Liquick Cor-BIL TOTAL MALLOY-EVELYN

DIAGNOSTIC KIT FOR DETERMINATION OF TOTAL BILIRUBIN CONCENTRATION

Kit name	Cat. No
Liquick Cor-BIL TOTAL MALLOY-EVELYN 500	2-350
Liquick Cor-BIL TOTAL MALLOY-EVELYN "bulk"	2-349

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.

Hiperbilirubinemia is usually the result of jaundice (mechanical, hemolytic), Dubin-Jonson syndrome, Gilbert's syndrome, Crigler-Najjar syndrome, bile ducts disease.

METHOD PRINCIPLE

Bilirubin and bilirubin glucuronate react with sulphodiazonium salt and form coloured derivative – azobilirubin. Bilirubin glucuronate is soluble in water and reacts directly, bilirubin associated with albumin must be previously hydrolysed with detergents. The colour intensity of formed azobilirubin is proportional to total bilirubin concentration in the sample.

REAGENTS

Package

	Liquick Cor- BIL TOTAL MALLOY-EVELYN 500	Liquick Cor- BIL TOTAL MALLOY-EVELYN "bulk"
1-BIL TOTAL MALLOY-EVELYN	3 x 400 ml	--*
2-BIL TOTAL MALLOY-EVELYN	1 x 300 ml	--*

*****reagent volume is printed on the label.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 10 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Working reagent preparation and stability

Assay can be performed with use of separate 1-BIL TOTAL MALLOY-EVELYN and 2-BIL TOTAL MALLOY-EVELYN reagents or with use of working reagent.

For working reagent preparation mix gently 4 parts of 1-BIL TOTAL MALLOY-EVELYN with 1 part of 2-BIL TOTAL MALLOY-EVELYN. Avoid foaming!

Stability of working reagent: 7 days at 2-8°C
1 day at 15-25°C

Protect from light and avoid contamination!

Concentrations in the test

sulphanilic acid	25.6 mmol/l
hydrochloric acid	40 mmol/l
sodium nitrite	1 mmol/l
detergent	49.6 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for intended purpose, by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- 1-BIL TOTAL MALLOY-EVELYN meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients:

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

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 530 nm (Hg 546, 550 nm);
- thermostat at 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 up to 3 days at 2-8ºC or up to 3 months at -70ºC. Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	530 nm (Hg 546, 550 nm)
temperature	37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvettes:

	test (T)	test blank (TB)	standard (S)	standard blank (SB)
1-BIL TOTAL MALLOY-EVELYN	-	1000 µl	-	1000 µl
working reagent	1000 µl	-	1000 µl	-

Bring up to the temperature of determination. Then add:

sample	50 µl	50 µl	-	-
calibrator	-	-	50 µl	50 µl

Mix well, incubate for 5 min. at 37°C. Then read absorbance of test (T) against test blank (TB) and standard (S) against standard blank (SB). The colour is stable for 30 min.

Calculation

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

Reagent Start method
The determination can be also performed with use of separate 1-BIL TOTAL MALLOY-EVELYN and 2-BIL TOTAL MALLOY-EVELYN reagents.
Pipette into the cuvettes:

	reagent blank (RB)	test (T)	standard (S)
1-BIL TOTAL MALLOY-EVELYN	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

calibrator	-	-	100 µl
sample	-	100 µl	-
distilled water	100 µl	-	-

Mix well and read the absorbance A1 of test (T) and standard (S) against reagent blank (RB). Then add:

2-BIL TOTAL MALLOY-EVELYN	250 µl	250 µl	250 µl

Mix well and after 5 min. of incubation read the absorbance A2 of test (T) and standard (S) against reagent blank (RB). The intensity of colour is stable for 30 minutes. Calculate ΔA (A2 – A1) for the test and standard.

Calculation

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

REFERENCE VALUES [3]

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 is recommended the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177).
The calibration curve should be prepared every 10 weeks, 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 25 mg/dl (428 µmol/l).

For higher bilirubin concentrations dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

- **Specificity / Interferences**

Haemoglobin up to 0.08 g/dl, ascorbate up to 62 mg/l and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	1.09	0.01	0.84
level 2	5.57	0.03	0.59

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	1.10	0.01	0.95
level 2	5.55	0.06	1.09

- **Method comparison**

A comparison between bilirubin total values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 46 samples gave following results:
y = 1.2164 x - 0.057 mg/dl;
R = 0.9995 (R - correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. Malloy H.T., Evelyn K.A.: J. Biol. Chem. 119, 481-490 (1937).
2. Pesce A.J., Kaplan L.A.: Methods in Clinical Chemistry 1105-1119 (1987).
3. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders, 1803, (1999).

Date of issue: 04. 2016

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04/16/04/16