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CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

REF

Bilirubin Total – Instructions for use

R3330000011 R1: 2 x 18 mL
R2: 2 x 4,5 mL

CE IVD

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

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, DCA
Wavelength:	546 nm (540 – 560 nm)
Temperature:	20 – 25 °C or 37 °C
Sample:	Serum, heparin plasma
Linearity:	up to 30 mg/dL
Sensitivity:	The lower limit of detection is 0.07 mg/dL

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
Reagent 1	
Phosphate buffer	50 mmol/L
NaCl	150 mmol/L
Reagent 2	
2,4-Dichlorophenyl-diazonium salt	5 mmol/L
HCl	130 mmol/L

SUMMARY [1,2]

Bilirubin is a breakdown product of haemoglobin. 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 glucoroniedes are 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) hyperbilirubinea 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.

TEST PRINCIPLE

In acidic solution, Bilirubin reacts with diazotized 2,4-dichloroaniline (DCA) to form a red colored azocompound. A specific mixture of detergents enables a safe determination of the total bilirubin.

REAGENT PREPARATION

Substrate Start:
The reagents are ready to use.
Sample Start:
Not possible.

REAGENT STABILITY AND STORAGE

Conditions:	Avoid contamination. Close immediately after use. Reagent 2 must be protected from light! Do not freeze the reagents.
Storage:	at 2 – 8 °C
Stability:	up to the expiration date

SAMPLE STABILITY AND STORAGE

It is very important to store the sample protected from light!

Stability [3]:	at 20 – 25 °C	1 day
	at 4 – 8 °C	7 days
	at - 20 °C *	6 months

* if frozen immediately!. Freeze only once!
Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.
Substrate Start:

Pipette into test tubes	Blank	Calibr.	Sample
Reagent 1	1000 µL	1000 µL	1000 µL
Sample	-	-	25 µL
Calibrator	-	25 µL	-
Mix. Incubate for 5 min. at 37 °C or 10 min. at 20 – 25 °C and read absorbance A1 against reagent blank. Then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix. Incubate for 5 min. at 37°C or 10 min. at 20 – 25 °C and read absorbance A2 against reagent blank. Calculate: ΔA = A2 – A1.			

CALCULATION

$$\text{Bilirubin [mg/dL]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times \text{conc. of cal. [mg/dL]}$$

UNIT CONVERSION

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

REFERENCE RANGE [1] *

		[mg/dL]	[µmol/L]
Neonates	24 h	< 8.8	< 150
	2nd day	1.3 - 11.3	22 – 193
	3rd day	0.7 - 12.7	12 – 217
	4th – 6th 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

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 30 mg/dL. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the results multiplied by 2.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 0.07 mg/dL

PRECISION (at 37°C)

Intra-assay 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

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Sample 3	4.83	0.05	0.95

Inter-assay 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

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	2000 mg/dL
Naproxen	1 mmol/L

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between this Bilirubin Auto Total (y) and a commercially available test (x) using 247 samples gave the following result: y = 1.003 x - 0.001 mg/dL; r= 1.000.

QUALITY CONTROL

All control sera with bilirubin values determined by this method can be used.
We recommend our serum controls Normal control serum (control serum with values in the normal range) and Pathological control serum (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

CALIBRATION

The assay requires the use of a Bilirubin Standard or Calibrator.
We recommend our Multicalibrator. The assigned calibrator values for total bilirubin have been made traceable to the NIST SRM 916 reference material.

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. Reagent 1 and 2: Warning.
   H290: May be corrosive to metals.
   H319: Causes serious eye irritation.
   P234: Keep only in original container.
   P280: Wear protective gloves/protective clothing/eye protection/face protection.
   P305+P351+P338: If in eyes: Rinse cautiously with water for seeral minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
   P337+P313: If eye irritation persists: Get medical advice/attention.
   P390: Absorb spillage to prevent material damage.
2. Reagent 2:
   P264: Wash hands and face thoroughly after handling.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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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 interferene in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

Symbols used on labels and packaging

IVD = In vitro diagnostic medical device
REF = Catalogue Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

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Revision A R3330000011 11-2018