

# Measurement of total bilirubin

By

Medical Biochemistry Unit

# Sensitivity

Sensitivity answers the following question:

If a person has a disease, how often will the test be positive (true positive rate)?

i.e.: if the test is highly sensitive and the test result is negative you can be nearly certain that the individuals don't have disease.

A Sensitive test helps **rule out** disease (when the result is negative).

**Sensitivity rule out or "Snout"**

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{true positive} + \text{false negative}} \times 100$$

## 2 X 2 Contingency Table

Test	Disease	
	+	-
+	True Positive (TP)	False Positive (FP)
-	False Negative (FN)	True Negative (TN)

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

# Specificity

Specificity answers the following question:

If a person does not have the disease how often will the test be negative (true negative rate)?

i.e., if the test result for a highly specific test is positive you can be nearly certain that the individuals actually have the disease.

A very specific test rules in disease with a high degree of confidence (when the result is positive).

## Specificity rule in or "Spin"

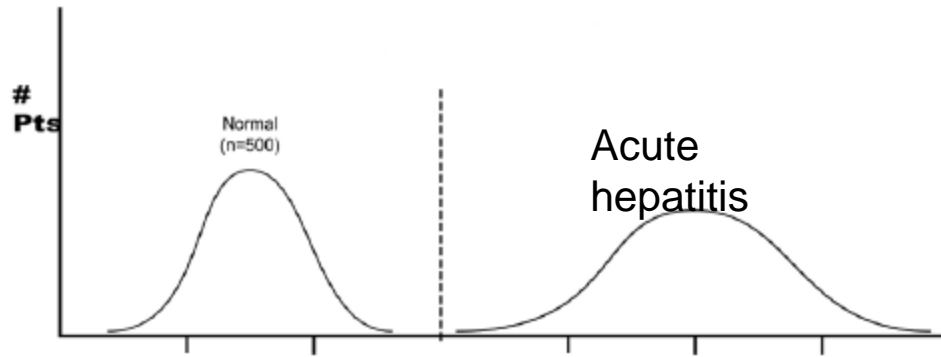
$$\text{Specificity} = \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \times 100$$

# 2 X 2 Contingency Table

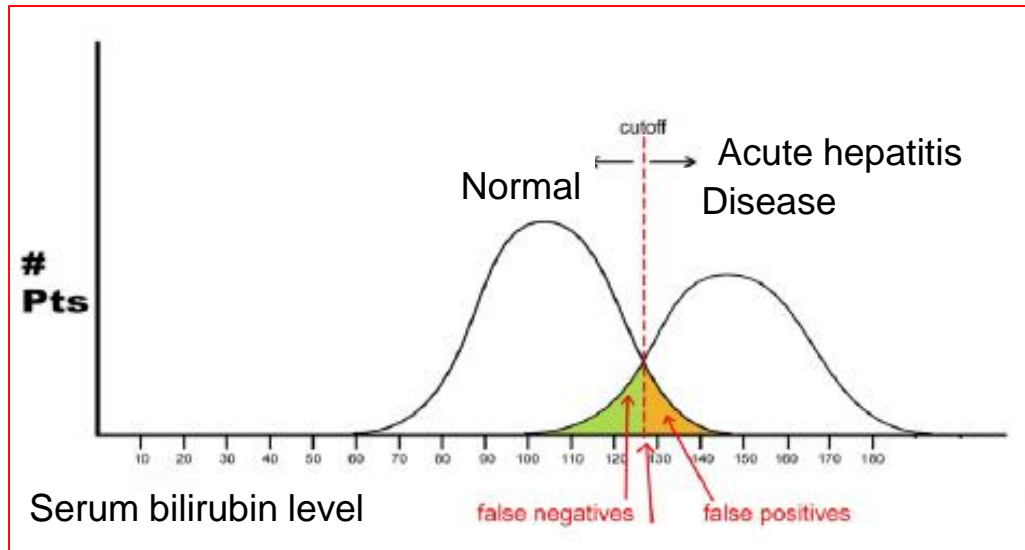
Test	Disease	
	+	-
+	<b>True Positive (TP)</b>	<b>False Positive (FP)</b>
-	<b>False Negative (FN)</b>	<b>True Negative (TN)</b>

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100$$

# An ideal diagnostic lab test results for many subjects (normal and patients)



- A perfect test for acute hepatitis:  
The test identifies ALL patients with disease and  
All subjects without disease 100% of the time.



