Liquick Cor-BIL DIRECT MALLOY-EVELYN

DIAGNOSTIC KIT FOR DETERMINATION OF DIRECT BILIRUBIN CONCENTRATION

Kit name	Cat. No
Liquick Cor-BIL DIRECT MALLOY-EVELYN "bulk"	2-290

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

Bilirubin glucuronate reacts directly with sulphodiazonium salt and forms coloured derivative – azobilirubin. The colour intensity of formed azobilirubin measured at 540-550 nm is proportional to direct bilirubin concentration in the sample.

REAGENTS

Package

	Liquick Cor-BIL DIRECT MALLOY-EVELYN "bulk"
1-BIL DIRECT MALLOY-EVELYN	--*
2-BIL DIRECT MALLOY-EVELYN	--*

*reagent volume is printed on the label.

Reagents preparation and stability

The reagents are ready to use.

The reagents are stable up to the kit expiry date printed on the package when stored at 2-25°C. The reagents are stable for 3 weeks on board the analyser at 2-10°C. Protect from light, avoid contamination!

Concentrations in the test

sulphanilic acid	27.74 mmol/l
hydrochloric acid	40 mmol/l
sodium nitrite	1.38 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- 1-BIL DIRECT MALLOY-EVELYN meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

1-BIL DIRECT MALLOY-EVELYN contains hydrochloric acid.

Danger

H314 Causes severe skin burns and eye damage.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor.

1-BIL DIRECT MALLOY-EVELYN contains sulfanilic acid. May produce an allergic reaction (EUH208)

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 546 nm or 550 nm;
- thermostat at 25°C or 37ºC;
- general laboratory equipment;

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 increased bilirubin concentration thus fasting specimen is recommended.

Because bilirubin is photooxidized when exposed to light, specimen should be protected from direct exposure to either artificial light or sunlight.

Serum can be stored at -20ºC at darkness. Nevertheless it is recommended to perform the assay with freshly collected samples!

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	546 nm (Hg 550 nm)
temperature	25°C / 37°C
cuvette	1 cm

Pipette into the cuvette:

	blank (B)	test (T)	standard (S)
1-BIL DIRECT MALLOY-EVELYN	800 µl	800 µl	800 µl

Bring up to the temperature of determination. Then add:

standard	-	-	50 µl
sample	-	50 µl	-
distilled water	50 µl	-	-

Mix well and after 4 minutes of incubation read the absorbance A1 of standard (S) and test (T) against blank (B). Then add:

2-BIL DIRECT MALLOY-EVELYN	100 µl	100 µl	100 µl

Mix well and after exactly 3 min. of incubation at 25°C or after exactly 2 min. of incubation at 37°C read the absorbance A2 of standard (S) and test (T) against blank (B). Calculate ΔA (A2 – A1) for the test and standard.

Calculation

Direct bilirubin concentration = ΔA(T) / ΔA(S) x standard concentration

REFERENCE VALUES

serum (adults)	< 0,3 mg/dl < 5,1 µmol/l

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

QUALITY CONTROL

For internal quality control for manual procedure it is recommended to use the control serum CORMAY SERUM HP (Cat. No 5-173) with each batch of samples, whereas the control serum CORMAY SERUM HN (Cat. No 5-172) should not be used.

For internal quality control for automatic analysers it is recommended to use followed control sera: the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.

For the calibration is recommended the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176).

The calibration curve should be prepared every 1 week, 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 Prestige 24i. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity / Limit of Quantitation:** 0.12 mg/dl (2.05 µmol/l).

- **Linearity:** up to 25 mg/dl (428 µmol/l).

- **Specificity / Interferences**

Haemoglobin and ascorbate interfere even with small amounts. 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	0.34	0.028	8.25
level 2	2.27	0.074	3.27

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.27	0.005	1.75
level 2	1.30	0.020	1.67

- **Method comparison**

A comparison between direct bilirubin values determined at Prestige 24i (y) and at COBAS INTEGRA 400 (x) using 27 samples gave following results:
y = 1.0985 x – 0.0003 mg/dl;
R= 0.9998 (R - correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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