Liquick Cor-BIL TOTAL

DIAGNOSTIC KIT FOR DETERMINATION OF TOTAL BILIRUBIN CONCENTRATION

Kit name	**Cat. No**
Liquick Cor-BIL TOTAL 500	2-296
Liquick Cor-BIL TOTAL "bulk"	2-272

INTRODUCTION

Bilirubin is a yellow pigment – product of heme degradation. For clinical purposes, bilirubin is expressed as two fractions: conjugated and unconjugated. In hepatocytes bilirubin is enzymatically conjugated with glucuronic acid residues. This form is called direct or conjugated. Bilirubin without glucuronic acid modification is bound to albumin and is termed unconjugated or indirect. Indirect bilirubin is calculated as the difference between total and direct bilirubin.

Serum bilirubin measurement is widely used as a screening test for liver functions. Hiperbilirubinemia is usually the result of jaundice (mechanical, hemolytic), Dubin-Jonson syndrome, Gilbert's syndrome, Crigler-Najjar syndrome, bile ducts disease.

METHOD PRINCIPLE

Method is based on chemical oxidation, utilizing vanadate as an oxidizing agent.

In the presence of detergent and vanadate in a acidic solution, total bilirubin (both conjugated – direct, and unconjugated bilirubin) is oxidized to produce biliverdin.

This oxidation reaction causes change of the yellow colour, which is specific to bilirubin to the green colour typical for biliverdin. Therefore, the total bilirubin concentration in the sample can be obtained by measuring the absorbance before and after the vanadate oxidation.

REAGENTS

Package

	Liquick Cor-BIL TOTAL 500	**Liquick Cor-BIL TOTAL "bulk"**
1-BIL TOTAL	3 x 417.0 ml	--*
2-BIL TOTAL	1 x 250.2 ml	--*

*reagent volume is printed on the label.

The reagents when stored at 10-25°C are stable up to expiry date printed on the package. On board stability of the reagents depends on type of analyser used for analysis. Do not freeze reagents. Protect from light and avoid contamination!

Concentrations in the test

1-BIL TOTAL	
citrate buffer (pH 2.8)	90 mmol/l
detergent	
2-BIL TOTAL	
phosphate buffer (pH 7.0)	4.6 mmol/l
sodium metavanadate	3.0 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Do not use after expiry date.
- Do not interchange caps.
- Reagent bottles should be shaken before use by gently inverting several times.
- The appearance of turbidity or control sera values outside the manufacturer's acceptable range may indicate of the reagents instability.
- Lack of significant changes in the color of the reaction mixture at the samples with low bilirubin concentration does not indicate the assay malfunction.
- 1-BIL TOTAL meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Warning.

H319 Causes serious eye irritation.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

SPECIMEN

Serum free from hemolysis.

Serum should be separated from red blood cells as soon as possible after blood collection. Lipemic specimens may show falsely decreased bilirubin concentration thus fasting specimen is recommended. It is recommended to follow CLSI procedures regarding specimen collecting and handling.

Because bilirubin is photooxidized when exposed to light, specimen should be protected from direct exposure to either artificial light or sunlight. Therefore it is essential to store specimens in the dark at 2-8°C, at the most 3 days.

Nevertheless it is recommended to perform the assay with freshly collected samples!

ADDITIONAL EQUIPMENT

- automatic analyser or photometer (monochromatic or bichromatic) able to read main wavelength at 420 nm (450 nm);
- thermostat at 37ºC;
- general laboratory equipment;

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	420 nm (450 nm)
temperature	37°C
cuvette	1 cm

Pipette into the cuvettes:

	test (T)	standard (S)
1-BIL TOTAL	1000 µl	1000 µl
calibrator	-	100 µl
sample	100 µl	-

Mix well and after 2 min. of incubation at 37ºC read the absorbance A1 of standard (S) and test samples (T). Then add:

2-BIL TOTAL	200 µl	200 µl

Mix well and after exactly 10 min. of incubation read the absorbance A2 of standard (S) and test (T). Calculate ΔA (A1 – A2) for the test and standard.

Calculation

$$\text{total bilirubin concentration} = \frac{\Delta A(T)}{\Delta A(S)} \times \text{calibrator concentration}$$

REFERENCE VALUES [2]

serum (adults)	0.3 – 1.2 mg/dl 5 – 21 µmol/l

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of manual assay the CORMAY MULTICALIBRATOR LEVEL 2 (Cat. No 5-175; 5-177) is recommended.
For the calibration of automatic analysers the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

Calibration stability depends on type of analyser used for analysis. The calibration curve should be prepared with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
These metrological characteristics have been obtained using the automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.20 mg/dl (3.42 µmol/l).

- **Linearity:** up to 59 mg/dl (1009 µmol/l).

- **Specificity / Interferences**
  Haemoglobin up to 0.25 g/dl, ascorbic acid up to 500 mg/l and triglycerides up to 250 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.93	0.01	1.03
level 2	4.21	0.04	0.83

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.91	0.03	3.61
level 2	4.10	0.12	2.85

- **Method comparison**

A comparison between total bilirubin values determined at Biolis 24i Premium (y) and at Olympus AU400 (x) using 74 samples gave following results:
y = 0.951 x + 0.019 mg/dl;
R = 0.9996 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. Tokuda K. Tanimoto K. New method of measuring serum bilirubin using vanadic acid. Jpn J Clin. Chem. 1993:22(2);116-122.
2. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders; 1999: 1803.
3. Tietz NW. Fundamentals of Clinical Chemistry, 4th ed. Edited by Burtis CA. and Ashwood ER. WB Saunders Company; 1996: 547.

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