Students Selective /S5/2025-2026
Basic of Biochemical Testing

Liver Function Tests

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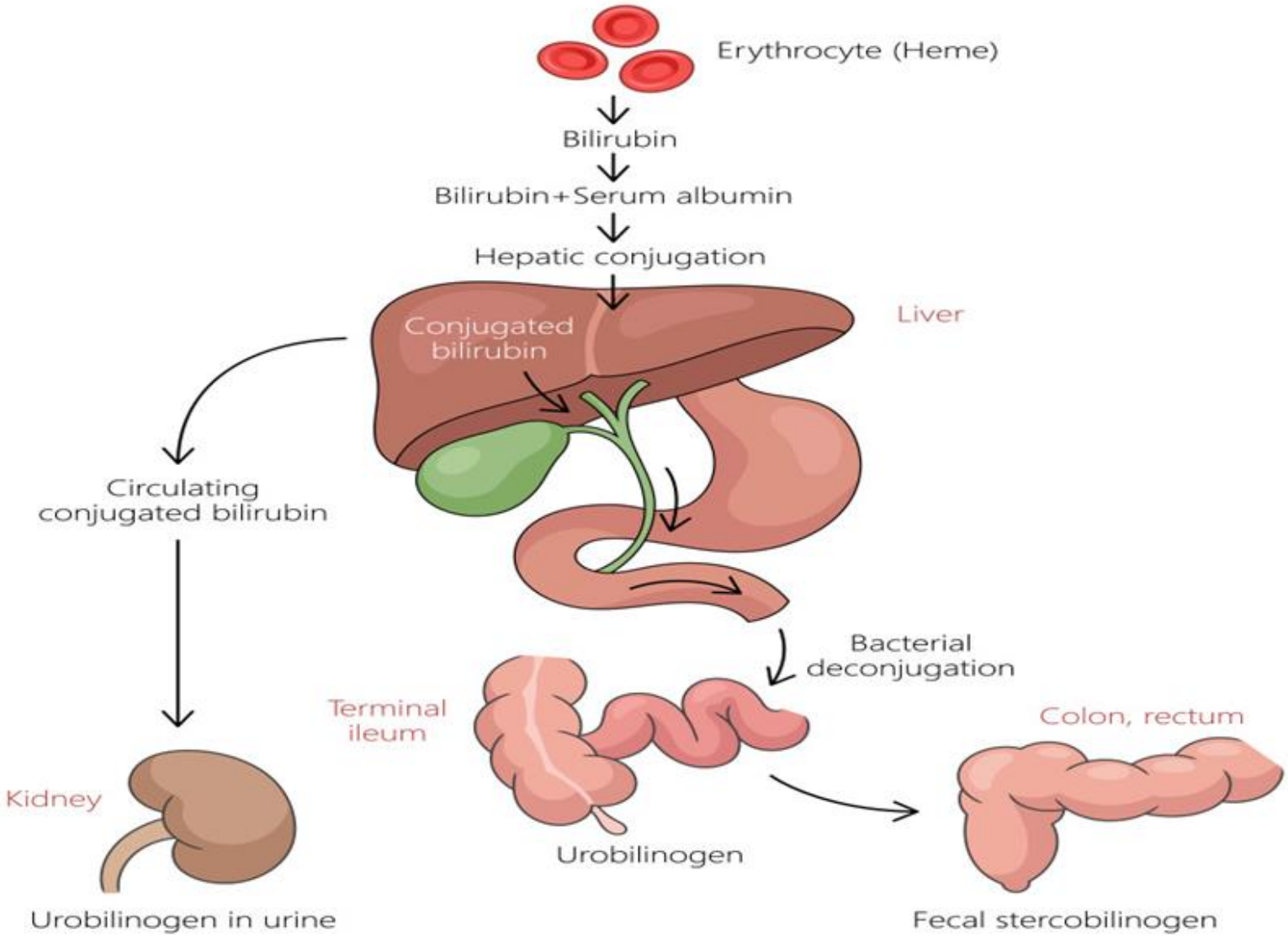
Jaundice (Icterus)

- Yellow pigmentation of skin, sclera, and mucous membranes due to **elevated serum bilirubin (>2–3 mg/dL)**.
- It indicates **disruption in bilirubin metabolism** at one or more stages: production, uptake, conjugation, or excretion

