

BILIRUBIN-DMSO (TOTAL + DIRECT)

Diagnostic reagent for the in-vitro quantitative determination of total or direct bilirubin in human serum and plasma on both automated and manual system.

REF: BS.1/BD01.050.0050
REF: BS.1/BD05.050.0250

50 test
250 test

REF: BS.1/BD02.050.0100

100 test

CLINICAL SIGNIFICANCE ^(1,2)

The average level of the bilirubin produced in humans from different sources ranges between 250 to 300 mg/day, of which 85% is derived from the heme moiety of the haemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system. The remaining 15 % is produced from erythrocytes destroyed in the bone marrow and from catabolism of other heme containing proteins such as cytochromes and myoglobin. Bilirubin is transported to the liver in association with albumin, where it is conjugated with glucuronic acid for solubilization and elimination through the bile duct via the digestive tract.

Ehrlich in 1884 describe a reaction in which bilirubin is coupled with diazotized sulfanilic acid (p-diazobenzenesulfonic acid) to produce an azo dye of pink color in an acid medium and blue in an alkaline one. The measurement of the blue form has been more popular because of greater sensitivity, and used to distinguish and quantitate two type of serum bilirubin. The direct form, consists of conjugated, water-soluble derivatives and reacts in the absence of an accelerating or solubilizing agent. The indirect form, consists of free, unconjugated bilirubin bound to serum albumin. This form only reacts in the presence of an accelerating agent. The sum of these two forms is termed total bilirubin. The differentiation between direct and indirect is important in diagnosing causes of hyperbilirubinemia.

Disease or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

METHOD PRINCIPLE ^(3,4)

The test principle depends on the conversion of bilirubin to colored diazotized sulfanilic acid (dialzo). Direct and indirect bilirubin couple with dialzo to produce azobilirubin, only direct bilirubin react freely in the aqueous solution, while the unconjugated bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect). The intensity of the color produced is directly proportional to the amount of total bilirubin concentration present in the sample.

REAGENT COMPOSITION

Reagents:	Composition
R1: Total Bilirubin	
- Sulfanilic acid	- 30 mmol/L
- DMSO	- 7 mol/L
- Hydrochloric acid	- 130 mmol/L
R2: Direct Bilirubin	
- Sulfanilic acid	- 30 mmol/L
- Hydrochloric acid	- 130 mmol/L
R3: Reagent 3	
- Sodium nitrite	- 0.74 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Bilirubin DMSO reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Bilirubin DMSO reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

On mass volume or automated system a working reagent can be prepare in the following ration: 20 volumes of R1 or R2 + 1 volume of R3. Prepared working reagent for total bilirubin is stable for 2 days at 2-8°C, and for direct bilirubin for 7 days at 2-8°C

Deterioration

The **BioScien** Bilirubin DMSO reagent can be damaged due to contamination or on exposure to light which is indicated by reagents turbidity. It is recommended that such reagent should be discarded or in case of control values out of the assigned range.

SPECIMEN COLLECTION AND PRESERVATION

Clean and dry glassware free from detergents must be used for sample collection, serum or blood samples drawn with heparin and oxalate anticoagulant are suitable. Do not use hemolyzed samples.

Stability in the specimen:

- 2 days at room temperature
- 4-7 days at 2-8°C

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature.

Automated Procedure

Refer to specific instrument application instructions

Manual Procedure

	Total Bilirubin		Direct Bilirubin	
	Blank	Assay	Blank	Assay
Reagent R1	1.0 ml	1.0 ml		
Reagent R2			1.0 ml	1.0 ml
Distilled water	50 µl		50 µl	
Reagent R3		50 µl		50 µl
Mix.				
Specimen	100 µl	100 µl	100 µl	100 µl

Mix and incubate for exactly 3 minutes at 37°C or 5 minutes at 15-25°C. Measure absorbance of assay "A" against its blank "A" at 546 nm (530 - 580 nm).

CALCULATION

Concentration (mg/dl) = absorbance (assay - blank) × Factor

Where the Theoretical Factor is calculated:

Bilirubin (total and direct) in mg/dl = 11.4

Bilirubin (total and direct) in µmol/l = 195

Conversion Factor : mg/dl × 17.1 = µmol/l

Note

For bilirubin determination in newborns use 50 µl sample and multiply the result by 2.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Bioscien** technical support.

PERFORMANCE CHARACTERISTICS

Precision

Within run (Repeatability)		Run to run (Reproducibility)	
Normal level	High level	Normal level	High level

Total Bilirubin

n	20	20	20	20
Mean mg/dl	0.78	4.23	0.67	4.52
SD. mg/dl	0.02	0.14	0.022	0.17
CV. %	2.54	3.52	3.27	3.78

Direct Bilirubin

n	20	20	20	20
Mean mg/dl	1.15	2.79	1.09	2.74
SD. mg/dl	0.022	0.015	0.028	0.09
CV. %	1.94	0.53	2.6	3.3

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from **Bioscien** Bilirubin DMSO reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.97.

Sensitivity

When run as recommended, the minimum detection limit of the assay is 0.1 mg/dl for total bilirubin and 0.04 mg/dl for direct bilirubin.

Linearity

The reaction is linear up to Bilirubin DMSO concentration of 18 mg/dl (308 µmol/L); specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result×5).

INTERFERING SUBSTANCES ⁽⁵⁾

Haemolysis

Erythrocyte contamination interfere with the test result.

lipemia

Lipemic specimens interfere with the method.

Others

A number of drugs and substances affect bilirubin results.

EXPECTED VALUES ⁽⁶⁾

Total Bilirubin	mg/dl	[µmol/L]
Newborn		
3-5 days	4.0 - 8.0	[68-137]
<48 hours	6.0 -10.0	[103-171]
<24 hours	2.0 - 6.0	[34-103]
Adults and infants >5 days		
>5 days - 65 years	0.3 -1.2	[5-21]
60 - 90 years	0.2 - 1.1	[3 -19]
> 90 years	0.2 - 0.9	[3 -15]
Direct Bilirubin	mg/dl	[µmol/L]
Adults and infants >5 days		
>5 days - 65 years	< 0.3	[< 5.1]
60 - 90 years	< 0.3	[< 5.1]
> 90 years	< 0.3	[< 5.1]

DYNAMIC RANGE

Total bilirubin: 0.1- 18 mg/dL (1.7 . 308 µmol/L)

Direct bilirubin: 0.04- 18 mg/dL (0.68 . 308 µmol/L)

REFERENCES

- Ehrlich, P., Zeitschr, Sur Anal. Chemie 23:275 (1884).
- Tietz N.W. Text book of clinical chemistry, 3rd ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999): 1133-1137.
- Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method. J Biol Chem. 1937;119:481-490.
- Walters M, Gerarde H, Microchem J (1970) 15 : 231-243
- Young, D.S., et al, Clin. Chem. 21:1D (1975).
- Gambino, S.R., et al, Bilirubin Assay (Revised), Commission on Continuing Education, Am. Soc. of Clin. Path., Chicago, (1968).
- Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders: 1987:729-761.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use		Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		



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