OBio...
Supporting Care to Cure
A Division of Orchard Medical

Bilirubin Total

Order Information

Cat. No.	Kit Configuration
OMR1044	Reagent 1: 2 x 40 mL
	Reagent 2: 1 x 10 mL

Summary

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucoronic acid and the resulting water soluble bilirubin glucoronides are excreted via the bile ducts. Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60-70% of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation. Common bilirubin methods detect either total bilirubin or direct bilirubin. Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Unconjugated bilirubin can therefore be estimated as the difference between total bilirubin and direct bilirubin.

Method

Modified DMSO method

Principle

Sulfanilic acid reacts with sodium nitrite to produce diazotized sulfanilic acid (diazo). Direct and indirect bilirubin couple with diazo to produce azobilirubin in the presence of dimethyl sulfoxide (DMSO). The intensity of the color produced is directly proportional to the amount of total bilirubin concentration present in the sample.

Reagents

Storage Instructions and Reagent Stability
Reagent up to the end of the indicated month of expiry, if stored at 2° – 30°C, protected from light and contamination is avoided. Do not freeze the reagents!

Reagent 1: DMSO
Solution Reagent 2:
Nitrite Solution

Composition

Sulphanilic acid 7 gm/L, Dimethyl sulphoxide 270 ml/L, Conc. HCL 270 ml/L, Sodium Nitrite 7.0 g/L.

Warning and Precautions

1. Keep out of reach of children. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
2. Take off immediately all contaminated clothing.
3. Wear suitable gloves and eye/face protection.
4. Always use safety pipettes to pull the reagents into a pipette.
5. Reagents may contain some non-reactive and preservative components. It is suggested to handle carefully, avoid direct contact with skin and do not swallow.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin, plasma or EDTA plasma separate at the latest 1h after blood collection from cellular contents.
Stability in
plasma/Serum 4 days
at 2°–8°C
30 days at –20°C

Assay Procedure

Wavelength	546nm
Opticalpath	1 cm
Temperature	37°C
Measurement	Against sample blank

Total Bilirubin

	Sample Blank	Test
Reagent 1	1000 µL	1000 µL
Reagent 2	---	50 µL
Sample	50 µL	50 µL
Mix, Incubate for 5 min. at 37˚C. Read absorbance against sample blank.		

Calculation

Note: Take ΔA (sample) and multiply by the corresponding factor from below:
ΔA (sample) = Sample Test (A2) – Sample Blank
(A1) Bilirubin (Total) mg/dL = ΔA (sample) x factor (21)

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in controlrecovery.

Performance Characteristics and Measuring Range

The test has been developed to determine bilirubin within a measuring range from 0.5-30 mg/dL. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

Specificity/Interferences

No interference was observed by triglycerides up to 800 mg/dL.

Sensitivity/Limit of Detection

The lower limit of detection is 0.5 mg/dL.

Linearity

The higher limit of detection is 30 mg/dL.

Precision

Total Bilirubin

Intra-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	1.59	0.05	2.91
Sample 2	4.09	0.07	1.79

Inter-assay n=20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample1	1.42	0.13	9.47
Sample 2	3.93	0.10	2.43

Method Comparison

A comparison of Nucleus Diagnosys Bilirubin (y) with a commercially available test (x) using 15 samples gave following results:

$y = 0.996x + 0.077$; $r^2 = 0.996$

Reference Range

Total Bilirubin		mg/dL
Neonates	24hr.	<8.8
	2nd day	1.3-11.3
	3rd day	0.7-12.7
	4th-6th day	0.1-12.6
Children	>1 month	0.2-1.0
Adults		0.1-1.2

Each laboratory should check if the references range are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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5. **Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.**

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