Normal Bilirubin Metabolism

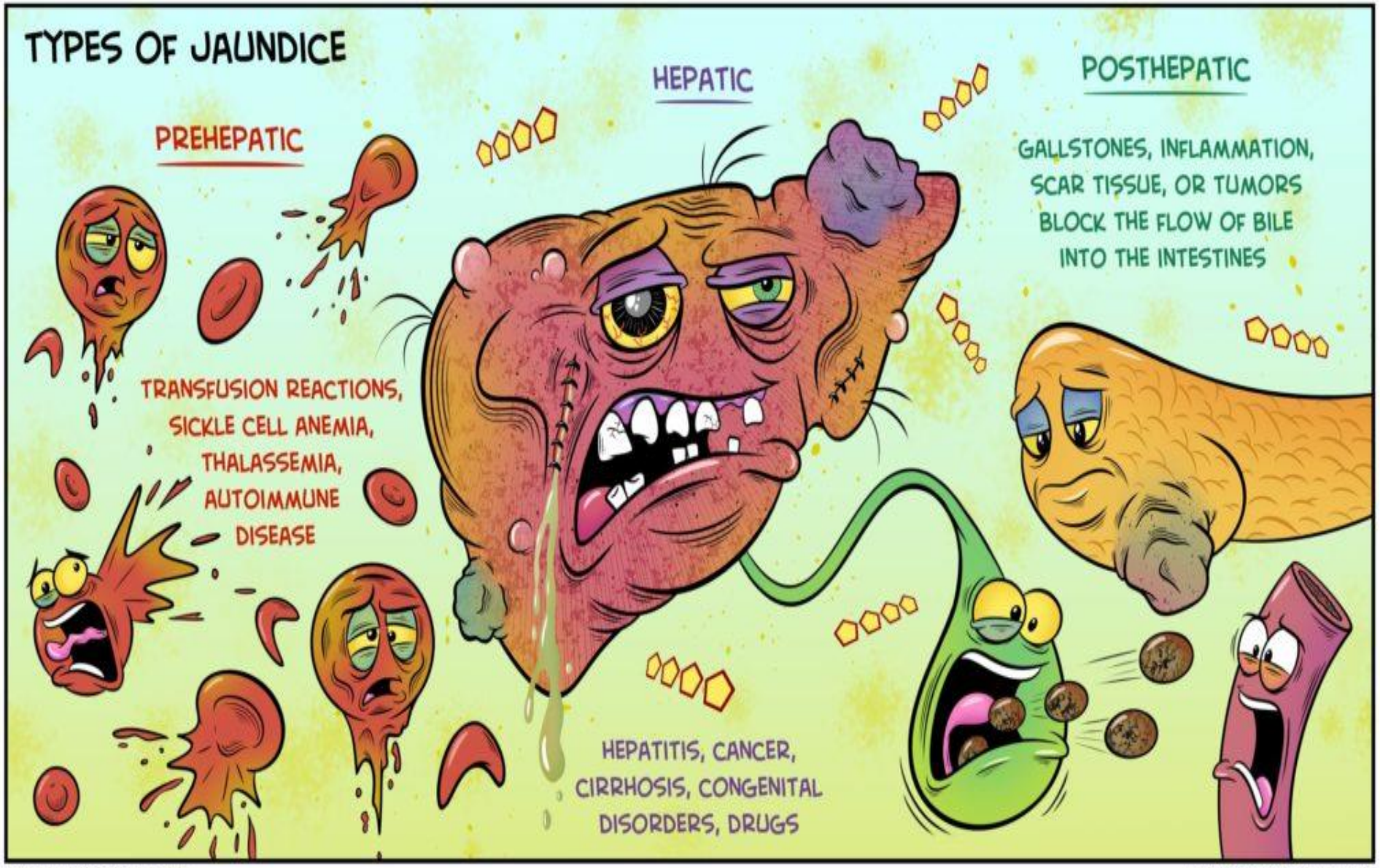
Step	Location	Process	Clinical Note
1	Reticuloendothelial system (spleen, bone marrow)	Breakdown of Hb → biliverdin → **unconjugated bilirubin (indirect)**	Unconjugated bilirubin is **lipid-soluble**, toxic, and **albumin-bound**
2	Hepatocyte uptake	Bilirubin-albumin complex dissociates; bilirubin enters liver cell	Impaired in some drugs (rifampicin)
3	Conjugation	Via **UDP-glucuronyl transferase → conjugated bilirubin (direct)**	Impaired in Gilbert's & Crigler-Najjar
4	Excretion into bile canaliculi	Active ATP-dependent transport into bile	Blocked in cholestasis
5	Intestinal conversion	→ **Urobilinogen** → excreted as stercobilin (stool color) & urobilin (urine color)	Absence → pale stool, dark urine

