Atlas Medical CE

Total & Direct Bilirubin

DMSO. Colorimetric

IVD For in vitro diagnostic use only.

Store at 2-8

INTENDED USE

For the determination of Total and Direct Bilirubin in human serum or plasma.

INTRODUCTION

Bilirubin is a breakdown product of hemoglobin, insoluble in water. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia:

Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, and drugs.

Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions presents in serum, bilirubin-glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulphoxide (DMSO) to react (bilirubin indirect). In the determination of indirect bilirubin, the direct is also determined, the results correspond to total bilirubin.

The intensity of the color formed is proportional to the bilirubin concentration in the sample.

REAGENTS

R1(Total Bilirubin)	Sulfanilic acid Hydrochloric acid (HCl)	30mmol/L 150mmol/L
R2(Direct Bilirubin)	Sulfanilic acid Hydrochloric acid (HCl) Dimethylsulphoxide (DMSO)	30mmol/L 150mmol/L 7 mol/L
R3	Sodium nitrite	29 mmol/L

EQUIPMENTS NEEDED BUT NOT PROVIDED

- Spectrophotometer or colorimete measuring at 555 nm
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

PRECAUTIONS

- Dimethylsulphoxide (DMSO): Irritant to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Hydrochloric acid (HCL): Irritant to eyes respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

PREPARATION

- All the reagents are ready to use.

STORAGE AND STABILITY

- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8^{0}C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.
- **Signs of reagent deterioration:**
  - Presence of particles and turbidity.
  - Color development in R 2.

SAMPLES

Serum or plasma, free of hemolysis. Protect samples from direct light. Stability: Bilirubin is stable at 2-8^{0}C for 4 days and 2 months at –20°C.

PROCEDURE

1. Assay conditions:
   Wavelength ..... …… 555 nm (530-580)
   Cuvette: ..... 1 cm light path
   Temperature ..... 15-25^{0}C
2. Adjust the instrument to zero with distilled water. Pipette into a cuvette:

TOTAL BILIRUBIN DETERMINATION:

	Blank	B. Total
R 1 (mL)	1.5	1.5
R 3 (µL)	--	50
Sample	100	100

- Read the absorbance (A). Mix and incubate for exactly **5 minutes** at room temperature.
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DIRECT BILIRUBIN DETERMINATION:

	Blank	B. Direct
R 2 (mL)	1.5	1.5
R 3 (µL)	--	50
Sample (µL)	100	100

- Mix and incubate for exactly **5 minutes** at room temperature.
- Read the absorbance (A).

CALCULATIONS

With Calibrator:

$$\frac{\text{(A)Sample – (A) Sample Blank}}{\text{(A) Calibrator – (A) Calibrator Blank}} \times \text{Conc. Calibrator} = \text{mg/dL bilirubin}$$

With Factor:

((A) Sample - (A) Sample Blank) x **Factor*** = mg/dL bilirubin in the sample

***Factor:**

$$\frac{\text{Concentration of Calibrator}}{\text{(A) Calibrator – (A) Calibrator Blank}}$$

Conversion factor: mg/dL x 17,1 = µmol/L.

QUALITY CONTROL

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

Bilirubin Total in adult Up to 1.10 mg/dL = Up to 18.81 µmol/L

Bilirubin Total in newborn <12mg/dL = <205.2 µmol/L

Bilirubin Direct Up to 0.25 mg/dL = Up to 4.27 µmol/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Bilirubin Total

Measuring range:

From *detection limit* of 0.00526 mg/L to *linearity limit* of 18 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	1.53	5.06	1.53	5.02
SD	0.03	0.05	0.03	0.11
CV (%)	1.73	1.01	1.92	2.18

Sensitivity:

1 mg/dL = 0.05074 A.

Bilirubin Direct

Measuring range*:*

From *detection limit* of 0.07 mg/L to *linearity limit* of 20 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0.96	2.48	0.96	2.50
SD	0.024	0.051	0.043	0.035
CV (%)	2.52	2.06	4.49	1.41

Sensitivity:

1 mg/dL = 0.06856 A.

Accuracy:

Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples for Bilirubin D were the following:

Correlation coefficient $(r)^2$: 0.96.

Regression equation: y= 0.71177x -0.05267.

The results obtained using 50 samples for Bilirubin T were the following:

Correlation coefficient $(r)^2$: 0.991.

Regression equation: y= 0.82743x -0.0382.

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

INTERFERENCES

Hemolysis causes decreased bilirubin values.

A list of drugs and other interfering substances with bilirubin determination has been reported.

NOTES

1. For bilirubin determination in newborns, pipette 50 µL of sample. Multiply the result by 2.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry