Bilirubin Auto Direct FS

Bilirubin Auto Direct Reagent Test Kit

DiaSys Diagnostic Systems

Intended Use

Diagnostic reagent for in vitro quantitative determination of direct bilirubin in Human serum or plasma on photometric analysers.

Order Information

Item Code	Pack Size
108219934840	R1 : 4 x 60 mL; R2 : 4 x 15 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 t he 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 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

Principle

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

Reagents

Components and Concentrations

R1	Hydrochloric Acid	15ml/L
	Sulphanilic Acid	7g/L
R2	Sodium Nitrite	5g/L
Preservatives & Stabilizers		q.s.

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. May be corrosive to metals. Keep only in original container. Absorb spillage to prevent material damage.
2. In very r are c ases, samples of patients with gammopathy might give falsified results [6].
3. 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.
4. 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
TruCal U (591009910064)
TruLab N (590009910061)
TruLab P (590509910061)

Specimen

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.		

$\Delta A = (A2 - A1)$ 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 the DiaSys TruCal U calibrator is recommended. This method has been standardized against the manual Jendrassik-Gróf test. For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

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)
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

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.33	0.01	2.28
Sample 2	1.16	0.02	1.72
Sample 3	1.37	0.02	1.09

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.75	0.02	2.18
Sample 2	1.41	0.03	1.82
Sample 3	0.60	0.01	1.50

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.

Result Interpretation
High bilirubin results can be observed in neonatal jaundice, hyperbilirubinemia. It is important to take advice with doctor for result interpretation.Results should be correlated clinically.

Limitations
Result interreference can be seen for the patient sample results with concentration of Triglyceride above 1000 mg/dL

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 Tex tbook of Clinical Chemis try. 3 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 n ew diazo me thod for t he 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.

Notes on Symbols and Marks

	Consult instruction for use
	Use-by date
LOT	Batch code
REF	Catalogue number
	Caution
	Manufacturer
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CONT.	The pack contains
	Recycle
	Date of manufacture

ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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