Cat. No 4-598

DIAGNOSTIC KIT FOR DETERMINATION OF DIRECT BILIRUBIN CONCENTRATION

HC – BIL DIRECT VANAD

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 vanadate at about pH 3, conjugated (direct) bilirubin is oxidized to produce biliverdin.

This oxidation reaction causes change of the yellow colour, which is specific to bilirubin. 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 69.3 ml
2-Reagent	6 x 17.7 ml

1-Reagent stored at 2-10°C and 2-Reagent stored at 2-35°C are stable until expiry date printed on the package. The reagents are stable for 9 weeks on board the analyser at 2-10°C. Protect from light, avoid contamination!

Concentrations in the test

1-Reagent	
tartrate buffer (pH 2.9)	0.1 mol/l
detergent	
2-Reagent	
phosphate buffer (pH 7.0)	10 mmol/l
sodium methavanadate	4 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel.
- Do not mix or use reagents with different lot numbers.
- Values of control sera outside the manufacturer's acceptable range or the presence of precipitates may indicate of reagent instability.
- Do not dilute the sera for analysis.

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.

Because bilirubin is degraded to biliverdin (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

The reagents are ready to use.

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) is recommended. **Calibrator and deionised water** should be used for calibration.

The calibration curve should be prepared every 9 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 an automatic analyser Hitachi 912. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.07 mg/dl (1.197 µmol/l).

- **Linearity:** up to 40 mg/dl (684 µmol/l).
  Do not dilute the sera for analysis.

- **Specificity / Interferences**
  Ascorbic acid up to 62 mg/l and triglycerides up to 500 mg/dl do not interfere with the test. Haemoglobin interferes even in small amount with the determination.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.37	0.006	1.59
level 2	1.99	0.012	0.59

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.40	0.012	3.11
level 2	2.10	0.031	1.48

- **Method comparison**

A comparison between direct bilirubin values determined at Hitachi 912 (y) and at ADVIA 1650 (x) using 21 samples gave following results:
y = 0.848 x + 0.0083 mg/dl;
R = 0.9991 (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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