

# Bilirubin-Direct

## DC-Test (5 + 1 - Reagent)



Cat.No	Package Size
113 116	7x10 mL / 2 x 8 mL
113 100	5 x 20 mL / 2 x 10 mL
113 102	Hit-I 4 x 50 mL / 2 x 20 mL
113 103	Hit-II 4 x 100 mL / 4 x 20 mL
113 106	AU 4 x 70 mL / 3 x 20 mL
113 125	LW 5 x 20 mL / 2 x 10 mL

### METHOD / TESTPRINCIPLE

Photometric test with stabilized 2,4-Dichlorophenyldiazoniumsalt ("DC"): Direct Bilirubin reacts with the DC-derivative in acid solution to form a red diazo dye.

### REAGENT COMPOSITION

<b>R1:</b>	EDTA-Na <sub>2</sub>	0,07 mmol/l
	NaCl	6,6 g/l
	Sulfaminic Acid	70 mmol/l
<b>R2:</b>	2,4-Dichlorophenyl-Diazoniumsalt	0,09 mmol/l
	HCl	130 mmol/l
	EDTA-Na <sub>2</sub>	0,02 mmol/l

**Calibrator(Cal) :** Use Greiner Multicalibrator

### PRECAUTIONS

- For in vitro diagnostic use only.
- Avoid direct exposure to light.
- Possible interferences with protein on surfaces of analyzer tubes can be avoided by rinsing with 0.1 N NaOH solution.
- Avoid contamination by using clean laboratory material (pipette, plastic vial for analyzers)

### STABILITY OF REAGENTS

When stored at 2-8° C and protected from light, the reagents are stable up to the expiry date stated on the labels.

### PREPARATION AND STABILITY OF WORKING REAGENTS

R1 and R2 are ready for use  
Stability after opening  
3 months at 2 – 8°C  
4 weeks at room temp

### SAMPLES

Serum free of hemolysis.  
Heparin or EDTA plasma.  
(Bilirubine is very light sensitive : Protect sample material from light !)

### REFERENCE VALUES

	[mg/dL]	[µmol/L]
Children and Adults	< 0,2	< 3,4

*Note: It is recommended for each laboratory to establish and maintain its own reference values. The given data are only an indication.*

### PROCEDURE

This reagent can be used manually (see method below) and on most analyzers. Applications are available on request.

**Wavelength :** 546 nm (540-560)  
**Temperature :** 37°C  
**Cuvette :** 1 cm light path  
**Read against reagent blank (RB)**

	Reagent Blank	Sample/Standard
<b>Sample/Standard</b>	-	100 µL
<b>Dist.Water</b>	100 µL	-
<b>Reagent 1</b>	1000 µL	1000 µL
Mix, incubate for 3 – 5 min read absorbance A <sub>1</sub> , then add		
<b>Reagent 2</b>	200 µl	200 µL
Mix, incubate for exactly 5 min and read A <sub>2</sub>		

$$\Delta A = [(A_2 - A_1) \text{ Sample/Calibrator}] - [(A_2 - A_1) \text{ RB}]$$

### CALCULATION

With calibrator :

$$\text{Bilirubin Direct [mg / dl]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times C \text{ [mg / dl]}$$

**C = Concentration Calibrator**

### CALIBRATORS & CONTROLS

For the calibration of automated analyzers Greiner Multicalibrator is recommended, for quality control use Greiner normal and abnormal control, Unitrol I and Unitrol II, for direct bilirubin the special bilirubin control .

## PERFORMANCE DATA (37°C)

### - Analytical range

The reagent is linear up to 10 mg/dL .

### - Detection limit

The detection limit is equal to 0,1 mg/dL

### - Precision

Within-run reproducibility

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

Between-run reproducibility

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

### - Correlation

A comparative study has been performed between the Greiner method and another commercial reagent on 85 human serum samples. The parameters of linear regression are as follows:

$$Y = 0.95 x - 0,04 \text{ mg/dl} \quad R = 0.995$$

## INTERFERENCES

Interferences are found according to literature.

For the manual method (with sample blanc) and the automated method ( two point method) interferences are eliminated

## BIBLIOGRAPHY

1. Thomas L ed. Clinical Laboratory Diagnostics. 1<sup>st</sup> 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. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
3. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962;6:570-8.

## SYMBOLS USED

IVD

For *in vitro* diagnostic medical use

LOT

Batch Code



Use by



Temperature limitation