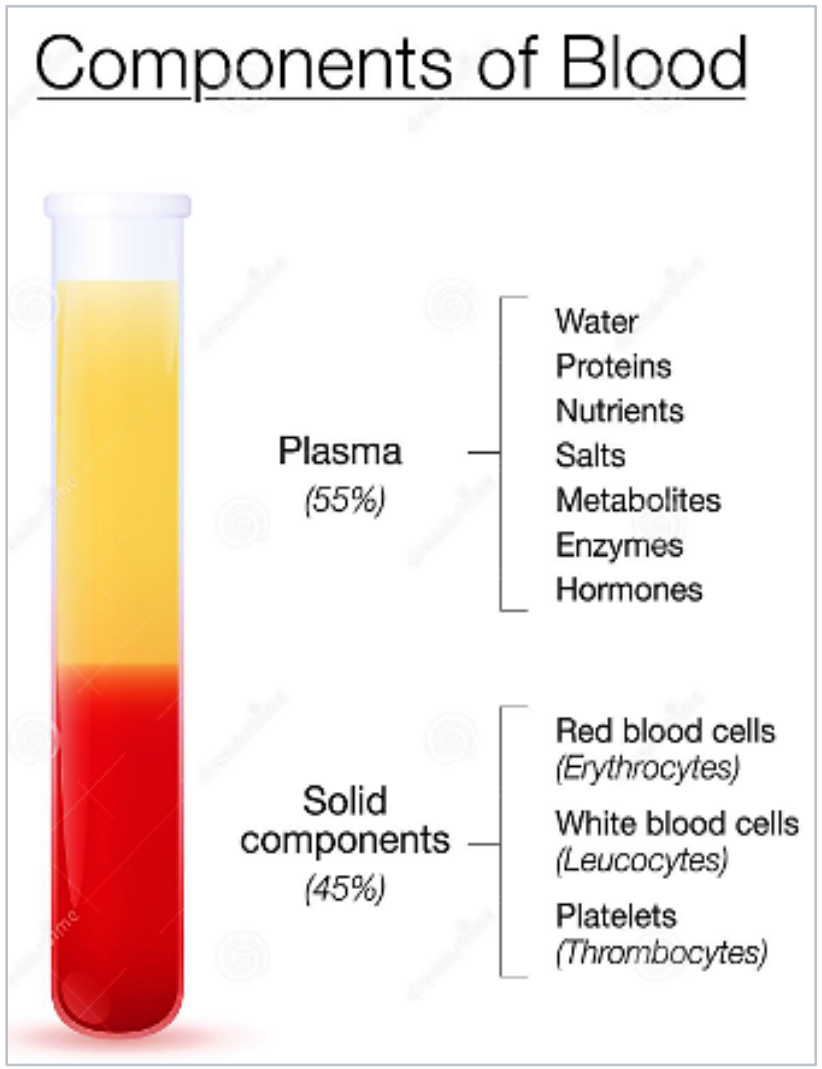
Blood: definition and composition

Blood is the red body fluid that delivers oxygen and nutrients to cells.

It removes metabolic waste products away from tissues.

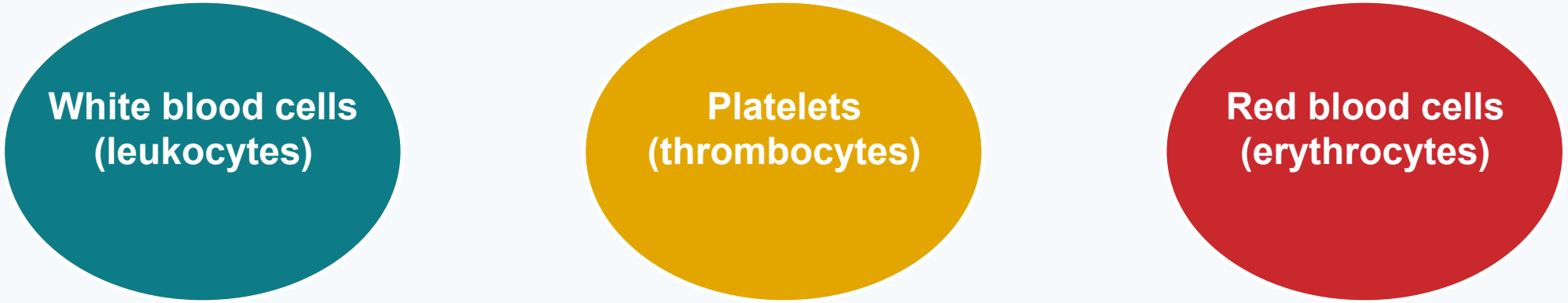
Approximate composition: 55% fluid fraction and 45% blood cells.

