

# DIRECT BILIRUBIN

## Colorimetric determination of Direct Bilirubin in serum and plasma

### TEST SUMMARY

The test is based on the modified Jendrassik-Gröf method.

Direct Bilirubin in acid solution reacts with diazotised sulfanilic acid, forming a coloured compound (azobilirubin) which absorbs at 555 nm. The colour intensity is proportional to the direct bilirubin concentration present in the sample.

### SAMPLES

Fresh and not hemolyzed serum or plasma.

Stability: 2 hours at room temperature in the dark or 12 hours at 2-8°C.

Direct light can cause a decrease in Direct Bilirubin in the sample up to 50% in one hour.

Gently shake and bring the samples to room temperature before use.

The turbidity in the sample due to the presence of macromolecules can interfere.

In that case are suggested the centrifugation or filtration with membrane filters 0.2 µ.

### REAGENTS

Reagent A: Sulfanilic acid 30 mmol/L, Chloridric acid 0,25 mol/L.

Reagent B: Sodium Nitrite < 10 mmol/L.

### MATERIAL REQUIRED BUT NOT SUPPLIED

Standard Serum with known concentration of Direct Bilirubin.

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatisation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Distilled water.

### PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

### REAGENTS PREPARATION

The reagents are stable until the expiration date indicated on the package, stored at 4-30°C if not contaminated during use.

Prepare the Working Reagent by mixing 15 parts of Reagent A with 1 part of reagent B (es.: 30 mL Reagent A + 2 mL Reagent B).

The working reagent is stable for 7 days at 2-8°C, protected from light if not contaminated.

### PROCEDURE

Kind of analysis:	End Point
Reading time:	5 minutes
Wavelength:	555
Temperature:	R.T.
Colour Stability:	30 minutes
Lightpath:	1 cm
Zero:	Demineralized water

REAGENTS	Standard BLANK	Standard	Sample BLANK	Sample
Standard	100 µl	100 µl	--	--
Sample	--	--	100 µl	100 µl
Reagent A	1 ml	--	1 ml	--
Working Reagent	--	1 ml	--	1 ml

Mix and incubate at room temperature. After 5 minutes EXACT read the absorbances against demineralized water at 555 nm. The colour is stable for 30 minutes at room temperature.

### CALCULATION

#### Against Standard

Direct Bilirubin mg/dl

$$\frac{A_{(\text{Sample})} - A_{(\text{SampleBlank})}}{A_{(\text{Standard})} - A_{(\text{StandardBlank})}} \times \text{standard value}$$

#### Against Factor

Direct Bilirubin mg/dl

$$A_{(\text{Sample})} - A_{(\text{SampleBlank})} \times 10$$

#### Conversion

Direct Bilirubin µmol/L

$$\text{Direct Bilirubin mg/dl} \times 17.1$$

### EXPECTED VALUES

Direct Bilirubin (mg/dl) = up to 0.25

Every laboratory should establish own reference intervals in accordance with own population.

### NOTES

- If the determination is not made against standard, the wavelength must be exactly collimated in instruments having a non-excessively large bandwidth. With instruments having lower characteristics, determine the factor using control sera as standard.
- Reaction volumes can be varied proportionally.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

### CALIBRATION/ QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

CC03100 10 x 5 ml

Control Serum normal values

CC03200 10 x 5 ml

Control Serum pathological values

### TEST PERFORMANCE

#### Precision

Intra-assay (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	0.688	0.010	1.52
Sample 2	1.774	0.012	0.69

Inter-assay (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	0.702	0.013	1.83
Sample 2	1.816	0.013	0.74

#### Sensitivity/limit of detection

The method is able to discriminate until 0.1 mg/dl.

#### Linearity

The method is linear up to 20 mg/dl. For concentrations higher than 20 mg/dl, repeat the analysis on a diluted 1:2 sample with saline solution and multiply the result by 2.

#### Interferences

The presence of hemoglobin and serum Hyperlipemic can give erroneous results due to interference.

In particular, the hemoglobin can lead to low results while the lipids can lead to high results.

Reaction is inhibited from reducing substances, so sera of patients under massive treatment with ascorbic acid could give low false values.

#### Methods comparison

A comparison with a commercial available product gave the following results in a comparison on 71 samples:

Direct Bilirubin LTA = x  
Direct Bilirubin competitor = y  
n = 50

$$y = 0.97608x + 0.02703 \quad r = 0,99744$$

### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

### PACKAGING

CODE CC00620	(400 TESTS)	
Reagent A	4 x 95 ml	(liquid)
Reagent B	1 x 25 ml	(liquid)

### REFERENCES

- Jirsa M., Vecerek B. – Bd. 311:87 (1958).  
Cherian G., Soldin St. Clin. Chem. 27/5:748 – 752(1981).  
Maringoni A., Federici G., Teruzzi A., Frigerio M. Communication 20th SIBioC, Roma (1988).  
Zilva JF, Pannall PR. "Liver Disease and Gall Stones" in Clinical Chemistry in Diagnosis and Treatment". Lloyd-Luke 1979; Chap XIII: 286-8.  
Tietz Textbook of Clinical Chemistry, Second Edition. Burtis CS and Ashwood ER (Eds), WB Saunders Company, 1994.  
Young DS. Effects of Drugs on Clinical Laboratory Tests, Third Edition. AACCPress, 1990.  
Watchel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine, 1995; 26: 593-7.  
National Committee for Clinical Chemistry Standards. User evaluation of Precision Performance of Clinical Laboratory Devices NCCLS; 1984, NCCLS Publication EP5-T.

### MANUFACTURER

LTA s.r.l.  
Via Milano 15/F, 20060 Bussero (Milan) - ITALY  
Tel: +39 02 95409034 - Fax: +39 02 95334185  
e-mail: info@LTAonline.it - Website: http://www.LTAonline.it

### SYMBOLS

- Only for IVD use
- Lot of manufacturing
- Code number
- Storage temperature interval
- Expiration date
- Warning, read enclosed documents
- Read the directions
- Biological risk

Mod. 01.06 (ver. 2.0 – 02/07/2018)