- The lab test results in normal and disease conditions overlap.
- To increase the overall accuracy of the test, the centermost point of overlapping is chosen as the cutoff value.
- There are some normal subjects who will have a positive results (False positives)
- There are some patients who will have negative results (False negatives)

# Example of calculation

**A Lab test to measure serum bilirubin was performed on 1000 individuals. The test gave the following results:**

- Number of positive results in patients with acute hepatitis: 440
- Number of positive results in normal subjects: 50
- Number of negative results in normal subjects: 450
- Number of negative results in patients with acute hepatitis: 60
- For this Serum bilirubin test, calculate the following quality measures:
  1. The sensitivity
  2. The specificity



Answer: draw a  
2 X 2 Contingency  
Table

Test	Disease	
	+	-
+	TP	FP
-	FN	TN

Test	Disease	
	+	-
+	440	50
-	60	450

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100 = \frac{440}{440 + 60} \times 100 = 0.88 \times 100$$

$$\text{Sensitivity} = 88\%$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100 = \frac{450}{450 + 50} \times 100 = 0.90 \times 100$$

$$\text{Specificity} = 90\%$$

**Q1. What are the liver function tests (LFTs)?**

# Q1. What are the liver function tests (LFTs)?

Liver chemistry test	Clinical implication of abnormality
Alanine aminotransferase (ALT)	Hepatocellular damage
Aspartate aminotransferase (AST)	Hepatocellular damage
Bilirubin	Cholestasis, impaired conjugation, or biliary obstruction
Alkaline phosphatase	Cholestasis, infiltrative disease, or biliary obstruction
Prothrombin time	Synthetic function
Albumin	Synthetic function
$\gamma$ -glutamyltransferase	Cholestasis or biliary obstruction
Bile acids	Cholestasis or biliary obstruction

**e.g. Viral hepatitis**

**Q2. What is bilirubin and how is it produced in the body?**

## **Q2. What is bilirubin and how is it produced in the body?**

- Bilirubin is a yellow bile pigment.
- It is produced from the degradation of heme; which is one of the breakdown products of red blood cells.

**Q3. Which form of bilirubin is carried to the liver and how?**

### **Q3. Which form of bilirubin is carried to the liver and how?**

- The **unconjugated** form of bilirubin is carried to the liver
- Unconjugated bilirubin forms a **complex with albumin** to be transported

Q4.

