



DIRECT BILIRUBIN LR liquid reagent

REF C1700620/ C1700620A 507 Test

CE C1700650 /C1700650A 1280 Test

IVD For in vitro medical device

"Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (mg/dl)	N = 0.82	H = 2.32
S.D.	N = 0.02	H = 0.06
C.V.%	N = 2.67	H = 2.62

"Inter-Assay" precision (between-Run)

Determined on 20 samples for each control (N-H).Results:

MEAN (mg/dl)	N = 0.82	H = 2.31
S.D.	N = 0.02	H = 0.05
C.V.%	N = 2.69	H = 1.96

Analytical sensitivity

The test sensitivity in terms of detection limit is 0.04 mg/dl.

Correlation

A study based comparing this method with a similar method on 2 samples has given a correlating factor $r = 0.99$

$$y = 1.0036x + 0.0333$$

Interferences

No interference was observed by the presence of

Triglycerides	≤ 500 mg/dl
Haemoglobin	≤ 200 mg/dl

For a comprehensive review of interfering substances, refer to the publication by Young.

Quality Controls

It's necessary, every time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

- Kaplan, L.A., Pesce, A.J.: "Clinical Chemistry", Mosby Ed. (1996).
- Jendrassik, L., Gróf, P., Biochem. Z., 297, 81 (1938).
- Fossati, P., Pontì, M., Principe, L., Tarenghi, G., Clin. Chem., 35, 173 (1989).
- Young D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Washington, DC 5th ed. 2000.

Simbols

CE CE Mark (requirement of 98/79 regulation)

IVD in vitro medical device

LOT Batch Code

Use by

Storage temperature limits

Read instruction for use

Gesana Production srl

Use

Kit for measurement of direct bilirubin in serum or plasma. Colorimetric method Jendrassik - Grof modified.

Summary

Direct bilirubin measurements are used in the diagnosis and treatments of various liver diseases, and metabolic disorders.

Principle

End point analysis. Direct (conjugated) bilirubin reacts with sulphanilic acid and giving a coloured azocompound (azobilirubin). The increase in absorbance due to the formation of azobilirubin is proportional to the direct bilirubin concentration in the sample.

Reagents

R1	Sulphanilic acid	22.0 mmol/l
	preservatives and surface-active agents not anionic	
R2	Nitrite sodium	0.35 mmol/l

Reagent Preparation

Reagents are liquid and ready to use. About using as monoreagent ("sample-starter") dissolve the content of **R2** vial in the **R1** vial. For minor use add to every 4 ml of **R1** reagent, 1 ml of **R2** reagent.

Storage And Stability

- Store the kit at 15-25°C. Do not freeze the reagents.
- After opening, the vials R1 and R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 14 days at 2-8°C.

Precaution In Use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with care, according to good laboratory practice. Caution: the reagents contain Caffeine. Avoid swallowing and contact with skin, eyes and mucous membranes.

Waste Management

Please refer to the local legal requirements.

Sample

- Serum or EDTA-Na₂ plasma.
- Do not use samples with haemolysis.
- Keep the samples far from light and heat because the bilirubin is a photosensitive pigment.
- Perform the samples as soon as possible.

Note

- The kit, according to this method, must be used in manual procedures. About automatic use follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the single vials.

Procedure

Wavelength	λ: 570 (550 – 580) nm
Working Temperature	37°C
Optical Path	1 cm
Reaction	"end point"

Monoreagent Procedure "sample starter"

Working Reagent	Blank	Sample
	1500µl	1500µl
Distilled Water	100 µl	-
Sample	-	100 µl

Mix, then incubate 5' at 37°C. Measure the absorbance of sample (EC) and blank (EBC) against water.

Bireagent Procedure "substrate starter"

Reagent R1	Blank	Sample
	1200µl	1200µl
Distilled Water	100 µl	-
Sample	-	100 µl

Mix, incubate at 37°C for 5' and then add:

Reagent R2		
	300 µl	300 µl

Mix, then incubate 5' at 37°C. Measure the absorbance of sample (EC) and blank (EBC) against water.

Calculation

$$\text{Direct bilirubin [mg/dl]} = (\text{EC}-\text{ECB}) \times 14.5$$

$$\text{Direct bilirubin [µmol/l]} = (\text{EC}-\text{ECB}) \times 248$$

The reagents performances are related to 37°C, 1 cm and 570 nm.

Conversion Factor

$$\text{Bilirubin [mg/dl]} \times 17.0 = \text{Bilirubin [µmol/l]}$$

Reference Values

Adults	0 – 0.2 mg/dl (0 - 3.4 µmol/l)
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Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

Analytical Performances

Linearity

Reaction is linear up to a concentration 10 mg/dl Samples with values exceeding 10 mg/dl must be diluted with saline solution. Then, multiply, the result for diluting factor.