Total & Direct Bilirubin

Jendrassik-Grof Method
Colorimetric Test

IVD CE

PRODUCT CODE
CS004

INTENDED USE
These reagents are intended for in vitro quantitative determination of Total and Direct Bilirubin in serum or plasma.

CLINICAL SIGNIFICANCE
Bilirubin is formed by the breakdown of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred to a direct bilirubin.
Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

PRINCIPLE
Bilirubin reacts with diazotized sulphanilic acid (DSA) to form a red azo dye, the intensity of which at 546 nm is directly proportional to the bilirubin concentration in the sample. Water-soluble bilirubin glucuronides react "directly" with DSA whereas the free or "indirect bilirubin" will only react with DSA in presence of an accelerator.
Total - Direct = Indirect bilirubin
Sulphanilic acid + sodium nitrite → DSA
Direct Bilirubin + DSA → DIRECT Azobilirubin
Total Bilirubin + DSA + accelerator → TOTAL Azobilirubin

REAGENT COMPOSITION
1-Total Bilirubin Reagent (R1)

Sulphanilic Acid	14 mmol/L
Hydrochloric Acid	250 mmol/L
Caffeine (accelerator)	200 mmol/L
Sodium Benzoate	420 mmol/L

2Total Bilirubin, Nitrite Reagent (R2)

Sodium Nitrite	6.5 mol/L

3-Direct Bilirubin Reagent (R1)

Sulphanilic Acid	14 mmol/L
Hydrochloric Acid	250 mmol/L

4-Direct Bilirubin, Nitrite Reagent (R2)

Sodium Nitrite	6.5 mmol/L

REAGENT PREPARATION
Both reagents and nitrites solutions are ready to use.

REAGENT STORAGE AND STABILITY
Both reagents and nitrite solution are stable to the given expiry date if stored at 15-25°C.

SPECIMEN
Fresh haemolysis- free serum or heparinized plasma may be used. Carefully protect from light until use. Bilirubin in sample is stable for '3' days when stored in the dark at 2-8° C.

PRECAUTION
To avoid contamination, use clean laboratory wares.
Avoid direct exposure of reagent to light.

ASSAY /TOTAL BILIRUBIN

Wavelength	:	546nm
Cuvette	:	1 cm light path
Temperature	:	20-25°C
Measurement	:	Against sample blank (without nitrite)

PROCEDURE

	Blank	Sample
Total Bilirubin Reagent (R1)	1000 µL	1000 µL
Total Bilirubin, Nitrite reagent (R2)	--	20 µL
Sample	100 µL	100 µL
Mix and stand for exactly '5' minutes at room temperature. Measure the absorbance of sample Blank (As)		

ASSAY /DIRECT BILIRUBIN

Wavelength	:	546nm
Cuvette	:	1 cm light path
Temperature	:	20-25°C
Measurement	:	Against sample blank (without nitrite)

PROCEDURE

	Blank	Sample
Direct Bilirubin Reagent (R1)	1000 µL	1000 µL
Direct Bilirubin, Nitrite reagent (R2)	--	20 µL
Sample	100 µL	100 µL
Mix and stand for exactly '5' minutes at room temperature. Measure the absorbance of sample Blank (As). For direct bilirubin assay it is very important to read the absorbance after exactly 5 minutes to obtain results.		

CALCULATION
Bilirubin Concentration = As X 13.2 mg/dL
To convert mg/dL to µmol/L. multiply 17.1

NORMAL RANGE
Total Bilirubin

At birth up to	5 mg/dL	85.5 µmol/L
5 days up to	12 mg/dL	205.0 µmol/L
1 month up to	1.5 mg/dL	25.6 µmol/L
Adults up to	1.1 mg/dL	18.8 µmol/L

Direct Bilirubin

At birth up to	0.25 mg/dL	4.30 µmol/L

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL
All control sera with Bilirubin value estimated by this method can be used

PERFORMANCE CHARACHTERISTICS
Sensitivity: 0.08 mg/dL for both total and direct Bilirubin.
Linearity: The assay is linear up to 10 mg/dL both total and direct Bilirubin, values exceeding 10 mg/dL dilute serum 1+4 with physiological saline (NaCl: 9g/L) and repeat the assay. Multiply the result by 5.

Precision Bilirubin Total

Description	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	1.60	4.23	1.62	4.33
SD	0.04	0.15	0.07	0.04
CV (%)	2.35	3.51	4.21	1.00

Precision Bilirubin Direct

Description	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	1.04	3.34	1.05	3.33
SD	0.03	0.05	0.03	0.03
CV (%)	2.41	1.43	3.23	1.00

Method Comparison:
Result obtained Bio Research(x) did not show systematic difference when compared with other commercial reagents(y).The results obtained using 30 samples where the following,
Total Bilirubin
Correlation coefficient $(r)^2$: 0.994
Regression equation: y= 0.973+0.038.
Direct Bilirubin
Correlation coefficient $(r)^2$: 0.997
Regression equation: y= 0.970+0.044
The results of the performance characteristics depend on the analyzer used.

NOTES
1- It is important to ensure the working reagent and nitrite reagent are thoroughly mixed before adding the sample.
2- For bilirubin values exceeding 10 mg/dL dilute serum 1+4 with physiological saline (NaCl: 9g/L) and repeat the assay. Multiply the result by 5.
3- Bilirubin levels may be reduced if the sample is exposed to light. Haemolytic sample will also show low value.

SYMBOL ON LABELS

Symbols	Signify	Symbols	Signify
REF	Catalogue Number	SIZE	Pack Size
	Expiry Date	VOL	Volume
	Storage Condition	LOT	Lot Number
	Instruction for Use	IVD	In Vitro Diagnostics
	Manufacturing Date		Manufacturer
	Number of Tests	2	For Single Use Only
EC REP	EC Representative	CE	European conformity

BIBLIOGRAPHY

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