

# BioMed-TOTAL BILIRUBIN



## D C A method

**REF:** 2 x 100 mL Total Bilirubin  
1 x10 mL Nitrite Solution

### INTENDED FOR USE:

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

### CLINICAL SIGNIFICANCE (1)

At least four distinct bilirubin species exist in the serum: Direct-reacting bilirubin (DB) consists of mono and diconjugated bilirubin ( and - Bilirubin) and the -fraction which is bilirubin tightly bound to albumin; unconjugated -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 (hemolytic 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 wch absorbance, directly proportional to the concentration of TB in the specimen, is measured at 550 nm (540-560).

### REAGENTS

<b>R1</b>	<b>TOTAL BILIRUBIN</b>
<b>R2</b>	<b>NITRITE SOLUTION</b>

### SAFETY CAUTIONS

- 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 advise.
  - 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

Reagents are ready for 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.
  - Discard any reagent if cloudy or if absorbance of the working reagent is > 0.100 at 550 nm.
  - Don't use 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 at MANUAL PROCEDURE.

### INTERFERENCES (3) (4)

Hemolysis 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)

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

- 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 BIOMED technical support or your local Agent

### EXPECTED VALUES (2)

Total Bilirubin	mg/dL	[Amol/L]			
	Full term	Premature	Full term	Premature	Newborn
Notes :	[< 34]	[< 34]	< 2.0	< 2.0	In cord

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]

Total bilirubin		
Adult (and child > 5 days)	mg/dL	[Amol/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 Mmol/L).

Above, do not dilute the specimen : perform procedure n°2. Procedure n°2 : up to 100 mg/dL (1710 Mmol/L)

Pediatric specimen : perform procedure n°2

## PERFORMANCES (PROCEDURE N°1)

High level	Medium level	Within run N = 20	High level	Medium level	Between run N = 20
5.28	1.23	Mean mg/dL	5.48	1.20	Mean mg/dL
0.090	0.018	S.D. mg/dL	0.15	0.059	S.D. mg/dL
1.7	1.5	C.V. %	2.79	4.87	C.V. %

Detection limit : approximately 0.1 mg/dL Sensitivity for 1 mg/dL : 70 mAbs at 550 nm.  
Comparison study with commercially available reagent :  $y = 1.0042x + 0.015$   $r = 0.9980$

## MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

### Procedure n1:

TOTAL BILIRUBIN		Pipette into well identified test tubes :
Blank	Assay	
1 mL	1 ml	Reagent R1
	50 ul	Reagent R2
100uL	100 uL	Specimen

Mix and read absorbances at 550 nm (540-560) after 5 minutes at 37°C against blank.

### Procedure n 2: Icteric or Pediatric Specimens

TOTAL BILIRUBIN		Pipette into well identified test tubes :
Blank	Assay	
1 mL	1ml	Reagent R1
	50 ul	Reagent R2
20 uL	20 uL	Specimen

Mix and read absorbances at 550 nm (540-560) after 15 minutes at room temperature against blank.

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 BIOMED technical support.

3. To take into account the colration of the working reagent, one should perform a reagent blank

## CALCULATION

Calculate the result as follows :

### With a calibrator (Procedure n1 only) :

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

### with factor :

$$\text{Procedure n°1 : mg/dL} = [\text{Abs. Assay} - \text{Abs. Blank}] \times 11.8^*$$

$$\text{Mmol/L} = [\text{Abs. Assay} - \text{Abs. Blank}] \times 253^*$$










$$\text{Procedure n°2 : mg/dL} = [\text{Abs. Assay} - \text{Abs. Blank}] \times 58.6^*$$

$$\text{Amol /L} = [\text{Abs. Assay} - \text{Abs. Blank}] \times 1173^*$$

\*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.

## 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, 4 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, 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®

	Consult Instructions for Use
	Caution, Consult accompanying Documents
	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
	Authorized Representative in the European Community
	Catalogue Number
	Batch Code
	Use by



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