Bilirubin Auto Direct FS*

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

Intended Use

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

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 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 postpartum 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. [1,2]

Method

Photometric test using 2,4-dichloroaniline (DCA)

Direct bilirubin in presence of diazotized 2,4-dichloroaniline forms a red colored azocompound in acidic solution. [3]

Reagents

Components and Concentrations

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

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

Warnings and Precautions

1. ⚠ Reagent 1 and 2: Warning. H290 May be corrosive to metals. P234 Keep only in original packaging. P390 Absorb spillage to prevent material damage.
2. In very rare cases, samples of patients with gammopathy might give falsified results [4].
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

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma

Protect sample from light.

Stability [5]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
6 months	at	–20°C

in case of immediate freezing.

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	545/658 nm
Temperature	37°C
Measurement	Endpoint
Sample/Calibrator	3.5 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 17/18 (230/244 s)
Absorbance 2	Cycle 41/42 (586/600 s)
Calibration	Linear

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

DiaSys TruCal U is recommended for calibration. Calibrator values have been made traceable to the manual Jendrassik-Gróf test. Use DiaSys TruLab N and P 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

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 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.	
Limit of detection**	0.01 mg/dL

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	30 mg/dL
Hemoglobin interferes at low concentrations.	
Lipemia (triglycerides)	600 mg/dL
For further information on interfering substances refer to Young DS [6,7].	

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	0.25	1.52	2.90
CV [%]	2.79	1.55	1.96
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	0.85	2.20	2.35
CV [%]	2.49	1.86	1.63

Method comparison (n=109)	
Test x	DiaSys Bilirubin Auto Direct FS (Hitachi 917)
Test y	DiaSys Bilirubin Auto Direct FS (BioMajesty® JCA-BM6010/C)
Slope	1.02
Intercept	–0.004 mg/dL
Coefficient of correlation	0.999

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

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

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* Fluid Stable