Bilirubin Auto Direct FS*

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

Order Information

Cat. No.	Kit size						
1 0821 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 0821 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 0821 99 10 023	R1	1 x	800 mL	+	R2	1 x	200 mL
1 0821 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL
1 0821 99 10 930	R1	4 x	20 mL	+	R2	2 x	10 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 glucoronic acid is 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. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin.

Method

Photometric test using 2,4-dichloroaniline (DCA)

Principle

Direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution.

Reagents

Components and Concentrations

R1:	EDTA-Na_2	0.1 mmol/L
	NaCl	150 mmol/L
	Sulfamic acid	100 mmol/L
R2:	2,4-Dichlorophenyl-diazonium salt	0.5 mmol/L
	HCl	900 mmol/L
	EDTA-Na_2	0.13 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.

Warning and Precautions

1. Reagents: Warning. H290 May be corrosive to metals. P234 Keep only in original container. P390 Absorb spillage to prevent material damage.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Eltrombopag medication leads to falsely low or high results in patient samples.
4. 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.
5. 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]: 2 days at 20 – 25°C
7 days at 4 – 8°C
6 months at –20°C
in case of immediate freezing.
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	-	50 µL
Dist. Water	50 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for 3 – 5 min. at 20 – 25°C/37°C, read absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for exactly 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

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

Conversion factor

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

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method has been standardized against the manual Jendrassik-Gróf test. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 X 5 mL
	5 9000 99 10 061	6 X 5 mL
TruLab P	5 9050 99 10 062	20 X 5 mL
	5 9050 99 10 061	6 X 5 mL

* fluid stable

Performance Characteristics

Measuring range

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 10 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, naproxen up to 1 mmol/L and lipemia up to 1000 mg/dL triglycerides. Interference by hemoglobin occurs starting at hemoglobin concentrations of 50 mg/dL.
For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 0.1 mg/dL.

Precision (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.36	0.01	3.12
Sample 2	0.76	0.01	1.46
Sample 3	2.07	0.03	1.30

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.35	0.01	3.34
Sample 2	0.75	0.01	1.00
Sample 3	2.13	0.02	0.71

Method Comparison

A comparison of DiaSys Bilirubin Auto Direct FS (y) with a commercially available test (x) using 85 samples gave following results: y = 0.95 x + 0.04 mg/dL; r = 0.995

Reference Range [1]

Adults and children ≤ 0.2 mg/dL (≤ 3.4 µmol/L)

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.

Manufacturer

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