Direct Bilirubin

DPD Method. Colorimetric

IVD CE

PRODUCT CODE
CS023

INTENDED USE
The reagent is intended for in vitro quantitative determination of Direct 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, neonatal jaundice, ineffective erythropoiesis and presence of drugs.
Direct bilirubin: Hepatic cholestasis, genetic, hepatocellular damage. Clinical diagnosis should not be made based on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE
Direct bilirubin (conjugated) couples with the diazo reagent in the presence of sulfamic acid 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 direct bilirubin concentration.

REAGENT COMPOSITION

Direct Bilirubin Reagent (R1)	
Sulphanilic Acid	10 mM
Direct Bilirubin, Nitrite Reagent (R2)	
2,4-DPD	0,5 mM
Hydrochloric acid (HCl)	0,3 M

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.

PROCEDURE:
Assay condition:
Wavelength: ……..…546nm (530-580)
Cuvette: ……..……1cm light path
Temperature: ……..37ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Calibrator blank	Sample blank
R 1 (µL)	800	800
Calibrator (µL)	50	-
Sample (µL)	-	50

4. Mix and incubate for 5 minutes at 37ºC.
5. Read the absorbance (A1) of the sample and calibrator.
6. Add:
Calibrator Sample
R 2 (⊥L) 200 200
7. Mix and incubate for 5 minutes at 37ºC.
8. Read the absorbance (A2) of the sample and calibrator against the blank.
9. Calculate the increase of the absorbance: ΔA= A2–A1.

PRECAUTION
R1: H314 - Irritation or skin corrosion. / R2: H290- Corrosive to metals.
H335 - May cause respiratory irritation.
H314 - Irritation or skin corrosion.
R2: contains HCl and 2,4-DPD.
Follow the safety advice given in MSDS and product label.

CALCULATIONS:
- **With calibrator :**

$$\frac{(\Delta A)\text{Sample}}{(\Delta A)Calibrator} \; x\; Calibrartor\; conc. = mg/dl\; of\; bilirubin\; in\; the\; sample$$

With Factor : $(\Delta A)\ \boldsymbol{Sample\ x\ Factor} = mg/dl\ of\ bilirubin\ in\ the\ sample$

Factor : $\frac{\text{Calibrator concentration}}{(\Delta A)Calibrator}$

Conversion factor : $mg/dl\ x\ 17.1\ \mu mol/L$

NORMAL RANGE
Direct bilirubin 0 – 0,2 mg/dL (0 – 3,42 µmol/L)
These values are for orientation purpose; each laboratory should establish its own reference range.

QUALITY CONTROL
All control sera with Direct Bilirubin value estimated by this method can be used.
It is recommended that each laboratory establishes its own reference range.

LINEARITY
From detection limit of 0,03 mg/dL to linearity limit of 9 mg/dL.
If the results obtained are greater than the linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

INTERFERENCES
Interferences from hemolysis, lipemia and ascorbic acid were evaluated for this direct bilirubin method on a Spintech 240 analyzer. Two concentrations of direct bilirubin were evaluated. No interferences were observed for lipemia (Intralipid) up to 350 mg/dL and ascorbic acid up to 40 mg/L. Hemolysis causes decreased direct bilirubin values, therefore hemolytic samples should be discarded. 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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