Liquick Cor-BIL DIRECT

DIAGNOSTIC KIT FOR DETERMINATION OF DIRECT BILIRUBIN CONCENTRATION

Kit name	Cat. No
Liquick Cor-BIL DIRECT 500	2-297
Liquick Cor-BIL DIRECT "bulk"	2-273

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.

Increased level of direct bilirubin is usually the result of mechanical jaundice, Dubin-Jonson syndrome, bile ducts or gallbladder diseases.

METHOD PRINCIPLE

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

In the presence of detergent and and vanadate in a acidic solution, conjugated (direct) 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 direct bilirubin concentration in the sample can be obtained by measuring the absorbance before and after the vanadate oxidation.

REAGENTS

Package

	Liquick Cor-BIL DIRECT 500	Liquick Cor-BIL DIRECT "bulk"
1-BIL DIRECT	3 x 360 ml	--*
2-BIL DIRECT	1 x 270 ml	--*

*reagent volume is printed on the label.

Reagents stored at 2-8°C are stable until 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 DIRECT

citrate buffer (pH 2.9)	100 mmol/l
detergent	

2-BIL DIRECT

phosphate buffer (pH 7.0)	4.6 mmol/l
sodium metavanadate	4.0 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 DIRECT meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

1-BIL DIRECT contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one, mixture (3:1).

Warning

H317 - May cause an allergic skin reaction.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 If skin irritation or rash occurs: Get medical advice.
P363 Wash contaminated clothing before reuse.

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 436 nm or 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	436 nm /450 nm
temperature	37°C
cuvette	1 cm

Pipette into the cuvette:

	blank (B)	test (T)	standard (S)
1-BIL DIRECT	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

calibrator	-	-	50 µl
sample	-	50 µl	-
distilled water	50 µl	-	-

Mix well and after 5 minutes of incubation read the absorbance A1 of standard (S) and test (T) against blank (B). Then add:

2-BIL DIRECT	250 µl	250 µl	250 µl

Mix well and after exactly 5 min. of incubation at 37°C read the absorbance A2 of standard (S) and test (T) against blank (B). Calculate ΔA for the test and standard:

$\Delta A = (0.8077 \times A1) - A2$

The 0.8077 coefficient compensate the decrease of absorbance after 2-BIL DIRECT addition.

Calculation

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

REFERENCE VALUES [3]

serum (adults)	< 0.4 mg/dl < 6.8 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 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) are 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 automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.05 mg/dl (0.855 µmol/l).

- **Linearity:** up to 40 mg/dl (684 µmol/l).

- **Specificity / Interferences**
  Ascorbic acid up to 62 mg/l and triglycerides up to 650 mg/dl do not interfere with the test. Haemoglobin interferes even in small amount with the determination.

- **Precision**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.34	0.01	2.93
level 2	2.15	0.01	0.58

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.35	0.00	1.18
level 2	2.01	0.02	0.78

- **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 43 samples gave following results:
y = 1.0058 x + 0.0007 mg/dl;
R = 0.996 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Tietz NW. Fundamentals of Clinical Chemistry, 4th ed. Edited by Burtis CA. and Ashwood ER. WB Saunders Company; 547 (1996).
2. Tokuda K. Tanimoto K. New method of measuring serum bilirubin using vanadic acid. Jpn J Clin. Chem. 1993:22(2); 116-122.
3. Akiyama, K. and Makino, I.: Rinsho-I, 19 (Supply.), 242-244 (Japanese) (1993).

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