Cat. No 4-548

DIAGNOSTIC KIT FOR DETERMINATION OF DIRECT BILIRUBIN CONCENTRATION

HC – BIL DIRECT

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

1-Reagent	6 x 70 ml
2-Reagent	6 x 18 ml

Reagents stored at 2-8°C are stable until expiry date printed on the package. The reagents are stable for 12 weeks on board the analyser at 2-10°C. Do not freeze reagents. Protect from light and avoid contamination!

Concentrations in the test

1-Reagent	
citrate buffer (pH 2.9)	100 mmol/l
detergent	
2-Reagent	
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.

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!

PROCEDURE
These reagents may be used in automatic analyser Hitachi 911/912.
Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:
1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.
4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

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 automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) is recommended. As a 0 calibrator deionized water should be used.
The calibration curve should be prepared every 12 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 the automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.04 mg/dl (0.684 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	0.36	0.01	2.54
level 2	2.01	0.01	0.47

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.37	0.01	2.68
level 2	1.98	0.03	1.42

- **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 56 samples gave following results:
y = 0.9518 x - 0.0181 mg/dl;
R = 1.000 (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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