The fluid fraction is mainly plasma; cellular elements include red cells, white cells, and platelets.



Centrifuged blood layers: plasma, buffy coat, red-cell pellet

Blood cells: major categories



- **Leukocytes contribute to immune defense and inflammation.**
- **Erythrocytes transport oxygen and carbon dioxide.**
- **Platelets participate in hemostasis and clot formation.**

Types of blood specimens

Specimen	How it is obtained	Key feature
Whole blood	**Venous, arterial, or capillary blood in anticoagulant tube**	**Cells remain present**
Serum	**Blood collected without anticoagulant, allowed to clot, then centrifuged**	**Clear fluid without fibrin/clotting factors**
Plasma	**Blood mixed with anticoagulant and centrifuged**	**Clear fluid with clotting inhibited**



Visual anchor: blood separates after centrifugation

Plasma vs serum

Plasma

Liquid, cell-free part of blood treated with anticoagulants.

Contains fibrinogen and clotting factors.

Obtained by centrifugation of anticoagulated blood.

Serum

Liquid part of blood after coagulation.

Devoid of clotting factors such as fibrinogen.

Obtained by centrifugation after clot formation.

Key rule: Serum = Plasma − Fibrinogen

Blood collection techniques

- ✓ **The process of collecting a blood sample is called phlebotomy.**
- ✓ **Capillary blood collection uses fingertip, earlobe, or heel puncture.**
- ✓ **Arterial collection is mainly used for blood gas analysis and requires skilled personnel.**
- ✓ **Venous collection is most common for clinical chemistry and serology.**



Venipuncture is the most commonly used clinical chemistry collection method

Capillary, arterial and venous samples

Technique	Typical source/use	Key point
Capillary	**Fingertip, earlobe, or heel; rapid tests, pediatric/geriatric use**	**Mixture of arterial, venous, and capillary blood**
Arterial	**Blood gas analysis**	Needs special training and skilled physician/personnel
Venous	Routine clinical chemistry and serology	**Most commonly used in practice**

Selection depends on test requirement, patient condition, and safety.

Venipuncture equipment

Antiseptic: 70% isopropyl alcohol

Cotton or gauze

Gloves and face mask

Tourniquet

Syringe or needle system

Appropriate collection tubes



Use equipment safely and select tube according to test requirements

Before blood collection: patient preparation

- Ask essential questions: full name, drug use, diet, venous state, chronic disease, and intravenous fluid.
- Ensure the patient is comfortable: sitting or lying on a bed.
- Never perform venipuncture on a standing patient.
- Extend the arm straight from wrist to shoulder.
- Wash hands and wear protective gloves and face mask.



Procedure: site selection and cleansing

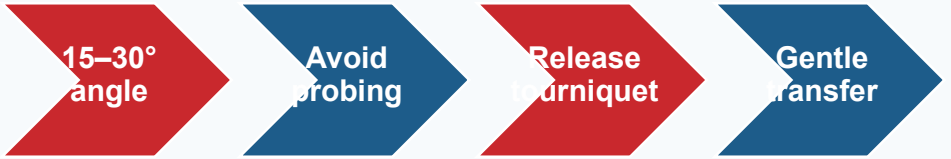
- ✓ **Select a suitable venipuncture site.**
- ✓ **Place the tourniquet 3–4 inches above the selected puncture site.**
- ✓ **Do not apply the tourniquet too tightly or leave it longer than one minute.**
- ✓ **Clean the area in a circular motion with alcohol and allow it to dry.**
- ✓ **After cleansing, do not touch or palpate the site again.**



Site preparation prevents contamination and hemolysis-related errors

Procedure: needle insertion and tube transfer

- Swiftly insert the needle through the skin into the lumen of the vein.
- Needle angle: approximately 15–30 degrees with the arm surface.
- Avoid excessive probing.
- Before removing the needle, remove the tourniquet and place cotton over the needle site.
- Transfer blood into the appropriate tube by gentle ejection.