Bilirubin Metabolism



Classification of Jaundice

Type	Site of Problem	Main Mechanism	Typical Causes
A. Pre-hepatic (Hemolytic)	Before liver	↑ RBC breakdown → ↑ unconjugated bilirubin	Hemolytic anemia, malaria, transfusion reaction, spherocytosis
B. Hepatic (Hepatocellular)	Within hepatocyte	Impaired conjugation/excretion	Viral hepatitis, cirrhosis, alcoholic hepatitis, drug-induced injury
C. Post-hepatic (Obstructive/Cholestatic)	After liver	Blocked bile outflow → regurgitation of conjugated bilirubin	CBD stone, carcinoma head of pancreas, PSC, PBC


TYPES OF JAUNDICE
PREHEPATIC
TRANSFUSION REACTIONS, SICKLE CELL ANEMIA, THALASSEMIA, AUTOIMMUNE DISEASE
HEPATIC
HEPATITIS, CANCER, CIRRHOSIS, CONGENITAL DISORDERS, DRUGS
POSTHEPATIC
GALLSTONES, INFLAMMATION, SCAR TISSUE, OR TUMORS BLOCK THE FLOW OF BILE INTO THE INTESTINES
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Clinical Presentation and Bedside Differentiation

Feature	Pre-hepatic	Hepatic	Post-hepatic
Onset	Gradual	Variable	Gradual or sudden
Stool color	Dark	Variable	Pale/clay-colored
Urine color	Normal	Dark	Very dark
Itching	Absent	Mild	**Severe (cholestatic)**
Hepatomegaly	Mild	Tender, enlarged	Smooth, possibly palpable GB (Courvoisier sign)
Other signs	Anemia, splenomegaly	Stigmata of CLD	Scratch marks, jaundice, palpable GB

Laboratory Differentiation: LFT Patterns

Parameter	Normal Range	Pre-hepatic	Hepatic	Post-hepatic
Total bilirubin	0.3–1.2 mg/dL	↑ (indirect)	↑ (mixed)	↑ (direct)
Direct bilirubin	< 0.3 mg/dL	Normal	↑	↑↑
Indirect bilirubin	—	↑↑	↑	Normal/mild ↑
ALT (SGPT)	< 40 IU/L	Normal	**↑↑ (1000+)**	Mild ↑
AST (SGOT)	< 40 IU/L	Normal	**↑↑**	Mild ↑
AST/ALT ratio	—	—	>2 in alcoholic hepatitis	<1 in viral hepatitis
ALP	30–120 IU/L	Normal	Mild ↑	**↑↑ (>3× normal)**
GGT	10–50 IU/L	Normal	Mild ↑	↑↑ (with ALP → biliary origin)
Albumin	3.5–5 g/dL	Normal	↓ in chronic cases	Normal
Prothrombin Time (PT/INR)	—	Normal	**Prolonged**	Normal or prolonged in prolonged obstruction

Special Laboratory Clues

Lab Test	Finding	Interpretation
Urine bilirubin	Present only if conjugated bilirubin ↑	Indicates hepatic or obstructive jaundice
Urine urobilinogen	↑ in hemolysis, ↓ in obstruction	Helps differentiate pre- from post-hepatic
Reticulocyte count	↑	Hemolysis
Viral serology (HBsAg, anti-HCV)	+ve	Hepatitis
Autoimmune markers (AMA, ANA, SMA)	+ve	PBC/Autoimmune hepatitis
Tumor markers (CA 19-9, AFP)	+ve	Cholangiocarcinoma, HCC

Imaging Approach

Step	Modality	Purpose
1	**Ultrasound abdomen**	First-line; detects biliary dilatation, stones, masses
2	**MRCP / ERCP**	Defines level and cause of obstruction
3	**CT / MRI liver**	Parenchymal disease, masses
4	**Liver biopsy**	Confirm hepatocellular disease, fibrosis, cirrhosis


