ARENA BioScien

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BILIRUBIN- Jendrassik (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/BL01.050.0050	50 test	REF: BS.1/BL02.050.0100	100 tests
REF: BS.1/BL05.050.0250	250 test		

CLINICAL SIGNIFICANCE

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

The total bilirubin concentration is determined in presence of caffeine by the reaction with diazotized sulphanilic acid to produce an intensely colored diazo dye (560-600 nm). The intensity of color of this dye formed is proportional to the concentration of total bilirubin.

Direct bilirubin is determined in absence of caffeine by the direct reaction with diazotized sulphanilic acid to form red-colored azobilirubin, the color intenisity of which measured at 546 nm is Proportional to the concentration of the direct bilirubin in the sample.

$$\text{Sulfanilic acid} + NaNO_2 \xrightarrow{HCl} \text{Diazotized sulfanilic acid}$$

$$\text{Bilirubin} + \text{Diazotized sulfanilic acid} \xrightarrow{\text{pH 1.4}} \text{Azobilirubin}$$

REAGENT COMPOSITION

Reagents:	Composition
R1: - Sulfanilic acid - HCL	- 31 mmol/L - 0.20 N
R2: - Sodium nitrite	- 28 mmol/L
R3: - Caffeine - Sodium benzoate	- 28 mol/L - 55 mol/L
R4: - Tartarate - Sodium hydroxide	- 99 mol/L - 2.0 N

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Reagent 4 contains caustic material. Corrosive (C)
R35 Causes severe burns.
R41 Risk of serious damage to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

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 reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Bilirubin reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright position at 15-25°C.

Deterioration

The ***BioScien*** Bilirubin 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

Total Bilirubin	*Blank*	*Assay*
Reagent R1	200 µl	200 µl
Reagent R2		1 drop
Reagent R3	1.0 ml	1.0 ml
Specimen	200 µl	200 µl
Mix. and incubate for 10 minutes at 20 – 25℃. then add		
Reagent R4	1.0 ml	1.0 ml

Mix and incubate for 5 minutes at 20 - 25°C. Measure absorbance of assay "A" against its blank "A" at 578 nm (560 - 600 nm). The color intensity is stable for 30 minutes.

Direct Bilirubin	*Blank*	*Assay*
Reagent R1	200 µl	200 µl
Reagent R2		1 drop
Saline 0.9% NaCl	2.0 ml	2.0 ml
Specimen	200 µl	200 µl

Mix and incubate for 5 minutes at 20 - 25°C. Measure absorbance of assay "A" against its blank "A" at 546 nm (530 - 560 nm). The color intensity is stable for 30 minutes.

CALCULATION

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

Where the Theoretical Factor is calculated:
Bilirubin (total) in mg/dl = 10.8
Bilirubin (direct) in mg/dl = 14.4

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	0.79	4.37	0.299	0.77
SD.	0.016	0.18	0.016	0.057
CV. %	2.13	4.12	5.41	7.4
Direct Bilirubin				
n	20	20	20	20
Mean mg/dl	0.82	4.52	0.32	0.82
SD.	0.02	0.27	0.023	0.062
CV. %	2.24	4.21	5.57	8.1

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

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** Bilirubin reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.975.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 0.1 mg/dl (1.7 µmol/L) for total bilirubin and direct bilirubin.

Linearity
The reaction is linear up to total bilirubin concentration of 30 mg/dl (513 µmol/L); and direct bilirubin concentration of 10 mg/dl (171 µmol/L)
specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result×5).

INTERFERING SUBSTANCES

Haemolysis
Avoid haemolysis since it interferes with the test.
lipemia
Lipemic specimens interfere with the method.

Drugs
Theophyllin and propranolol may cause artificially low total bilirubin levels.

EXPECTED VALUES

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 >1 month	0.2 - 1.0	[5 - 21]
Newborns premature (3-5 d)	10 -14	[171 - 239]
Direct Bilirubin	0 - 3	[0 - 51]

DYNAMIC RANGE

Total bilirubin: 0.1 - 30 mg/dL (1.7 - 513 µmol/L)
Direct bilirubin: 0.1 - 18 mg/dL (1.7 - 171 µmol/L)

REFERENCES

1. Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders: 1987:729-761.
2. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method. J Biol Chem.1937:119:481- 490.
3. Tietz NW, ed. Clinical guide to laboratory tests. 3rd ed.Philadephia: WB saunders; 1995:268-273.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	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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CE