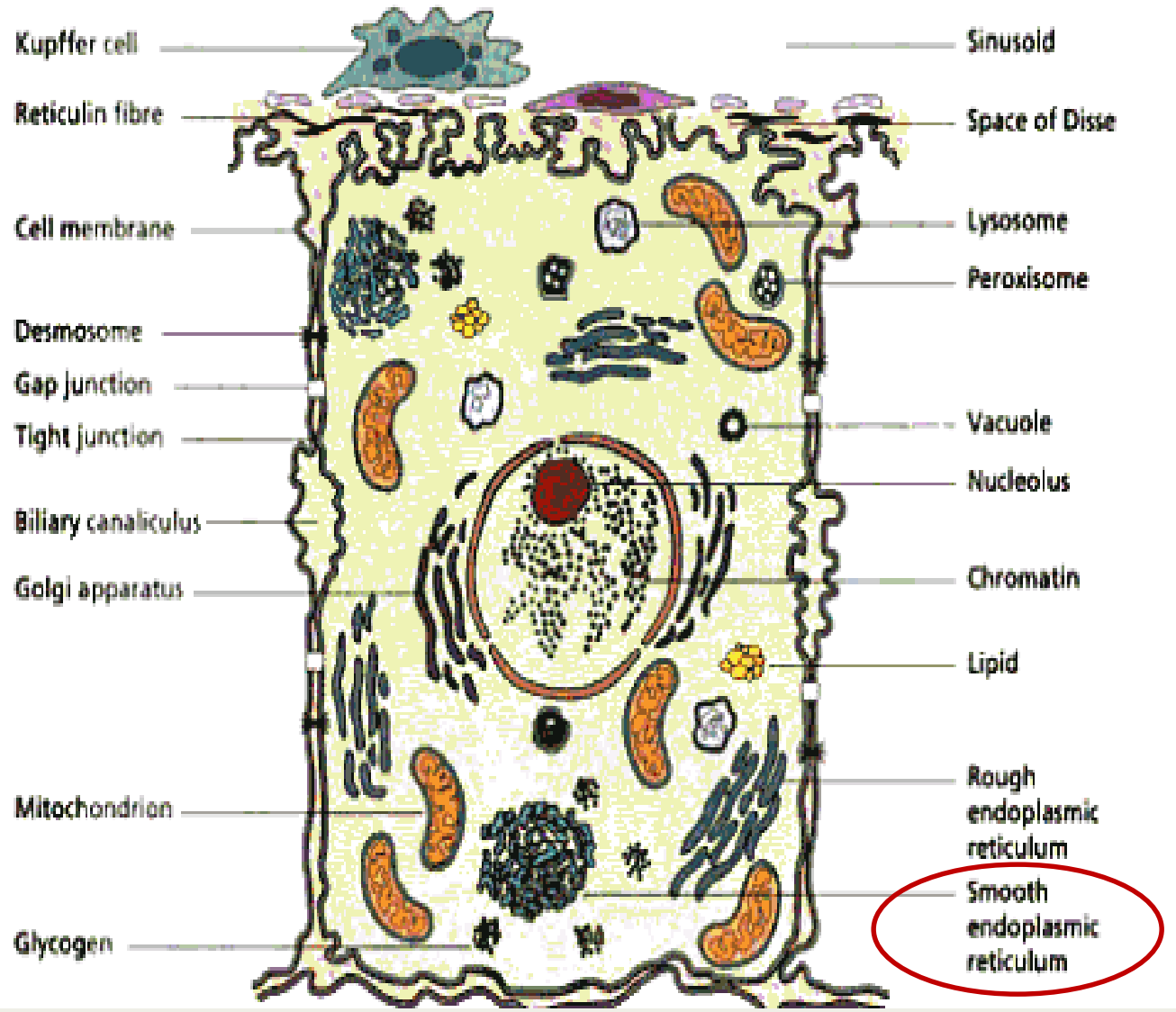
- How & why is bilirubin conjugated?
- On the picture below, mark the intracellular location for the process of conjugation?
- Mention 2 syndromes due to congenital deficiency of the conjugating enzyme (bilirubin glucuronyl-transferase).





# How & why is bilirubin conjugated?

- Bilirubin is conjugated by addition of glucuronic acid in hepatocytes
- The conjugated-bilirubin is water soluble and can be excreted in the urine and feces
- This prevents precipitation and deposition in tissues.