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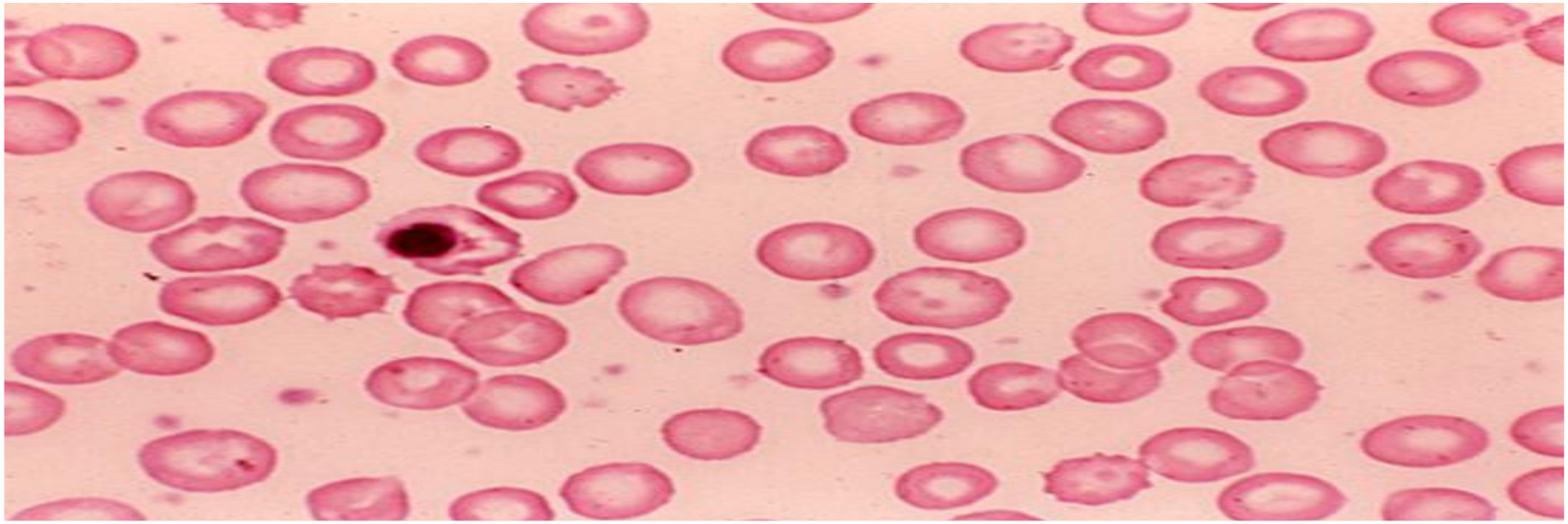
Case 1 – Hemolytic Jaundice

25-year-old male, mild jaundice, pallor, splenomegaly
Dark stool, normal-colored urine.

- Labs:
  - ✓ ↑ indirect bilirubin (4.5 mg/dL).
  - ✓ ALT, ALP normal.
  - ✓ Reticulocytes 6%.
  - ✓

→ Diagnosis: Pre-hepatic (hemolytic)

