TOTAL BILIRUBIN

Colorimetric determination of Total Bilirubin in serum and plasma

TEST SUMMARY

In acid solution in presence of diazotised Sulfanilic acid and DMSO, the Total Bilirubin reacts forming a coloured azocompound (azobilirubin) which intensity is proportional to the concentration of Total Bilirubin present in the sample.
Direct Bilirubin (conjugated with glucuronic acid) reacts directly with diazotate sulfanilic acid. The presence of Dimethyl sulfoxide (DMSO) as an accelerator allows the indirect Bilirubin to react.

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

REAGENTS

Reagent A:	Sulfanilic acid 6 mM, DMSO 7 mM, surfactants and preservatives
Reagent B:	Sodium Nitrite 20 mmol/L.

MATERIAL REQUIRED BUT NOT SUPPLIED

Standard Serum with known concentration of Total Bilirubin.
Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. 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 20 parts of Reagent A with 1 part of reagent B (es.: 40 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 for 5 minutes at room temperature.
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

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

Against Factor
Direct Bilirubin mg/dl

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

Conversion
Direct Bilirubin µmol/L

Direct Bilirubin mg/dl x 17.1

EXPECTED VALUES

Total Bilirubin (mg/dl) = 0.1 - 1.2 mg/dl

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	1.357	0.016	1.18
Sample 2	5.104	0.020	0.39

Inter-assay (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	1.348	0.025	1.85
Sample 2	5.111	0.021	0.41

Sensibility/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 = 0.98595x + 0.02322 r = 0,9974

WASTE DISPOSAL

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

PACKAGING

CODE CC00720	(400 TESTS)	
Reagent A	4 x 100 ml	(liquid)
Reagent B	1 x 20 ml	(liquid)

REFERENCES

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SYMBOLS

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

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