TOTAL BILIRUBIN LR liquid reagent

REF C1600620/C1600620A 6x20 ml
CE **C1600650/C1600650A** 6x50 ml
IVD For in vitro medical device

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

Summary
Total bilirubin measurements are used in the diagnosis and treatments of various liver diseases, and haemolytic and haematology disorders.

Principle
End point analysis. Total bilirubin reacts with sulphanilic acid, giving a coloured azocompound (azobilirubin). The increase in absorbance due to the formation of azobilirubin is proportional to the total bilirubin concentration in the sample.

Reagents

R1	Sulphanilic acid	12.5	mmol/l
	citric acid	1.00	mol/l
	caffeine	0.18	mol/l

preservatives and surface-active agents not anionic

R2	Nitrite sodium	0.52	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.
- 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 caution, 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-Na_2 plasma.
- Do not use samples with haemolysis.
- Do not use lipemic sample.
- 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 using 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 singles vials.

Procedure

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

Monoreagent Procedure "sample starter"

	Blank	Sample
Working Reagent	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"

	Blank	Sample
Reagent R1	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
Total bilirubin [mg/dl] = (EC-ECB) x 14.5
Total bilirubin [µmol/l] = (EC-ECB) x 248

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

Conversion Factor
Bilirubin [mg/dl] x 17.0 = Bilirubin [µmol/l]

Reference Values
Adults 0.3 – 1.2 mg/dl (5.1 - 17.1 µmol/l)

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 25 mg/dl Samples with values exceeding 25 mg/dl must be diluted with saline solution. Then, multiply, , the result for diluting factor.

"Intra-Assay" precision (within-Run)
Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (mg/dl)	N = 1.32	H = 4.10
S.D.	N = 0.03	H = 0.11
C.V.%	N = 2.38	H = 2.71

"Inter-Assay" precision (between-Run)
Determined on 20 samples for each control (N-H).Results:

MEAN (mg/dl)	N = 1.32	H = 4.01
S.D.	N = 0.04	H = 0.08
C.V.%	N = 2.71	H = 1.89

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 20 samples has given a correlating factor **r = 0.99**

y = 1.0077x + 0.034

Interferences
No interference was observed by the presence of

Triglycerides	≤ 200 mg/dl
Haemoglobin	≤ 500 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).
Jakobs, D.S., Kasten, Jr. B.L., Demmott, W.R., Wolfson, W.L.: "Laboratory Test Handbook", Lexi-Comp and Williams & Wilkins Ed. 2nd Edition (1990).
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
	Gesan Production srl

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