Total Bilirubin

DPD Method, Colorimetric

IVD CE

PRODUCT CODE
CS022

INTENDED USE
The reagent is intended for in vitro quantitative determination of Total Bilirubin in serum or plasma.

CLINICAL SIGNIFICANCE
Bilirubin is caused by the degradation of hemoglobin and exists in two forms. Unconjugated bilirubin is transported to the liver bound by albumin where it becomes conjugated (direct) with glucuronic acid and excreted. Hyperbilirubinemia is the result of an increase of bilirubin in plasma. Possible causes:
Total bilirubin: Increase hemolysis, genetic alteration, neonatal anemia, erythropoiesis alterations and presence of drugs.
Direct Bilirubin: cholestasis liver, liver abnormalities and genetic. Clinical diagnosis should not be made based on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE
Bilirubin (both conjugated and unconjugated) couples with the diazo reagent in the presence of a surfactant to form azobilirubin. The intensity of color formed is proportional to the bilirubin concentration in the sample tested. The increase of absorbance at 546 nm is directly proportional to the total bilirubin concentration.

REAGENT COMPOSITION

Total Bilirubin Reagent (R1)	
Surfactants	<1%
Hydrochloric acid (HCl)	160 mM
Total Bilirubin, Nitrite Reagent (R2)	
2,4-DPD	≥2 mM
Hydrochloric acid (HCl)	120 mM
Surfactant	<1%

REAGENT PREPARATION
Both reagents are ready to use.

REAGENT STORAGE AND STABILITY
The reagents are stable until the expiry date stated on the label when stored at 2-8ºC, protected from light and contaminations are prevented during their use. Do not use reagents over the expiration date.
Signs of reagent deterioration: Presence of particles and turbidity

SPECIMEN
Fresh hemolysis-free serum or heparinized plasma may be used. Carefully protect from light until use. Bilirubin in sample is stable for '4' days when stored in the dark at 2-8° C and 2 months at –20ºC.

PRECAUTION
- R1/ R2: H290- Corrosive to metals. H314 - Irritation or skin corrosion.
- R1: contains HCl and Triton X-114. R2: contains HCl and 2,4-DPD.
- To avoid contamination, use clean laboratory wares.

PROCEDURE

1. Assay condition:

Wavelength	546 nm (530-580)
Temperature	37 °C
Measurement	Against zero distilled water

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Calibrator Blank	Sample
Total Bilirubin Reagent (R1)	800 µL	800 µL
Calibrator	40 µL	-
Sample		40 µL

4. Mix and incubate for 5 minutes at 37 °C.
5. Read the absorbance (A1) of the sample and calibrator.
6. Add:

	Calibrator	Sample
R2 (μL)	200	200

7. Mix and incubate for 5 minutes at 37 °C.
8. Read the absorbance (A1) of the sample and calibrator against the blank.
9. Calculate the increase of the absorbance: $\Delta A = A2 - A1$

CALCULATION
With calibrator:
$\frac{(\Delta A)\ \text{Sample}}{(\Delta A)\ \text{Calibrator}}$ x Calibrator conc. = mg/dL of bilirubin in the sample

With Factor: (ΔA) Sample x Factor* = mg/dL bilirubin in the sample

*Factor: $\frac{\text{Calibrator concentration}}{(\Delta A)\ \text{Calibrator}}$
Conversion factor: mg/dL x 17,1 = µmol/L.

REFERENCE VALUES
Total bilirubin 0,2-1,2 mg/dL (3,4 – 20,5 ∟mol/L)
These values are for orientation purpose; each laboratory should establish its own reference range.

LINEARITY
From quantification limit of 0,1 mg/dL to linearity limit of 30 mg/dL.
If the results obtained were greater than the linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

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

INTERFERENCES
from hemolysis, lipemia and a. ascorbic were evaluated for this total bilirubin method. Two concentrations of total bilirubin were evaluated. No interferences were observed for lipemia (Intralipid) up to 1800 mg/dL, hemoglobin up to 2000 mg/dL and ascorbic acid up to 40 mg/L. A list of drugs and other interfering substances with bilirubin has been reported by Young et. al 4,5.

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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