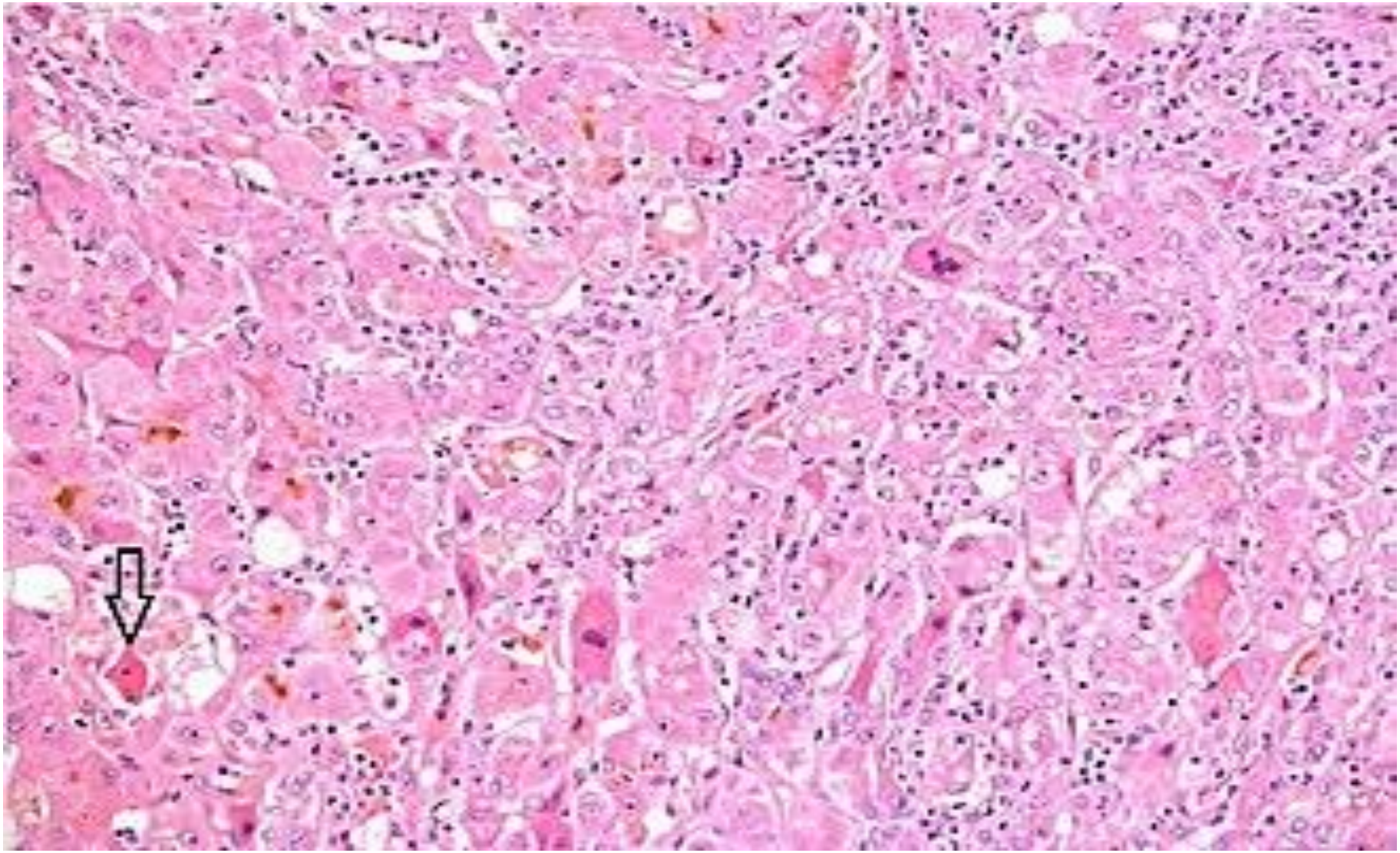


Case 2 – Acute Hepatitis

30-year-old, fever, malaise, yellow eyes
Dark urine, normal stool.

- Labs:
  - ✓ ALT 1200 IU/L, AST 900 IU/L, ALP 150 IU/L.
  - ✓ Mixed bilirubin elevation.

