BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

TOTAL BILIRUBIN
DCA method

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

REF 97443	**R1**	2 x 200 mL	Total Bilirubin
	R2	1 x 10 mL	Nitrite Solution

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD USAGE IN VITRO

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 TB and diazotised dichloroaniline leads, when a solvent or a detergent is present, to a compound, azobilirubin wich absorbance, directly proportional to the concentration of TB in the specimen, is measured at 550 nm (540-560).

REAGENTS

Vial R1 **TOTAL BILIRUBIN**

Brij 35	49	g/L
2-4 Dichloroaniline	2.7	mmol/L
Hydrochloric acid	290	mmol/L

Vial R2 **NITRITE SOLUTION**

Sodium Nitrite	2.3	mmol/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.
- For further information, 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 50 volumes of vial R1 with 1 volume of vial R2 **and allow standing at 2-8°C for 15 minutes before use.**

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.
- Once reconstituted, working reagent is stable for 5 days at 18-25°C and 3 weeks at 2-8°C, when free from contamination.
- 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 pediatric specimens: see § **MANUAL PROCEDURE**.

INTERFERENCES (3) (4)

Haemolysis leads to under-estimated results.
The bilirubin diazo reaction is temperature sensitive and should be carried out at a constant temperature.
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 the 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 serum and repeat the test.
3. **With factor:** Verify analysis parameters (Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor).
4. Use a new vial of reagent and repeat the test.
5. **With a calibrator:** If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
6. If control is still out of range, calibrate again with a new vial of reagent and repeat the test.
7. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Total Bilirubin	**mg/dL**		**[µmol/L]**	
Newborn	Premature	Full term	Premature	Full term
In cord	< 2.0	< 2.0	[< 34]	[< 34]
0-1 day	< 8.0	1.4-8.7	[< 137]	[24-149]
1-2 days	< 12.0	3.4-11.5	[< 205]	[58-197]
3-5 days	< 16.0	1.5-12.0	[< 274]	[26-205]

Adult (and child > 5 days)	**Total bilirubin mg/dL**	**[µmol/L]**
>5 days-60 years	0.3-1.2	[5-21]
60-90 years	0.2-1.1	[3-19]
> 90 years	0.2-0.9	[3-15]

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 (PROCEDURE N°1)

Within run N = 20	*Medium level*	*High level*
Mean mg/dL	1.23	5.28
S.D. mg/dL	0.018	0.090
C.V. %	1.5	1.7

Between run N = 20	*Medium level*	*High level*
Mean mg/dL	1.20	5.48
S.D.mg/dL	0.059	0.15
C.V. %	4.87	2.79

Detection limit: approximately 0.1 mg/dL

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

Comparison study with commercially available reagent:

y = 1,0042 x + 0.015 r = 0,9980

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Procedure n°1:

Pipette into well identified test tubes:	**TOTAL BILIRUBIN**	
	Blank	**Assay**
Reagent R1	1 mL	
Working Reagent		1 mL
Specimen	100 µL	100 µL
Mix and read absorbance at 550 nm (540-560) after 5 minutes at 37°C against blank.		

Procedure n°2: Icteric or Pediatric Specimens

Pipette into well identified test tubes:	**TOTAL BILIRUBIN**	
	Blank	**Assay**
Reagent R1	1 mL	
Working Reagent		1 mL
Specimen	20 µL	20 µL
Mix and read absorbance at 550 nm (540-560) after 5 minutes at 37°C against blank.		

Notes:

1. The colour developed by the reaction is stable for 1 hour away from light.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.
3. To take into account the coloration of the working reagent, one should perform a reagent blank

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