



BILIRUBIN Direct DCA

Colorimetric method with dichloroaniline (DCA) for the quantitative determination of Direct Bilirubin in plasma or serum



ORDER INFORMATION

REF	Kit size
GA4245 00	4x50 + 1x50 ml
KL4245 00	2x40 + 2x10 ml
BK4245 00	2x(60+15 ml)

INDICATION

Determination of total and direct bilirubin is used for the diagnosis and monitoring of hepatic (hepatitis, cirrhosis) and hemolytic and biliary disorders.

In particular, high levels of direct bilirubin (or "conjugated" bilirubin) that are generally absent or present only in trascurable quantities, are rilevable in the following cases:

- extra hepatic biliar disorders (for example gallbladder and choledochal calculi, pancreas tumor)
- inter hepatic biliar disorders (for example cirrhosis, hepatitis and liver tumor).

METHOD PRINCIPLE

Direct Bilirubin present in the sample reacts with diazotized Dichloroaniline to form a coloured azocomplex whose intensity at 546 nm (540-560 nm) is directly proportional to the analyte concentration.

COMPOSITION

REAGENT A:

EDTA-Na ₂	0.07 mmol/l
NaCl	6.6 g/l
Sulfamine acid	70 mmol/l

REAGENT B:

2.4-Dichlorophenyldiazoniumsalt	0.09 mmol/l
HCl	130 mmol/l
EDTA-Na ₂	0.02 mmol/l

Preparation

The reagents are liquids, ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l
- Calibrator (GD CAL Ref. GD8577 00)

SAMPLES

Serum, eparine or EDTA plasma. Do not use contaminated samples. It is very important to store the sample protected from light.

Stability:

at 15 - 25 °C:	1 day
at 2 - 8 °C:	7 days
at - 20 °C:	3 months

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known direct bilirubin concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	20-25 °C or 37 °C
Wavelength	546 nm (540-560) nm
Optical path	1 cm
Reaction	End point (increase)

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Calibrator	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Calibrator	-	100 µl	-
Sample	-	-	100 µl

Mix and incubate for 3-5 minutes (37 °C/20-25 °C) and read absorbance A₁ for each cuvette against Blank.

Then add:

Reagent B	250 µl	250 µl	250 µl
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Mix and incubate for 3-5 minutes (37 °C/20-25 °C) and read absorbance A₂ for each cuvette against Blank.

Calculate $\Delta A = A_2 - A_1$

Note:

- Reaction volumes can be proportionally changed.
- For concentration > 10 mg/dl, the sample should be diluted with NaCl solution (0.9 g/l) and result multiplied by utilized dilution factor.

CALCULATION OF RESULTS

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

Conversion factor:

$$\text{Direct Bilirubin [mg/dl]} \times 17.1 = \text{Direct Bilirubin } [\mu\text{mol/l}]$$

REFERENCE VALUES

Up to 0.25 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different direct bilirubin concentrations. The obtained results are reported in the following tables:

Within Run n =20	Mean (mg/dl)	SD (mg/dl)	%CV
Sample 1	0.34	0.01	3.24
Sample 2	0.73	0.01	1.51
Sample 3	2.05	0.03	1.27

Between Run n =20	Mean (mg/dl)	SD (mg/dl)	%CV
Sample 1	0.33	0.01	3.33
Sample 2	0.72	0.01	0.97
Sample 3	2.10	0.02	0.71

Linearity

The assay is linear up to 10 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.1 mg/dl.

Correlation

A correlation study comparing the present method and a commercial one gave the following results:

$$y = 0.95x + 0.04 \text{ mg/dl} \quad r = 0.995$$

Interferences

Hemoglobin > 50 mg/dl
Ascorbic acid > 30 mg/dl
Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentrations of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

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2. Henry, J, Cannon, D.C, Winkelmann, J.V. Clinical Chemistry, Principles and Tecnics, Verlag Chemie 1042 (1974).
3. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
4. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC