# Direct Bilirubin

DPD. Colorimetric

Quantitative determination of direct bilirubin

CE IVD

### *PACKAGING*

| | |
|---|---|
| Ref.: 101-0783 | Cont.: 1 x 240 / 1 x 60 mL |

Store at 2-8º C

### *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 OF THE METHOD*

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.

### *REAGENTS*

| | | |
|---|---|---|
| **R 1** | Sulfamic acid | 100 mM |
| **R 2** | 2,4-DPD<br>Hydrochloric acid (HCl) | 0.5 mM<br>0.3 M |

### *PRECAUTIONS*

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.

### *PREPARATION*

The reagents are provided in a ready to use format.

### *STORAGE AND STABILITY*

The reagents are stable until the expiry date stated on the label when stored at 2-8º C, 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.

### *ADDITIONAL EQUIPMENT*

- Spectrophotometer or analyzer capable of measuring absorbance at 546 nm.
- Cuvettes with 1,0 cm light path.
- General laboratory equipment.

### *SAMPLES*

Serum or plasma, free of hemolysis. Protect samples from light.
Stability of the sample: 4 days at 2-8º C or 2 month at –20º C.

### *PROCEDURE*

1. Assay conditions:
   Wavelength: ……546nm (530-580)
   Cuvette: ……..1cm light path
   Temperature: ……..37º C
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 37º C.
5. Read the absorbance (A1) of the sample and calibrator.
6. Add:

| | Calibrator | Sample |
|---|---|---|
| R 2 (µL) | 200 | 200 |

7. Mix and incubate for **5 minutes** at 37º C.
8. Read the absorbance (A2) of the sample and calibrator against the blank.
9. Calculate the increase of the absorbance: ΔA= A2–A1.

### *CALCULATIONS*

– **With calibrator:**

$$\frac{(\Delta A)\,\text{Sample}}{(\Delta A)\,\text{Calibrator}} \times \text{Calibrator conc.} = \text{mg/dL of bilirubin in the sample}$$

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

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

**Conversion factor:** mg/dL x 17.1 = µmol/L.

### *QUALITY CONTROL*

Control sera are recommended to monitor the performance of assay procedures: Contro-N (Ref. 101-0252 and 101-0083) and Contro-P (Ref. 101-0253 and 101-0084). 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*

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.

### *PERFORMANCE CHARACTERISTICS*

**Measuring range**: 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.
**Precision:**

| | Inter assay (n= 40) | | Intra assay (n= 80) | |
|---|---|---|---|---|
| Mean (mg/dL) | 0.7458 | 2.444 | 0.7458 | 2.444 |
| SD | 0.05868 | 0.0550 | 0.0276 | 0.024 |
| CV (%) | 7.9 | 2.2 | 3.7 | 1.0 |

**Sensitivity:** 1 mg/dL = 0,.040 Abs. units
**Accuracy:** Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x) on a Spintech 240 analyzer. The results obtained using 53 samples ranging from 0.06 a 9 mg/dL (1.02 to 153.9 µmol/L) were:
Correlation coefficient (r): 0.9986
Regression equation: y = 1.0056 x – 0.1046
The results of the performance characteristics depend on the analyzer used.

### *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].

### *NOTES*

1. **CHRONOLAB has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

### *BIBLIOGRAPHY*


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