Bilirubin

DC-Test (5 + 1 - Reagent)

Cat.No	Package Size
112 216	7 x10 mL / 2 x 8 mL
112 200	5 x 20 mL / 2 x 10 mL
112 202 (Hit I)	4 x 50 mL / 2 x 20 mL
112 203 (Hit II)	4 x 100 mL / 4 x 20 mL
112 206 (AU)	4 x 70 mL / 3 x 20 mL
112 225 (LW)	5 x 20 mL / 2 x 10 mL

METHOD / TESTPRINCIPLE

Photometric test with stabilized 2,4-Dichlorophenyldiazoniumsalt ("DC") :
Bilirubin forms with DC-derivative in acidic solution a red diazo dye. Bound bilirubin is released by detergents.

REAGENT COMPOSITION

R1:	Phosphate-Buffer	40 mmol/L
	NaCl	9 g/L
	Detergent, Stabilisors	
R2:	2,4-Dichlorophenyldiazoniumsalt	0.09 mmol/L
	HCl	30 mmol/L
	Detergent, Stabilisors	

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]
Newborns	24 h	< 8,8	< 150
	2. Day	1,3 - 11,3	22 - 193
	3. Day	0,7 - 12,7	12 - 217
	4. – 6. 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

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 also manually (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/ Calibrator
Sample/Calibrator	-	20 µL
Reagent 1	1000 µL	1000 µL
Mix, incubate for 3 – 5 min , read absorbance A_1 , then add		
Reagent 2	200 µl	200 µL
Mix, incubate for exactly 5 min and read A_2		

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

CALCULATION

With calibrator

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

C = concentration calibrator

CALIBRATORS & CONTROLS

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

Greiner Diagnostic GmbH - Unter Gereuth 10 **–** D-79353 Bahlingen – Germany
www.greiner-diagnostic.com

PERFORMANCE DATA (37°C)

- Analytical range
The reagent is linear up to 30 mg/dL .

- Detection limit
The detection limit is equal to 0,01 mg/dL

- Precision
Within-run reproducibility
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

Between-run reproducibility
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

- Correlation
A comparative study has been performed between the Greiner method and another commercial reagent on 247 human serum samples. The parameters of linear regression are as follows:

y = 1.00 x - 0,01 mg/dl r = 1.000

INTERFERENCES

Interferences are found according to literature.

For the manual method (with sample blanc) and the automated method (two point method), interferences are eliminated through the measuring technique.

BIBLIOGRAPHY

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. Rand RN, di Pasqua A. A new diazo method for the dtermination of bilirubin. Clin Chem 1962;6:570-8.

SYMBOLS USED

For *in vitro* diagnostic medical use

Batch Code


Use by


Temperature limitation

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