BIOLABO
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DIRECT BILIRUBIN
DCA method

Reagent for quantitative determination of direct bilirubin in human serum or plasma

REF 97553	**R1**	2 x 200 mL	Direct Bilirubin
	R2	1 x 100 mL	Nitrite Solution

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (**DB**) consists of mono and diconjugated bilirubin (β and γ-Bilirubin) and the δ-fraction which is bilirubin tightly bound to albumin; unconjugated α-bilirubin which is water soluble and bound to albumin. **Total bilirubin (TB)** is the sum of these different species.
There are icteruses in which unconjugated bilirubin predominates (hemolitic icteruses, Biermer disease, Thalassemia...); icteruses in which conjugated bilirubin predominates (extra or intra-hepatic bile ducts obstruction, viral hepatitis...); finally, icteruses in which both species of bilirubin are present without any predominance (cirrhosis, Dubin-Johnson disease).

PRINCIPLE (4) (5)

Method based on Rand and Di Pasqua principle automated by Golub and al.
The reaction between DB and diazotised dichloroaniline leads to a compound, azobilirubin which absorbance, directly proportional to the concentration of DB in the specimen, is measured at 550 nm (540-560).

REAGENTS

Vial R1 **DIRECT BILIRUBIN**

NaCl	7.2	g/L
Sulfanilic Acid	72	mmol/L
EDTA	756	µmol/L

Vial R2 **NITRITE SOLUTION**

2-4 Dichloroanilin	125	µmol/L
Sodium Nitrite	125	µmol/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENT PREPARATION

Reagent R1 is ready for use.

Preparation of the working reagent: Mix 4 volumes of vial R1 with 1 volume of vial R2.

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8° C.

- Reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert and free from contamination.
- When free from contamination, working reagent is stable for 5 days at 18-25°C and 3 weeks at 2-8°C.
- Discard any reagent if cloudy or if absorbance of the working reagent is > 0.100 at 550 nm.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2) (6)

Unhemolysed serum or plasma.
Bilirubin is photolabile. Store the specimen away from light.

- Stability in the specimen: 4 to 7 days at 2-8° C.
  2 days at room temperature.

Icteric or paediatric specimens: see § **MANUAL PROCEDURE**.

INTERFERENCES (3) (4)

The bilirubin diazo reaction is temperature sensitive and should be carried out at a constant temperature.
Ascorbic acid: No interference up to 25 mg/dL.
Glucose: No interference up to 1200 mg/dL
Triglycerides: No significant interference up to 3.9 µmol/L.
Haemoglobin: No significant interference up to 250 µmol/L.
Above, haemolysis leads to under-estimation.
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION (7)

- Use the experimental factor indicated § **CALCULATION** (Procedure n°1 and n°2)
- Or BIOLABO Multicalibrator REF 95015 traceable to SRM 916a (Procedure n°1 only)
- or a calibrator traceable to a reference method or material (Procedure n°1 only).

The calibration frequency depends on proper instrument functions and on preservation of reagents.
It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of ranges, even after using a new vial of fresh serum

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- BIOLABO PAEDIATRIC CONTROL REF 95403
- Assayed control sera referring to the same method and to the selected procedure.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Direct Bilirubin	mg/dL	[µmol/L]
Adult (and children over 5 days)	< 0.2	[< 3.4]

Each laboratory should establish its own normal ranges for the population that it serves.

LINEARITY

Procedure n°1: up to 20 mg/dL (342 µmol/L).

Above, **do not dilute the specimen:** perform procedure n°2.

Procedure n°2: up to 100 mg/dL (1710 µmol/L)

Paediatric specimen: perform procedure n°2

PERFORMANCES CHARACTERISTICS (PROCEDURE N°1)

Within run: N = 20	*Medium level*	*High level*
Mean mg/dL	1.13	2.54
S.D. mg/dL:	0.019	0.029
C.V. %:	1.68	1.15

Between run N = 20	*Medium level*	*High level*
Mean mg/dL	0.74	1.75
S.D.mg/dL:	0.017	0.032
C.V. %:	2.3	1.84

Detection limit: approximately 0.1 mg/dL

Sensitivity for 1 mg/dL: 70 mAbs at 550 nm.

Comparison studies with commercially available reagent:

y = 1.0084x – 0.527 r = 0.9926

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Procedure n°1:

Pipette into well identified test tubes:	DIRECT BILIRUBIN	
	Blank	**Assay**
Reagent R1	1 mL	
Working Reagent		1 mL
Specimen or calibrator	100 µL	100 µL
Mix and read absorbance at 550 nm (540-560) after 1 minute at 37° C (2 minutes maximum) against blank.		

Procedure n°2: Icteric or Pediatric Specimens

Pipette into well identified test tubes:	DIRECT BILIRUBIN	
	Blank	**Assay**
Reagent R1	1 mL	
Working Reagent		1 mL
Specimen or calibrator	20 µL	20 µL
Mix and read absorbance at 550 nm (540-560) after 1 minute at 37° C (2 minutes maximum) against blank.		

Notes:

1. After 2 minutes, indirect bilirubin reacts slowly with diazotised dichloroanilin and leads to over-estimated values.
2. To take into account the coloration of the working reagent, one should perform a reagent blank.
3. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With a calibrator (Procedure n°1 only):

$$\text{Result} = \frac{\text{Abs (Assay - Blank) specimen}}{\text{Abs (Assay - Blank) calibrator}} \times \text{calibrator concentration}$$

with factor:

Procedure n°1: mg/dL = [Abs. Assay – Abs. Blank] x 14.8*
µmol/L = [Abs. Assay – Abs. Blank] x 253*

Procedure n°2: **mg/dL = [Abs. Assay – Abs. Blank] x 68.6***
µmol /L =[Abs. Assay – Abs. Blank] x 1173*

*These factors should be used as a guide only and may vary with instrument and the batch of reagent used. It is recommended to verify it with elevated control serum.
(Procedure n°1: use BIOLABO EXATROL-P, Procedure n°2: use BIOLABO PAEDIATRIC CONTROL).

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1133-1137.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 172-177*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-90 to 3-110*
(4) *RAND R. N. and DI PASQUA A. A new diazo method for determination of bilirubin. Clin. Chem. (1962) 8, n°6, p. 570-578*
(5) *GOLUB M.: An automated method for determination of serum bilirubin. Clin. Chem. (1964) 10, p. 399-405*
(6) *Henry RJ, Clin Chem: Principles and technics. Harper and Row. p.592(1965)*
(7) *SRM: Standard Reference Material ®*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with