Correct technique reduces sample damage

Timing of blood collection

Timing is important for analytes with diurnal variation such as cortisol.

Timing matters for therapeutic drug monitoring samples.

Glucose and alcohol concentrations may change later; delayed measurement can alter results.

Fasting sample: best for many biochemical investigations after 12–14 hours or overnight fast.

Post-prandial sample: taken 2 hours after a meal.

Random sample: can be taken anytime.



Timed sampling supports valid interpretation

Haemolysis of sample

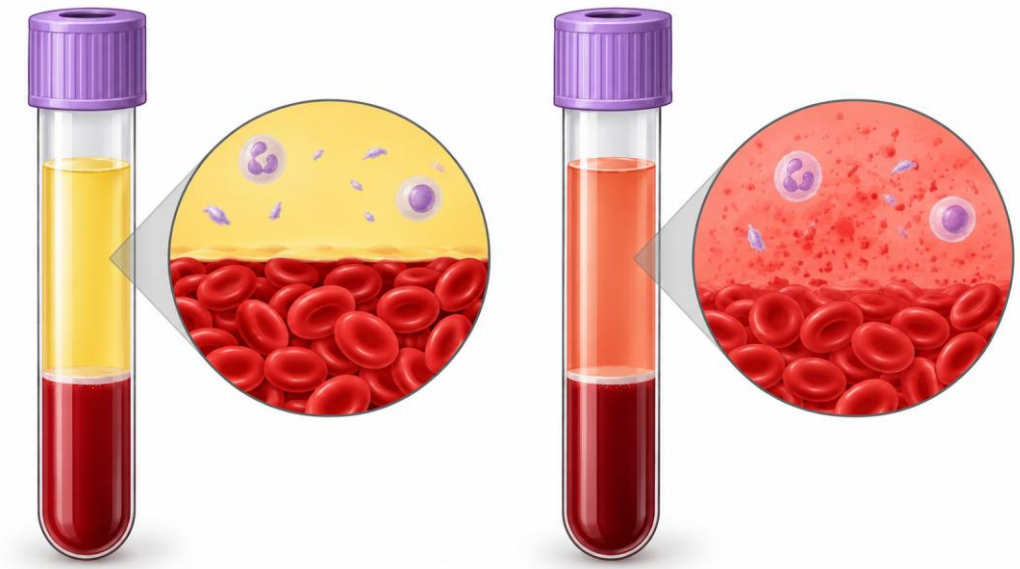
Haemolysis is rupture of red blood cells with release of intracellular contents into the surrounding fluid.

It should be avoided because it can make the sample invalid.

It may dilute constituents that are lower in RBCs than plasma, such as sodium.

It may falsely increase constituents that are higher in RBCs, such as potassium, AST, and LDH.

Released heme can interfere with spectrophotometric measurement, such as bilirubin.



Normal sample vs hemolyzed sample

Common causes of haemolysis

1. Moisture in collecting tube, syringe, or during collection
2. Vigorous mixing or rapid expansion of blood in tube/centrifuge
3. Incorrect needle size: too small or too large
4. Bad separation process

Preventing haemolysis is a core pre-analytical quality step.

Kinds of anticoagulants

Anticoagulant	Mechanism / use	Important note
EDTA	**Chelates calcium molecules**	**Common for hematology; prevents clotting**
Heparin	**Prevents fibrin formation from fibrinogen**	**Least interference among many anticoagulants**
Citrate	**Chelates calcium**	**May interfere with AST, ALT, ALP assays**
Oxalate	**Forms insoluble complex with calcium ions**	**May interfere with ALP, amylase, LDH**
Sodium fluoride	**Weak anticoagulant; preservative**	**Used mainly for blood glucose samples**

Collection times for microbiology specimens

Optimal timing depends on the infectious disease process and the laboratory's ability to process samples.

