



Instructions For Use

Bilirubin (Jendrassik & Groff (T&D))

Cat no.	size
1102 101	265 ml

INTENDED USE

Bilirubin Reagent is intended for in vitro Quantitative diagnostic determination of total and direct bilirubin.

DIAGNOSTIC CHARACTERISTICS

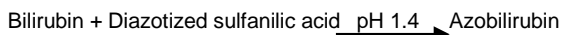
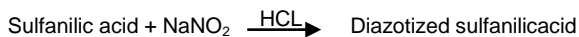
Bilirubin is a waste product derived from the heme moiety of the hemoglobin released from senescent or damaged erythrocytes, that are destroyed in the reticuloendothelial cells. After production, bilirubin is transported to the liver in association with albumin. Inside the hepatocytes bilirubin is conjugated with glucuronic acid and it is excreted into bile. A number of inherited and acquired diseases affect production, uptake, metabolism, and excretion of bilirubin, resulting in hyperbilirubinemia.

Unconjugated hyperbilirubinemia is seen in newborns (physiological jaundice), in increased red cell destruction (hemolytic anemia, extensive hematomata), in ineffective erythropoiesis and in some rare genetic diseases (Gilbert's syndrome, Crigler-Najjar syndrome). Conjugated hyperbilirubinemia is associated to a decreased excretion of bile due to liver diseases (hepatitis or cirrhosis) or to intrahepatic or extra hepatic cholestasis.

Jaundice is a clinical manifestation of hyperbilirubinemia, consisting of deposition of bile pigments in the skin, resulting in a yellowish staining of the skin and mucous membranes.

PRINCIPLE OF THE METHOD

In the presence of caffeine, the total bilirubin concentration is determined by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin. Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red colored azobilirubin; the color intensity of which measured at 546 nm is proportional to the concentration of the direct bilirubin in the sample.



COMPOSITION

Sulphanilic Reagent (R₁)	Sulphanilic acid	5 mmol/L
	HCL	0.3 N
SOD Nitrite Reagent (R₂)	Sodium nitrite	3 mmol/L
Caffeine Reagent (R₃)	Caffeine	45 mmol/L
	Sodium benzoate	20 mmol/L
Alkaline Reagent (R₄)	Sodium tartarate	25 mmol/L
	Sodium hydroxide	1.5 N

STORAGE

Store at 20-25°C.

Reagents are stable until the expiry date shown on the vial label.

REAGENT PREPARATION

Reagents provided are ready to use.

ADDITIONAL EQUIPMENT

Analyzer, spectrophotometer or photometer able to read at 578nm and 546nm.

SPECIMEN

1. Serum collected by standard procedures.

2. Bilirubin in serum is stable for 2 days at 2-8°C if serum is protected from light.

3. Discard contaminated specimens.

Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

Symbols in Product Labeling

EC REP	Authorized Representative		Expiration date
IVD	For in-vitro diagnostic use		CAUTION, consult instructions for use
REF	Catalogue number		Manufactured by
LOT	Lot number		

PROCEDURE:

Total Bilirubin		
	Serum Blank	Sample
Sulphanilic Reagent (R₁)	200 µL	200µL
Sod Nitrite Reagent (R₂)	-	1 drop
Caffeine Reagent (R₃)	1.0 mL	1.0 mL
Sample	200 µL	200 µL
Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C), then add		
Alkaline Reagent (R₄)	1.0 ml	1.0 ml
Mix and incubate for 5 minutes at 20 – 25°C. Measure the absorbance (A) of the Sample at 578 nm against the sample blank. The color is stable for at least 30 minutes .		

Direct Bilirubin		
	Serum Blank	Sample
Sulphanilic Reagent (R₁)	200 µL	200µL
Sod Nitrite Reagent (R₂)	-	1 drop
Saline 0.9% NaCl	2.0 ml	2.0 ml
Sample	200 µL	200 µL
Mix and incubate for exactly 5 minutes at 20 – 25°C. Measure absorbance of sample (A sample) against sample blank at 546 nm.		

CALCULATIONS

The Bilirubin concentration in the sample is calculated using the following general formula:

$$\text{Total bilirubin (mg/dl)} = (\text{A sample} - \text{A sample blank}) \times 10.8$$

$$\text{Direct bilirubin (mg/dl)} = (\text{A sample} - \text{A sample blank}) \times 14.4$$

REFERENCE VALUES

Total Bilirubin	Up to 1.0 mg/dL = 17.0 µmol/L
Direct Bilirubin	Up to 0.2 mg/dL = 3.4 µmol/L

QUALITY CONTROL

It is recommended to use the Control Serum level I and II to verify the performance of the measurement procedure.

METROLOGICAL CHARACTERISTICS

- Detection limit: (Total Bilirubin): 0.03 mg/dL

(Direct Bilirubin): 0.02 mg/dL

- Linearity limit: 20.0 mg/dL.

- Repeatability (within run):

Total Bilirubin			Direct Bilirubin		
Mean Concentration	cv	n	Mean Concentration	cv	n
0.59 mg/dL	3 %	20	0.77 mg/dL	1.2 %	20
6.74 mg/dL	1%	20	1.36 mg/dL	0.5%	20

- Reproducibility (run to run):

Total Bilirubin			Direct Bilirubin		
Mean Concentration	cv	n	Mean Concentration	cv	n
0.59 mg/dL	3.6 %	20	0.77 mg/dL	2.3 %	20
6.74 mg/dL	3.3%	20	1.36 mg/dL	0.9%	20

INTERFERENCES

Hemoglobin (10 g/L) does not interfere.

Lipemia (triglycerides > 15 g/L) may interfere.

Other drugs and substances may interfere

BIBLIOGRAPHY

1. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.