ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

# Bilirubin Direct – Instructions for use

R1: 2 x 18 mL R3330000010 REF
R2: 2 x 4,5 mL

CE IVD

**Diagnostic reagent for quantitative in vitro determination of direct bilirubin in human serum or plasma on photometric systems.**

## TEST PARAMETERS

| | |
|---|---|
| Method: | Colorimetric, endpoint, increasing reaction, DCA |
| Wavelength: | 546 nm (540 – 560 nm) |
| Temperature: | 20 – 25 °C or 37 °C |
| Sample: | Serum, heparin plasma |
| Linearity: | up to 10 mg/dL |
| Sensitivity: | The lower limit of detection is 0.1 mg/dL |

## SUMMARY [1,2]

Bilirubin is a breakdown product of haemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucoronic acid and the resulting water soluble bilirubin glucoroniedes are excreted via the bile ducts.
Hyperbilirubinemia can be caused by increased bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intra-hepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinea called Gilbert's syndrome is quite frequent in the population. High levels of total bilirubin are observed in 60 – 70 % of neonates due to an increased postpartal breakdown of erythrocytes and because of delayed function of enzymes for bilirubin degradation.

## TEST PRINCIPLE

Direct Bilirubin reacts with diazotized 2,4-dichloroaniline (DCA) to form a red colored azocompound in acidic solution.
Determinations of direct bilirubin measure mainly conjugated, water soluble bilirubin. Therefore, the value of unconjugated bilirubin may be estimated from the difference between total bilirubin and direct bilirubin.

## REAGENT COMPOSITION

| COMPONENTS | CONCENTRATION |
|---|---|
| **Reagent 1** | |
| EDTA-$Na_2$ | 0.1 mmol/L |
| NaCl | 150 mmol/L |
| Sulfamic acid | 100 mmol/L |
| **Reagent 2** | |
| 2,4-Dichlorophenyl-diazonium salt | 0.5 mmol/L |
| HCl | 900 mmol/L |
| EDTA-$Na_2$ | 0.13 mmol/L |

## REAGENT PREPARATION

**Substrate Start:**
The reagents are ready to use.
**Sample Start:**
Not possible.

## REAGENT STABILITY AND STORAGE

| | |
|---|---|
| Conditions: | Avoid contamination.<br>Close immediately after use.<br>Reagent 2 must be protected from light!<br>Do not freeze the reagents. |
| Storage: | at 2 – 8 °C |
| Stability: | up to the expiration date |

## SAMPLE STABILITY AND STORAGE

It is very important to store the sample protected from light!

| | | |
|---|---|---|
| Stability [3]: | at 20 – 25 °C | 2 days |
| | at 4 – 8 °C | 7 days |
| | at - 20 °C * | 6 months |

* in case of immediate freezing. Freeze only once!
Discard contaminated specimens.

## MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

## MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.
**Substrate Start:**

| Pipette into test tubes | Blank | Calibr. | Sample |
|---|---|---|---|
| Reagent 1 | 1000 µL | 1000 µL | 1000 µL |
| Sample | - | - | 50 µL |
| Calibrator | - | 50 µL | - |
| Dist. water | 50 µL | - | - |
| Mix. Incubate for 3 – 5 min. (20 – 25 °C / 37 °C) and read absorbance A1 against reagent blank. Then add: | | | |
| Reagent 2 | 250 µl | 250 µl | 250 µl |
| Mix. Incubate for 5 min. (37°C) or 10 min. (20 – 25 °C) and read absorbance A2 against reagent blank. Calculate: ΔA = A2 – A1. | | | |

## CALCULATION

$$\text{Bilirubin [mg/dL]} = \frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times \text{conc. cal. [mg/dL]}$$

## UNIT CONVERSION

Bilirubin [mg/dL] x 17.1 = Bilirubin [µmol/L]

## REFERENCE RANGE [1] *

Adults and children ≤ 0.2 mg/dL (≤ 3.4 µmol/L)

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

## PERFORMANCE CHARACTERISTICS

***LINEARITY, MEASURING RANGE***

The test has been developed to determine bilirubin concentrations within a measuring range from 0.1 – 10 mg/dL. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the results multiplied by 2.

**SENSITIVITY/LIMIT OF DETECTION**
The lower limit of detection is 0.1 mg/dL

**PRECISION (at 37°C)**

| Intra-assay n = 20 | Mean [mg/dL] | SD [mg/dL] | CV [%] |
|---|---|---|---|
| Sample 1 | 0.36 | 0.01 | 3.12 |
| Sample 2 | 0.76 | 0.01 | 1.46 |
| Sample 3 | 2.07 | 0.03 | 1.30 |

| Inter-assay n = 20 | Mean [mg/dL] | SD [mg/dL] | CV [%] |
|---|---|---|---|
| Sample 1 | 0.35 | 0.01 | 3.34 |

**I.S.E S.r.l.**
Via Delle Driadi, 45 – 00133 Roma - Italy
Tel.+39 0774579365; FAX +39 0774353085
E-mail: info@logotech-ise.com
www.logotech-ise.com

**Revision A R3330000010 11-2018**

| | | | |
|---|---|---|---|
| Sample 2 | 0.75 | 0.01 | 1.00 |
| Sample 3 | 2.13 | 0.02 | 0.71 |

**SPECIFICITY/INTERFERENCES**

no interference up to:

| | |
|---|---|
| Ascorbic acid | 30 mg/dL |
| Hemoglobin | 50 mg/dL |
| Triglycerides | 1000 mg/dL |
| Naproxen | 1 mmol/L |

For further information on interfering substances refer to Young DS [5].

**METHOD COMPARISON**

A comparison between this Bilirubin Auto Direct (y) and a commercially available test (x) using 85 samples gave following results: y = 0.95 x + 0.04 mg/dL; r= 0.995.

## CALIBRATION

The assay requires the use of a Bilirubin Standard or Calibrator. We recommend our Multicalibrator. This method has been standardized against the manual Jendrassik-Gróf test.

## QUALITY CONTROL

All control sera with bilirubin values determined by this method can be used.
We recommend our serum controls Normal control serum (control serum with values in the normal range) and Pathological control serum (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

## AUTOMATION

Special adaptations for automated analyzers can be made on request.

## WARNINGS AND PRECAUTIONS

1. Reagent 1 and 2: Warning.
   H290: May be corrosive to metals.
   P234: Keep only in original container.
   P390: Absorb spillage to prevent material damage.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
4. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

## WASTE MANAGEMENT

Please refer to local legal requirements.

## REFERENCES

1. Thomas L ed. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998: p.192 – 202.
2. Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p. 1125-77
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 18-9.
4. Rand RN, di Pasqua A. A new diazo method for the determination of bilirubin. Clin Chem 1962; 6:570-8.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interferene in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

**Symbols used on labels and packaging**

IVD = In vitro diagnostic medical device
REF = Catalogue Number
LOT = Lot Number
= Manufacturer
= Expiration date
= Temperature limitation
= Instruction for use

**I.S.E S.r.l.**
Via Delle Driadi, 45 – 00133 Roma - Italy
Tel.+39 0774579365; FAX +39 0774353085
E-mail: info@logotech-ise.com
www.logotech-ise.com