→ **Diagnosis: Hepatocellular jaundice (viral hepatitis)**

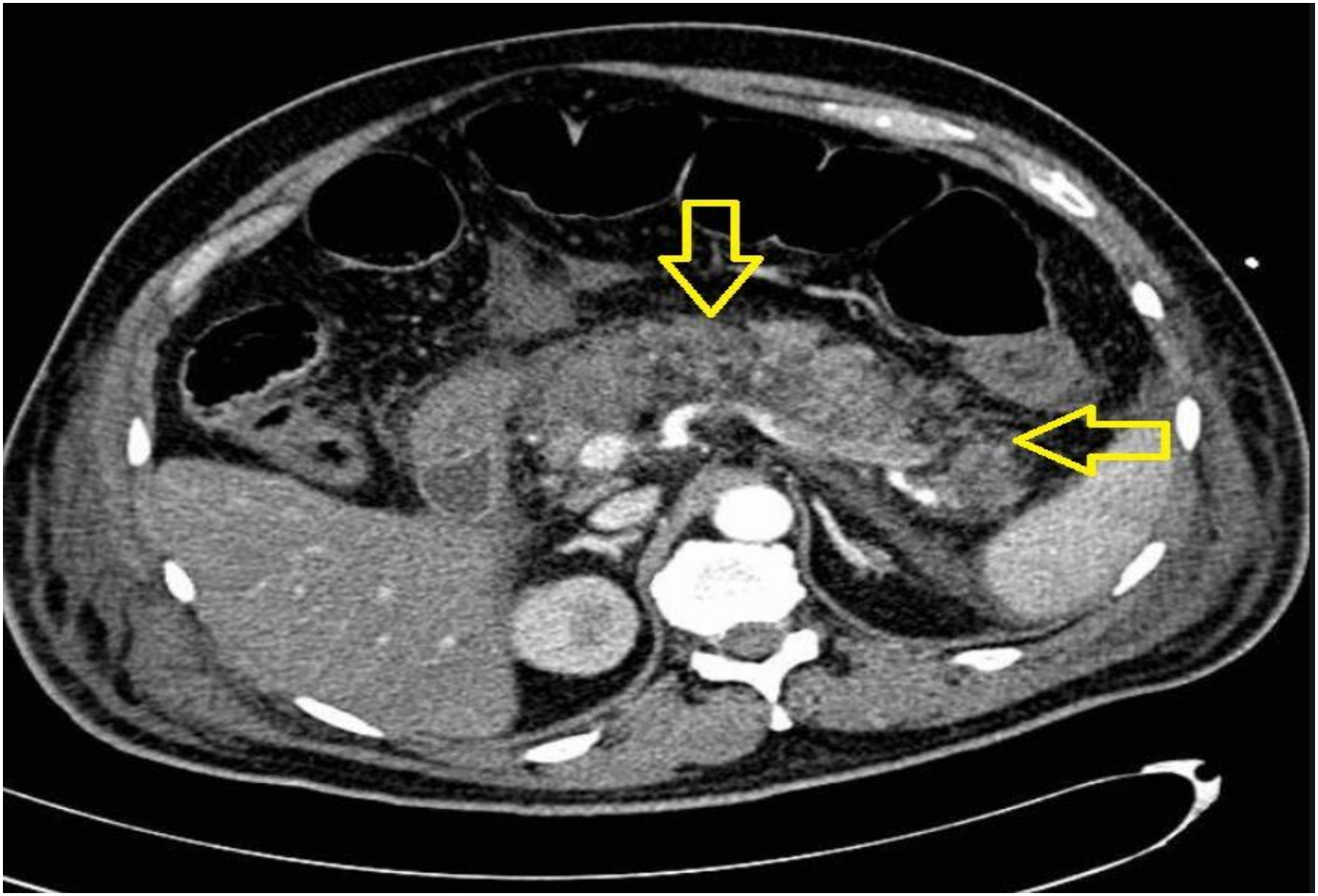


Case 3 – Obstructive Jaundice

65-year-old female, painless jaundice, itching, pale stool, palpable GB.

- Labs:
  - ✓ Bilirubin 12 mg/dL (direct 10 mg/dL).
  - ✓ ALP 650 IU/L, GGT 400 IU/L.
  - ✓ ALT/AST mild elevation.
  - ✓ USG: Dilated bile ducts.

    → **Diagnosis: Post-hepatic (CBD obstruction / pancreatic head mass).**



Interpretation Strategy (Clinician's Rule of Thumb)

1.Check bilirubin fractionation → direct vs indirect.

2.Check enzymes:

- ALT/AST → hepatocellular.
- ALP/GGT → cholestatic.

3.Check synthetic function:

- Albumin, PT/INR

4.Correlate with clinical features & imaging.

Function	Test	Meaning
Synthetic	Albumin, PT	Decreased in failure
Excretory	Bilirubin	Elevated in obstruction
Detoxification	Ammonia (not routine)	Elevated in hepatic encephalopathy
Enzyme leakage	ALT/AST/ALP/GGT	Indicates hepatocellular damage or cholestasis

Patterns of Enzyme Elevation

Pattern	AST/ALT	ALP/GGT	Typical Conditions
Hepatocellular injury	↑↑	Mild ↑	Viral hepatitis, drugs
Cholestatic injury	Mild ↑	↑↑	CBD obstruction, PBC
Mixed pattern	↑↑ & ↑↑	Both moderate ↑	Drug-induced, alcoholic hepatitis
Isolated ALP rise	Normal ALT	↑ ALP/GGT	Bone disease, infiltrative lesions

Special Syndromes of Jaundice

Syndrome	Defect	Type of Bilirubin (increased)	Clinical Note
Gilbert's	↓ UDP-glucuronyl transferase activity	Unconjugated	Benign, mild jaundice during stress
Crigler-Najjar I/II	Absent/deficient conjugation enzyme	Unconjugated	Type I fatal in infancy
Dubin–Johnson	Impaired excretion of conjugated bilirubin	Conjugated	Black liver on biopsy
Rotor syndrome	Defective hepatic storage	Conjugated	Mild, benign

Management Principles:

