DIAGNOSTICA PRODUCTS

Liquid Reagents – ready to use

BILIRUBIN DIRECT

DCA WITH ATCS

2 Reagents

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

Ref.No.	Kit Size	Content
DIA010281	62.5 ml	2x25 ml R1 + 1x12.5 ml R2
DIA010282	5 x 20 ml	4x20 ml R1 + 1x20 ml R2
DIA010283	5 x 25 ml	4x25 ml R1 + 1x25 ml R2
DIA010284	5 x 50 ml	4x50 ml R1 + 1x50 ml R2

Additionally offered:

DIA040012	1 x 3 mL	**Diacal Auto** (Calibrator)
DIA030012	1 x 5 mL	**Diacon N** (Control Normal)
DIA030022	1 x 5 mL	**Diacon P**(Control Abnormal)

*** Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia**

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 10 mg/dL

Sensitivity: The lower limit of detection is 0.1 mg/dL

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 increasedbilirubin production due to hemolysis (pre-hepaticjaundice), by parenchymaldamages of the liver (intra-hepaticjaundice) or by occlusion of bile ducts (post-hepaticjaundice). A chroniccongenital (predominantlyunconjugated) hyperbilirubineacalledGilbert'ssyndromeisquitefrequent in the population. High levels of totalbilirubin are observed in 60 – 70 % of neonates due to an increasedpostpartal breakdown of erythrocytes and because ofdelayedfunction of enzymes for bilirubindegradation.

TEST PRINCIPLE

Direct Bilirubin reacts with diazotized 2,4-dichloroaniline (DCA) to form a red colored azocompound in acidic solution.
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.

REAGENT PREPARATION

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

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1		
EDTA-Na_2	0.1	mmol/L
NaCl	150	mmol/L
Sulfamicacid	100	mmol/L
Reagent 2		
2,4-Dichlorophenyl-diazoniumsalt	0.5	mmol/L
HCl	900	mmol/L
EDTA-Na_2	0.13	mmol/L

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
*in case of immediate freezing. 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	-	-	50 µL
Calibrator	-	50 µL	-
Dist. water	50 µL	-	-
Mix. Incubate for 3 – 5 min. (20 – 25 °C / 37 °C) and read absorbance A1 against reagent blank. Then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix. Incubate for 5 min. (37°C) or 10 min. (20 – 25 °C) and read absorbance A2 against reagent blank. Calculate: ΔA = A2 – A1.			

CALCULATION

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

UNIT CONVERSION

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

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.

PERFORMANCE CHARACTERISTICS

LINEARITY, 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 results multiplied by 2.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.1 mg/dL

PRECISION (at 37°C)

Intra-assay 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

Inter-assay 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

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SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Hemoglobin	50 mg/dL
Triglycerides	1000 mg/dL
Naproxen	1 mmol/L

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

METHOD COMPARISON

A comparison between Diagnostica Bilirubin Auto Direct (y) and a commercially available test (x) using 85 samples gave following results: y = 0.95 x + 0.04 mg/dL; r= 0.995.

CALIBRATION

The assay requires the use of a Bilirubin Standard or Calibrator.
We recommend the Diagnostica multi calibration serum **Diacal Auto**.
This method has been standardized against the manual Jendrassik-Gróf test.

QUALITY CONTROL

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

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.
   P234: Keep only in original container.
   P390: Absorb spillage to prevent material damage.
2. In very rare cases, 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.
4. 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

Please refer to local legal requirements.

REFERENCES

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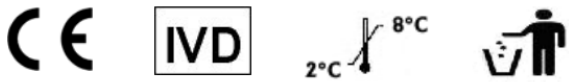

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