Daytime collection is often preferred because laboratories are usually better staffed.

First early-morning sputum and urine are optimal for recovery of acid-fast bacteria, fungi, and other pathogens.

Early morning secretions are more concentrated and more likely to contain the etiologic agent.

Blood culture timing depends on the clinical condition and physician schedule.

Blood cultures: usually no more frequently than ½ hour apart; three cultures per 24 h may be sufficient for many septicemia evaluations.

Specimen transport

Microorganisms are living: they grow, reproduce, and die.

Transport media are designed to prevent or slow these changes before culture.

Delayed or improper transport can lead to incomplete or misleading laboratory data.

Work cannot begin until the specimen arrives; prompt delivery matters.



Transport media and packaging maintain specimen integrity

Transport media: key differences

Medium	Main use / feature
Stuart's medium	**Originally formulated for Neisseria gonorrhoeae; non-nutritive; charcoal swabs may complicate Gram stain interpretation**
Amies medium	**Modified Stuart's medium; balanced salt solution; better transport system for most specimens**
Cary–Blair medium	**Modified for fecal specimens; higher pH; good for stool specimens; useful for enteric pathogens**
Buffered glycerol saline	**Designed for stool specimens; good for mailing fecal specimens; high pH favors fecal pathogens; not for suspected Campylobacter**

Specimen refrigeration

Can be refrigerated before inoculation

Urines

Respiratory exudates

Stools / feces

Wounds

Cannot be refrigerated before inoculation

Spinal fluids and other body fluids

Genital / cervical specimens for Gonococcus isolation

Blood

If processing is delayed, spinal fluids should be held at 35°C.

Specimen processing in bacteriology

Processing depends on specimen type, expected normal flora, and suspected pathogens.

Culture media and incubation conditions are selected to recover likely organisms while minimizing contaminants.

Gram stain is frequently used as an initial interpretive tool for many clinical specimens.

Some sites are normally sterile; any growth may be potentially significant.



Clean culture media illustration: blood, MacConkey-style and chocolate agar

Processing examples by specimen type

Specimen	Typical media / condition	Expected pathogens / interpretation
Throat	**Blood agar; chocolate agar for pediatric cases**	**Group A beta-hemolytic streptococci, H. influenzae, C. diphtheriae, B. pertussis**
Respiratory	**Blood agar, chocolate agar, MacConkey; sometimes anaerobic media**	**S. pneumoniae, H. influenzae, S. aureus, Enterobacteriaceae, Pseudomonas**
Urine	**Blood agar, MacConkey; 35°C aerobic; colony count**	**E. coli and other enterics, enterococci, Pseudomonas, staphylococci**
Feces	**MacConkey, XLD, selenite F; selective media for Campylobacter**	**Salmonella, Shigella, Yersinia, Campylobacter, E. coli**

Additional processing examples

Specimen	Media / condition	Common pathogens / comment
Genital for GC only	**Modified Thayer-Martin, 35°C, CO_2**	**Neisseria gonorrhoeae; Gram stain may be used**
Routine vaginal/cervical	**Blood agar, enriched chocolate, MTM, MacConkey, anaerobic blood agar**	**N. gonorrhoeae, Candida albicans, Gardnerella vaginalis, Group A/B streptococci, Listeria**
Sterile body fluids / CSF	**Blood agar, chocolate agar, MacConkey, thioglycollate**	**S. pneumoniae, N. meningitidis, H. influenzae, Gram-negative rods; identify all isolates**
Blood	**Two bottles: one aerobic and one anaerobic at 35°C**	**Any isolate is potentially significant**
Wound	**MacConkey, blood agar, chocolate agar; anaerobic media as needed**	**S. aureus, beta-hemolytic streptococci, Pseudomonas, Enterobacteriaceae, anaerobes**

Additional processing examples

Thank you