BIOLABO
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TOTAL AND DIRECT BILIRUBIN

Sulfanilic Acid Method

Reagent for quantitative determination of total bilirubin (DMSO as accelerator) or direct bilirubin in human serum and plasma

REF 80403:	**R1** 1 x 200 mL	Total bilirubin
	R2 1 x 200 mL	Direct bilirubin
	R3 1 x 40 mL	Nitrite Solution
REF 80443:	**R1** 2 x 200 mL	Total bilirubin
	R3 1 x 40 mL	Nitrite Solution
REF 80553:	**R2** 2 x 200 mL	Direct bilirubin
	R3 1 x 40 mL	Nitrite Solution

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (6)

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)

Reaction between bilirubin and diazotised sulfanilic acid which leads to a compound, the azobilirubin, coloured in very acid or basic medium.

Malloy-Evelyn principle modified by Walters and al: in an aqueous solution, only DB reacts. To enable the assay of TB, it is necessary to break the link between unconjugated bilirubin and albumin. This step is done by adding dimethylsulfoxide (DMSO).

The absorbance of azobilirubin thus produced is proportional to the concentration of bilirubin and is measured at 550 nm (530-580).

REAGENTS COMPOSITION

Vial R1 **TOTAL BILIRUBIN**

Sulfanilic acid	30	mmol/L
DMSO	7	mol/L
Hydrochloric acid	130	mmol/L

Vial R2 **DIRECT BILIRUBIN**

Sulfanilic acid	30	mmol/L
Hydrochloric acid	130	mmol/L

Vial R3 **NITRITE SOLUTION**

Sodium Nitrite	0.74	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.
- 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.

REAGENTS PREPARATION

Reagents are ready for use.
In case of important runs or automated system one can prepare single vial working reagent as follows: R1 or R2 (20 volumes)+R3 (1 volume).

STABILITY AND STORAGE

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

- When used and stored as described in the insert, reagents (vial R1, R2, R3) are stable, without contamination, until expiry date stated on the label.
- Working reagent TB is stable for 2 days at 2-8°C.
- Working reagent DB is stable for 7 days at 2-8°C.

Discard any reagent if cloudy or if absorbance at 550 nm > 0.100.

Don't use working reagents after expiry date stated on the label of the kit.

SPECIMEN COLLECTION AND HANDLING (2) (7)

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)

Hemoglobin: under-evaluation above 100 µmol/L (160 mg/dL) of haemoglobin.
Turbidity: No significant interference with TB.
No significant interference with DB up to triglycerides concentration equivalent to 4.6 mmol/L.

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 (8)

1. Use the experimental factor (see § **CALCULATION**)
2. Or a calibrator traceable to a reference method or material, BIOLABO Multicalibrator REF 95015 traceable to SRM 916a.

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

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

Made in France
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QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- BIOLABO PAEDIATRIC CONTROL REF 95403
- Other assayed control sera referring to the same method and 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.
1. 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]	**Direct Bilirubin** mg/dl	[µmol/L]
>5 days-60 years	0.3-1.2	[5-21]	< 0.2	[< 3.4]
60-90 years	0.2-1.1	[3-19]	< 0.2	[< 3.4]
> 90 years	0.2-0.9	[3-15]	< 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 specimen:** perform procedure n°2.
Procedure n°2: up to 100 mg/dL (1710 µmol/L)
Pediatric specimen: perform procedure n°2

PERFORMANCES CHARACTERISTICS (PROCEDURE N°1)

TOTAL BILIRUBIN

Within run: N = 23	*Normal level*	*High level*
Mean mg/dL	0.68	4.13
S.D. mg/dL:	0.02	0.104
C.V. %:	2.94	2.52

Between run N = 20	*Normal level*	*High level*
Mean mg/dL	0.67	3.85
S.D.mg/dL:	0.022	0.07
C.V. %:	3.27	1.78

DIRECT BILIRUBIN

Within run: N = 20	*Medium level*	*High level*
Mean mg/dL	1.15	2.79
S.D. mg/dL:	0.022	0.015
C.V. %:	1.94	0.53

Between run N = 20	*Medium level*	*High level*
Mean mg/dL	1.09	2.74
S.D. mg/dL:	0.028	0.09
C.V. %:	2.6	3.3

Detection limit: TB: approximately 0.13 mg/dL
DB: approximately 0.18 mg/dL

Sensitivity for 1 mg/dL: 88 mAbs at 550 nm.

Comparison study with commercially available reagent:

TB: y = 1.0145 x + 0.00513 r = 0.9976

DB: y = 1.0002 x – 0.00796 r = 0.9972

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Procedure n°1:

Pipette into well identified test tubes:	**TOTAL BILIRUBIN**		**DIRECT BILIRUBIN**	
	Blank	**Assay**	**Blank**	**Assay**
Reagent R1	1 mL	1 mL		
Reagent R2			1 mL	1 mL
Distilled water	50 µL		50 µL	
Reagent R3 (Nitrite)		50 µL		50 µL
Mix				
Specimen	100 µL	100 µL	100 µL	100 µL

Mix well and start a timer when adding specimen.
Read absorbances at 550 nm (530-580) against blanks
TB: reading after ≥ 3 minutes at 37°C or ≥ 5 minutes at room temperature.
DB: reading at **exactly** 3 minutes at 37°C or 5 minutes at room temperature.

Procedure n°2: Icteric or Pediatric Specimens

Pipette into well identified test tubes	**TOTAL BILIRUBIN**		**DIRECT BILIRUBIN**	
	Blank	**Assay**	**Blank**	**Assay**
Reagent R1	1 mL	1 mL		
Reagent R2			1 mL	1 mL
Distilled water	50 µL		50 µL	
Reagent R3 (Nitrite)		50 µL		50 µL
Mix				
Specimen	20 µL	20 µL	20 µL	20 µL

Well mix and start a timer when adding specimen.
Read absorbance at 550 nm (530-580) against blanks
TB: reading after ≥ 3 minutes at 37°C or ≥ 5 minutes at room temperature.
DB: reading at **exactly** 3 minutes at 37°C or 5 minutes at room temperature.

Notes:

1. Zero on distilled water and drain well the cuvette. Read first all blanks of one run then all the assays, well draining cuvette between each tubes. But **DO NOT RINSE WITH WATER** as it could produce streaks on the cuvette and lead to false results.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With 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 11.4*
µmol/L = [Abs. assay – Abs. Blank] x 195*

Procedure n°2: **mg/dL = [Abs. assay – Abs. Blank] x 53.0***
µmol/L = [Abs. assay – Abs. Blank] x 906*

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

REFERENCES

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