

Direct + Total BILIRUBIN

Modified Jendrassik method

R1(D): 1x100 ml + R1(T): 1x100 ml + R2 2x10 ml

CL12-220

INTENDED USE

Kit for quantitative determination of Direct and Total Bilirubin in serum.

CLINICAL MEANING

Analysis of total and direct bilirubin is made to determine the presence of hepatic damages or diseases e.g. obstruction of bile ducts, haemolytic anemias, metabolic issues, stones. A typical sign of high bilirubin levels is jaundice, which manifests with yellow skin and sclerae.

PRINCIPLE

Direct bilirubin reacts in an acid medium with diazotized sulphanilic acid to form a diazo pink compound (azobilirubin), whose intensity is proportional to the concentration of direct bilirubin present in the sample.

In presence of quaternary ammonium salt in an acid medium, total bilirubin reacts with diazotized sulphanilic acid to form a diazo pink compound (azobilirubin), whose intensity is proportional to the concentration of total bilirubin present in the sample.

SAMPLE

Non hemolyzed serum.

Analyze the samples within 2 hours from collection. Protect from light.

REAGENTS

Only for in Vitro diagnostics. Liquid reagents ready to use.

Package content	CL12-220
REAGENT 1(D) Sulphanilic acid 1,75 mmol/L, hydrochloric acid 0,015 mmol/L	1 x 100 ml
REAGENT 1(T) Sulphanilic acid 3,5 mmol/L, hydrochloric acid 0,09 mmol/L, CTAB 7 g/L	1 x 100 ml
REAGENT 2 Sodium nitrite 7 mmol/L	2 x 10 ml

Stability: store at 15-30°C and protect from light to keep the reagents stable up to the expiration date on the label. Keep bottles closed when not in use.

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

MANUEL ASSAY PROCEDURE

Wavelength	546 nm
Optical path	1 cm
Reading	Against blank sample
Temperature	37°C
Method	Increasing endpoint
Reaction time	10 minutes
Sample/reagent Ratio	1/16

Bring the reagents to the chosen temperature for the analysis.

Pipette in cuvette:

	Direct Bilirubin		Total Bilirubin	
	Blank sample	Sample	Blank sample	Sample
Reagent 1 (D)	1,5 ml	1,5 ml	---	---
Reagent 1 (T)	---	---	1,5 ml	1,5 ml
Reagent 2	---	100 µl	---	100 µl
De-min. water	100 µl	---	100 µl	---
Sample	100 µl	100 µl	100 µl	100 µl

Mix carefully. After exactly 10 minutes of incubation at 37°C, read the sample absorbance (AbsS) against the blank sample (AbsSB). The color is stable for about 60 minutes at room temperature and protected from direct light.

Reaction volumes can be proportionally varied without any change in calculation.

CALCULATION

Calculate the concentration in the sample using the following formula:

- direct bilirubin:

$$[\text{mg/dl}] = (\text{AbsS} - \text{AbsSB}) \times 14,5$$

$$[\mu\text{mol/l}] = (\text{AbsS} - \text{AbsSB}) \times 239$$

- total bilirubin:

$$[\text{mg/dl}] = (\text{AbsS} - \text{AbsSB}) \times 20,4$$

$$[\mu\text{mol/l}] = (\text{AbsS} - \text{AbsSB}) \times 349$$

REFERENCE VALUES

- direct bilirubin: up to 0,25 mg/dl (4,3 µmol/L)

- total bilirubin: 0,2 ÷ 1,2 mg/dl (3,4 ÷ 20,5 µmol/L)

Each laboratory should define its own reference values for this method.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:

PRE-NORM serums with normal values

PRE-PATH serums with pathological values

If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 0,05 mg/dl.

Linearity: up to 25 mg/dl (427 µmol/l).

For higher values, dilute the samples with saline solution and multiply the result by the dilution factor.

Precision:

Within-run n=10	Direct Bilirubin		Total Bilirubin	
	Sample 1	Sample 2	Sample 1	Sample 2
Average [mg/dl]	0,6	2,30	1,05	5,22
SD	0,009	0,08	0,024	0,172
CV %	1,50	3,48	2,37	3,30

Within-run n=20	Direct Bilirubin		Total Bilirubin	
	Sample 1	Sample 2	Sample 1	Sample 2
Average [mg/dl]	0,6	2,38	1,04	5,30
SD	0,015	0,101	0,016	0,118
CV %	2,50	4,24	1,57	2,23

Interferences: up to 150 mg/dl of hemoglobin does not interfere.

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives these equations:

- direct bilirubin:

$$Y = 1,0453X + 0,0062 \quad r = 0,9844$$

- total bilirubin:

$$Y = 0,975X + 0,042 \quad r = 0,9994$$

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided.

REFERENCES

- Pearlman F.C., Lee R.T.Y., Clin. Chem. 20, 447, (1974)
- Blumenfeld T.A. et al., Am. J. Clin. Path. 69, 388 (1978)

MANUFACTURER

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KEY SYMBOLS

	In Vitro diagnostic medical device
	batch number
	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

Issue 01 - Jan 2021 RR