Bilirubin (Total)

DiaSystem Scandinavia AB

Diagnostic reagent for quantitative in vitro determination of total bilirubin in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size						
01 00009 70 04 0125	R1	5 x	20 mL	+	R2	1 x	25 mL
01 00009 70 04 0500	R1	5 x	80 mL	+	R2	1 x	100mL
01 00009 70 10 0180	R1	4 x	36 mL	+	R2	4 x	9 mL
01 00009 70 02 0180	R1	4 x	36 mL	+	R2	4x	9 mL
CDT-Bil T	R1	3 x	30 mL	+	R2	2 x	11.3 mL

Summary [1,2]

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) hyper-bilirubinemia 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

Photometric test using 2,4-dichloroaniline (DCA)

Principle

In acidic solution, direct bilirubin forms a red colored azocompound with diazotized 2,4-dichloroaniline. A specific mixture of detergents enables a safe determination of the total bilirubin.

Reagents

Components and Concentrations

R1:	Phosphate buffer	50 mmol/L
	NaCl	150 mmol/L
R2:	2,4-Dichlorophenyl-diazonium salt	5 mmol/L
	HCl	130 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and contamination is avoided. Do not freeze the reagents! Reagent 2 must be protected from light!

Warnings and Precautions

1. Reagent 1 and 2: Warning. H290 May be corrosive to metals. H319 Causes serious eye irritation. P234 Keep only in original container. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention. P390 Absorb spillage to prevent material damage.
2. Reagent 2: P264 Wash hands and face thoroughly after handling.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Eltrombopag medication leads to falsely low or high results in patient samples
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum or heparin plasma
It is very important to store the sample protected from light!

Stability [3]:	1 day	at	20 - 25 °C
	7 days	at	4 - 8 °C
	6 months	at	-20 °C

If frozen immediately! Freeze only once!
Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	546 nm (540 - 560 nm)
Optical path	1 cm
Temperature	20 – 25 °C/37 °C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	25 µL
Dist. Water	25 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for 5 min at 37 °C or 10 min at 20 - 25 °C, read absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for 5 min at 37 °C, or 10 min at 20 - 25 °C, then read absorbance A2.		

ΔA = (A2 – A1) sample or calibrator

Calculation

With calibrator

$$Bilirubin\ [mg/dL] = \frac{\Delta A\ Sample}{\Delta A\ Cal.} \times Conc.\ Cal.\ [mg/dL]$$

Conversion factor

Bilirubin [mg/dL] x 17.1 = Bilirubin [µmol/L]

Bilirubin (Total)

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Calibrators and Controls

For the calibration of automated photometric systems the DiaSystem UniCal CC calibrator is recommended. The assigned calibrator values for total bilirubin have been made traceable to the NIST SRM 916 reference material. For internal quality control DiaSystem UniLab P and UniLab N controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 30 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, hemoglobin up to 500 mg/dL, naproxen up to 1 mmol/L and lipemia up to 2000 mg/dL triglycerides when measured using a triglyceride concentrate and up to 1000 mg/dL triglycerides when measured using Intralipid. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 0.07 mg/dL.

Precision (at 37 °C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.89	0.03	3.05
Sample 2	1.02	0.02	2.32
Sample 3	4.83	0.05	0.95

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.87	0.02	2.74
Sample 2	1.15	0.04	3.49
Sample 3	4.65	0.13	2.86

Method Comparison

A comparison of DiaSystem Bilirubin (Total) (y) with a commercially available test (x) using 247 samples gave following results: y = 1.003 x - 0.001 mg/dL; r = 1.000.

Reference Range [1]

		[mg/L]	[µmol/L]
Neonates	24 h	< 8.8	< 150
	2nd day	1.3 – 11.3	22 - 193
	3rd day	0.7 – 12.7	12 - 217
	4th – 6th day	0.1 – 12.6	1.7 - 216
Children	>1 month	0.2 – 1.0	3.4 - 17
Adults		0.1 – 1.2	1.7 - 21

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

Literature

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998. p 192-202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
4. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962; 6: 570-8.
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.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

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