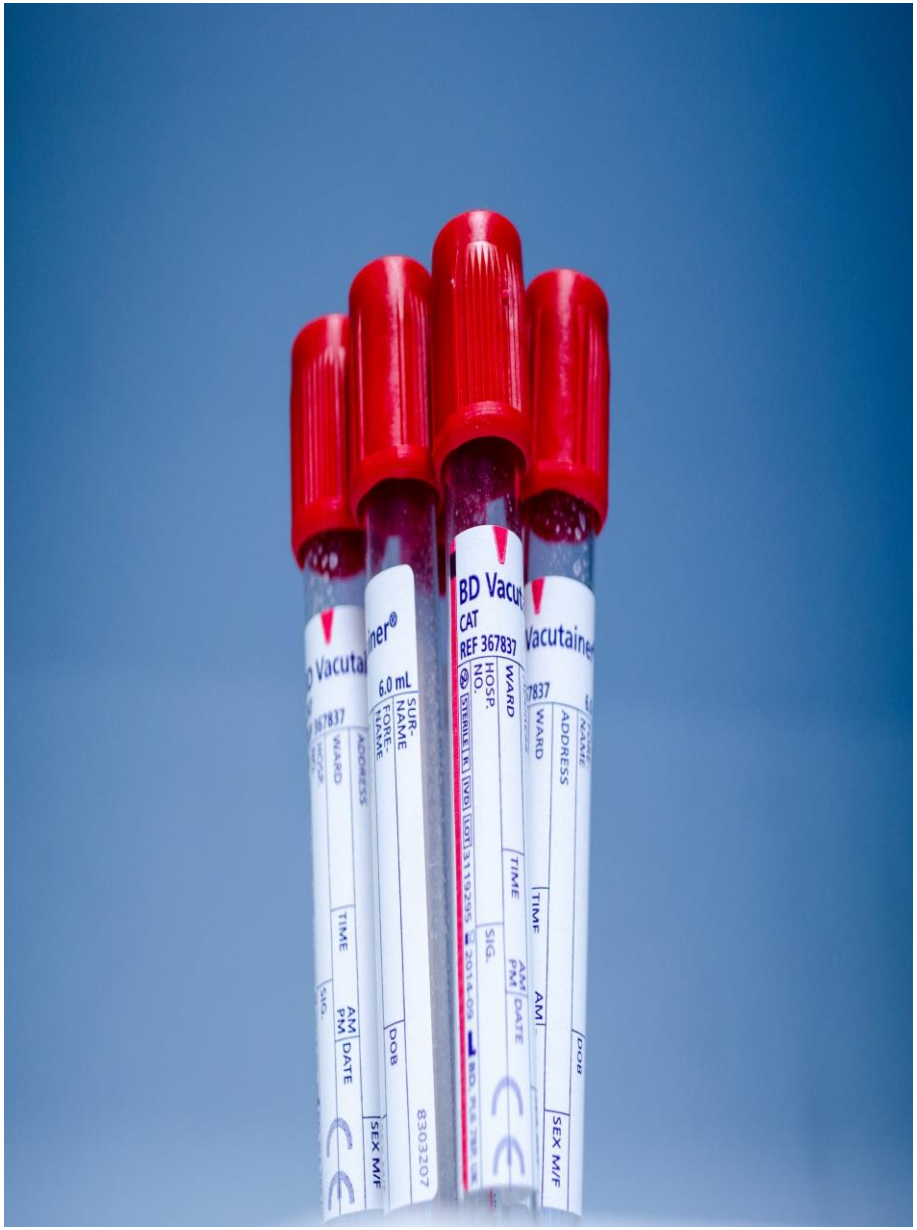
- **Pre-hepatic:** Treat underlying hemolysis (transfusion, steroids, stop offending drugs).
- **Hepatic:** Rest, avoid hepatotoxic drugs, treat viral/autoimmune cause, monitor LFTs.
- **Post-hepatic:** Relieve obstruction (ERCP stone removal, stenting, surgery).

summary

Feature	Pre-hepatic	Hepatic	Post-hepatic
Type of bilirubin	Indirect	Mixed	Direct
Urine bilirubin	Absent	Present	Present
Urine urobilinogen	↑	Variable	↓ or absent
Stool color	Dark	Normal	Pale
ALP	Normal	Mild ↑	Marked ↑
ALT/AST	Normal	Marked ↑	Mild ↑
GGT	Normal	Mild ↑	Marked ↑
Albumin/PT	Normal	↓/↑	Normal/slightly ↑

Laboratory points

Parameter	Tube Type	Sample Type	Volume	Handling/Storage	Notes
Bilirubin (Total & Direct)	Plain (red top) or serum separator (yellow top)	Serum	2–3 mL	Protect from light (bilirubin is light-sensitive)	Analyze within 2 hours
ALT, AST, ALP, GGT	Plain (red top) or gel tube	Serum	2–3 mL	Store at 2–8°C if delayed	Hemolysis falsely elevates AST
Albumin, Total Protein	Plain (red) or gel tube	Serum	2–3 mL	Stable at 2–8°C for 1 week	Avoid gross hemolysis
Prothrombin Time (PT/INR)	Blue top (citrate tube, 3.2%)	Plasma	2.7 mL (with 0.3 mL citrate)	Mix gently; test within 2 hrs	Do not refrigerate before testing
Ammonia (if required)	Purple top (EDTA)	Plasma	2 mL	Keep on ice, analyze within 30 min	Used for hepatic encephalopathy
Gamma-glutamyl transferase (GGT)	Plain or gel tube	Serum	2 mL	Stable at 2–8°C for 3 days	Alcohol and enzyme inducers increase GGT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Methods of Measurement