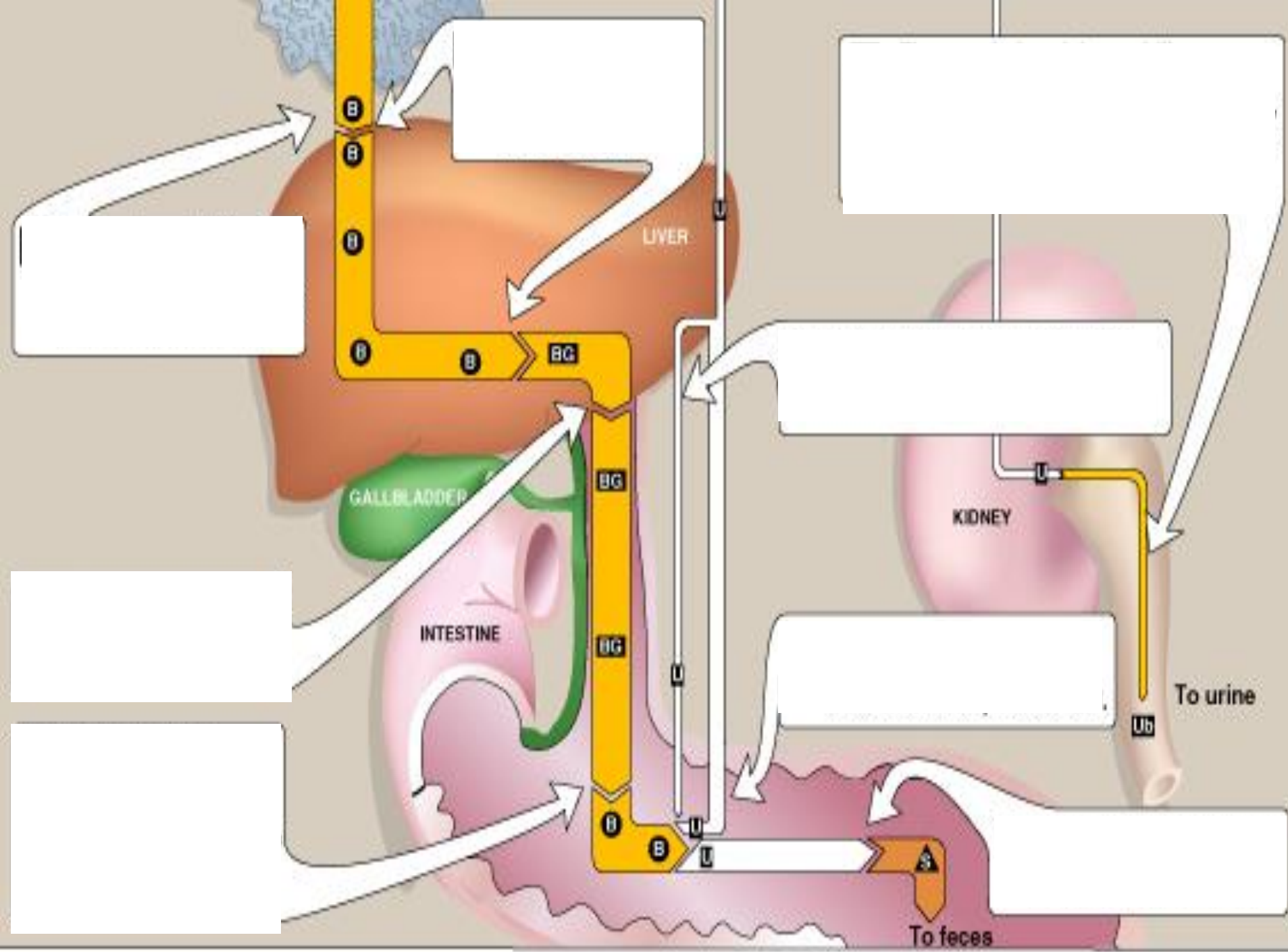


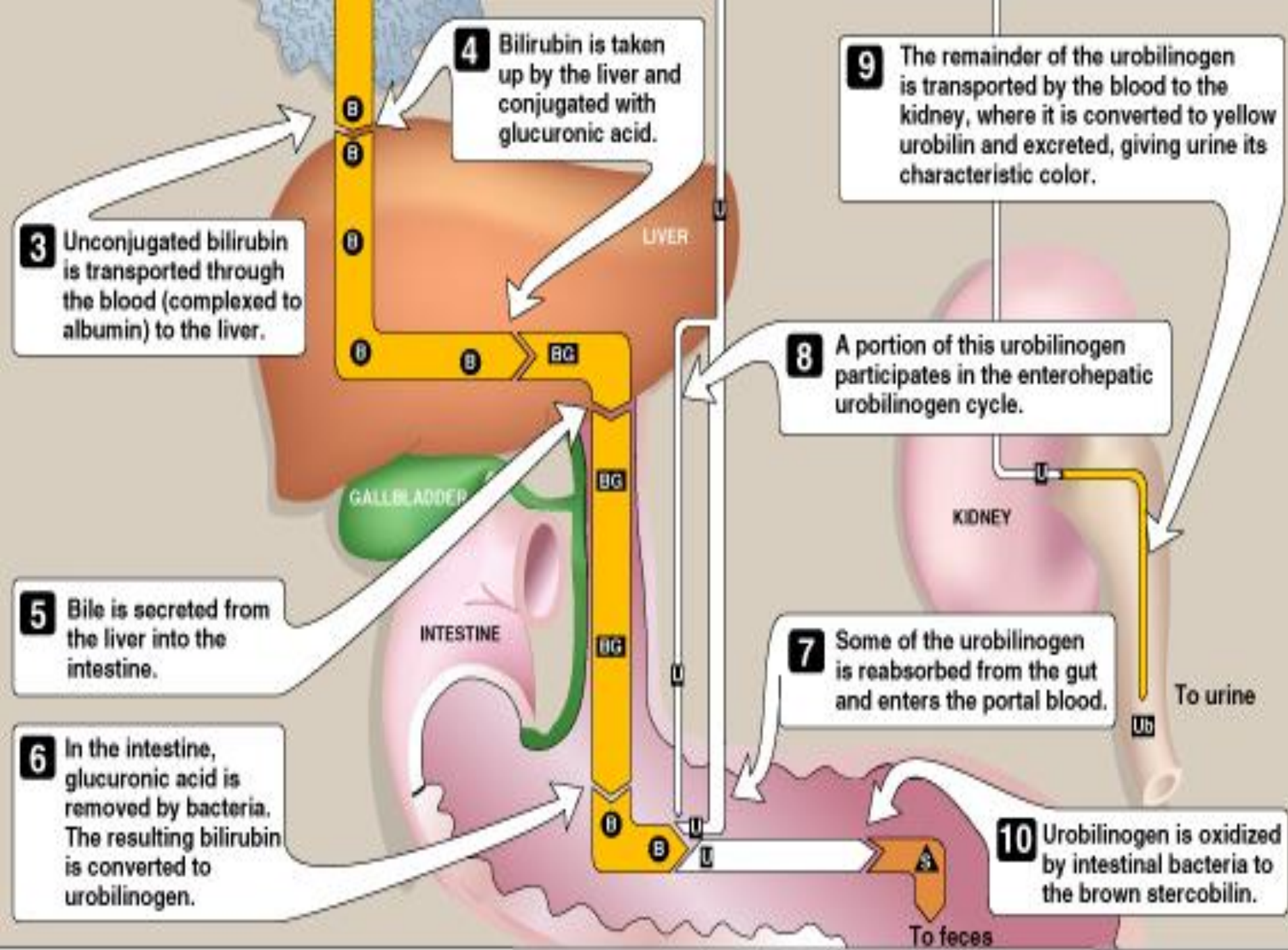
# Defective enzymatic conjugation of bilirubin

- Examples of clinical conditions due to congenital deficiency of the conjugating enzyme ( bilirubin glucuronyl transferase)
  - Crigler-Najjar syndrome
  - Gilbert syndrome

**Q5.**

- A. How is bilirubin eliminated from the body?**
- B. What are the fates of bilirubin in the intestine?**





# Measurement of Total Bilirubin

1

Add 200 $\mu$ l  
Reagent 1  
To blank and test

2

Add 50 $\mu$ l  
Reagent 2  
**Only in test**

3

Add 1 ml  
Reagent 3  
To blank and test

4

Add 200 $\mu$ l serum



Mix and wait for  
5-10 min at RT

5

Add 1 ml Reagent 4  
To blank and test

Mix and  
wait for  
5-20 min at  
RT

6

Transfer (Pour) to  
cuvette



7

Using blank set  
the base line at  
wavelength 578 nm

8

Read test (A) at  
578 nm



# Calculation of total bilirubin concentration

**Conc. of serum total bilirubin:**

$$A \times 185 = \dots \mu\text{mol/L}$$

***Note- (Normal range: 2 – 17  $\mu\text{mol/L}$ )***