Test	Method	Principle	Key Notes
Total Bilirubin	**Diazo (Jendrassik–Grof)**	Reaction with diazotized sulfanilic acid forms azobilirubin measured spectrophotometrically	Direct reacts immediately; total requires accelerator (e.g., caffeine)
ALT (SGPT)	**Kinetic UV method**	Catalyzes conversion of alanine + α-ketoglutarate → pyruvate + glutamate; rate of NADH oxidation measured at 340 nm	Avoid hemolysis
AST (SGOT)	**Kinetic UV method**	Aspartate + α-ketoglutarate → oxaloacetate + glutamate; NADH oxidation at 340 nm	Elevated in liver and muscle diseases
ALP	**Colorimetric (p-Nitrophenyl phosphate method)**	Hydrolysis of pNPP → yellow p-nitrophenol measured at 405 nm	ALP ↑ in bone and liver disorders
GGT	**Kinetic colorimetric (γ-glutamyl-p-nitroanilide method)**	GGT transfers γ-glutamyl group; color change read at 405 nm	Very sensitive for alcohol or biliary disease
Albumin	**BCG (Bromocresol green)**	Albumin binds to dye forming green complex measured at 628 nm	Low in malnutrition, CLD
Total Protein	**Biuret method**	Cu^{2+} reacts with peptide bonds to form violet color (540 nm)	Not specific for liver
Prothrombin Time (PT/INR)	**Coagulometric**	Measures clotting time after adding thromboplastin + Ca^{2+}	Reflects synthetic capacity of liver
Ammonia	**Enzymatic (glutamate dehydrogenase)**	NH_3 + α-ketoglutarate → glutamate; NADH oxidation at 340 nm	Requires immediate analysis

Interfering Factors & Precautions

Factor	Effect	Prevention
Hemolysis	↑ AST, ↑ total protein (false), ↑ bilirubin (interference)	Avoid traumatic venipuncture
Lipemia	Optical interference in colorimetric methods	Centrifuge or use blank correction
Icterus	High bilirubin interferes with ALP, ALT, AST readings	Automated analyzers apply correction
Prolonged tourniquet	↑ total protein falsely	Release tourniquet early
Light exposure	Degrades bilirubin	Keep tubes wrapped in foil
Delay in separation	Leakage of enzymes	Centrifuge within 30 min of collection

Hemolyzed sample

Lipidemic sample

Icterus sample

Reference Ranges (Typical Adult Values)

Test	Normal Range	Unit
Total Bilirubin	0.3 – 1.2	mg/dL
Direct Bilirubin	0.0 – 0.3	mg/dL
Indirect Bilirubin	< 1.0	mg/dL
ALT (SGPT)	< 40	IU/L
AST (SGOT)	< 40	IU/L
ALP	30 – 120	IU/L
GGT	10 – 50	IU/L
Total Protein	6.0 – 8.0	g/dL
Albumin	3.5 – 5.0	g/dL
Globulin	2.5 – 3.5	g/dL
PT (Control ± 3 sec)	11 – 13	sec

Quality Control and Calibration

- ✓ Use **bilevel control sera** (normal & pathological) daily.
- ✓ Calibrate analyzer regularly using **traceable standards (IFCC, WHO)**.
- ✓ Maintain **temperature (37°C)** in kinetic enzyme assays.
- ✓ Report results with **method used** for clinical correlation.

Sample Rejection Criteria

Hemolyzed samples (for ALT/AST, bilirubin).

Lipemic samples (for colorimetric tests).

Clotted plasma in citrate tubes (for PT).

Insufficient sample volume.

Automation and Analyzers

Modern labs use **automated biochemistry analyzers** (e.g., Roche Cobas, Beckman, Abbott Architect).
They use:

- **Photometric (UV-visible)** measurement.
- **Kinetic enzyme analysis.**
- **Ion-selective and immunoturbidimetric** assays.

Automation ensures accuracy, precision, and sample traceability.

Reporting and Interpretation

- ✓ Report results with **reference range** and **units**.
- ✓ Provide **interpretive comments** (e.g., hepatocellular vs cholestatic pattern).
- ✓ Suggest **repeat testing or confirmatory imaging** if pattern abnormal.

Summary Table: LFT Lab Essentials

Parameter	Tube	Sample	Method	Light Protection	Hemolysis Effect
Bilirubin	Red/Yellow	Serum	Diazo (colorimetric)	✓ Yes	False ↑
ALT	Red/Yellow	Serum	UV kinetic	✗ No	↑
AST	Red/Yellow	Serum	UV kinetic	✗ No	↑
ALP	Red/Yellow	Serum	pNPP colorimetric	✗ No	Slight
GGT	Red/Yellow	Serum	Kinetic colorimetric	✗ No	None
Albumin	Red/Yellow	Serum	BCG dye-binding	✗ No	None
Total Protein	Red/Yellow	Serum	Biuret	✗ No	None
PT/INR	Blue (Citrate)	Plasma	Coagulometric	✗ No	Invalid if clotted
Ammonia	Purple (EDTA)	Plasma	Enzymatic UV	✓ Ice	False ↑ if delayed


THANK
YOU