Cat. No 9-472

DIAGNOSTIC KIT FOR DETERMINATION OF TOTAL BILIRUBIN CONCENTRATION

OS – BIL TOTAL 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.

Serum bilirubin measurement is widely used as a screening test for liver functions. Hiperbilirubinemia is usually the result of jaundice (mechanical, hemolytic), Dubin-Jonson syndrome, Gilbert's syndrome, Crigler-Najjar syndrome, bile ducts disease.

METHOD PRINCIPLE

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

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

REAGENTS

Package

1-Reagent	6 x 49 ml
2-Reagent	4 x 20.5 ml

The reagents when stored at 10-25°C are stable up to 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 2.8)	90 mmol/l
detergent	
2-Reagent	
phosphate buffer (pH 7.0)	4.6 mmol/l
sodium metavanadate	3.0 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 analysers Olympus AU400/AU640.

1-Reagent and 2-Reagent are ready to use.

For reagent blank deionized water is recommended.

APPLICATION

Reagent ID: **501**

Specific Test Parameters

General | LIH | ISE | Range

Test name: **BilTV** Type: **Serum** Operation: **Yes**

Sample: Volume	10	µL	Dilution	0	µL	Pre-Dilution Rate:	1	
Reagents: R1 Volume	280	µL	Dilution	0	µL	Min OD	Max OD	
R2 Volume	70	µL	Dilution	0	µL	L -2.0000	H 2.5000	
						Reagent OD Limit:		
Wavelength: Pri.	450		Sec.	540		First L -2.0000	First H 2.5000	
Method:	END					Last L -2.0000	Last H 2.5000	
Reaction Slope:	-					Dynamic Range:		
Measuring Point 1: First	0		Last	27		L 0.13	H 69	
Measuring Point 2: First	0		Last	10		Correlation Factor:		
Linearity:		%				A 1.000	B 0.000	
No-Lag-Time:						On-board Stability Period:	84	

Specific Test Parameters

General | LIH | ISE | Range

Test name: **BilTV** Type: **Serum**

Value/Flag: # Level L: # Level H: #

Normal Ranges:

	Sex	Age L Year	Age L Month	Age H Year	Age H Month	L	H
1.	#	#	#	#	#	#	#
2.	#	#	#	#	#	#	#
3.	#	#	#	#	#	#	#
4.	#	#	#	#	#	#	#
5.	#	#	#	#	#	#	#
6.	#	#	#	#	#	#	#
7. None Selected						#	#
8 Out of Range						#	#

Panic Value: L # H # Unit: **mg/dl** Decimal Places: **2**

Calibration Specific

General | ISE

Test name: **BilTV** Type: **Serum**

Calibration Type: **2AB** Formula: **Polygonal** Counts: **3** Process: **CONC**

	Cal. No.	OD	CONC	Factor/OD-L	Factor/OD-H
Point 1:	#		*	-2.0000	2.5000
Point 2:	#		*	-2.0000	2.5000
Point 3:					
Point 4:					
Point 5:					
Point 6:					
Point 7:					

1-Point Cal.Point: with CONC-0 Slope Check: **None** Advanced Calibration: #

MB Type Factor: Calibration Stability Period: **42**

\# User defined

* Calibrator value

REFERENCE VALUES [2]

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 of automatic analysers systems CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) are recommended.

The calibration curve should be prepared every 6 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 Olympus AU400. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.13 mg/dl (2.22 µmol/l).

- **Linearity:** up to 69 mg/dl (1180 µmol/l).

- **Specificity / Interferences**
  Haemoglobin up to 0.25 g/dl, ascorbic acid up to 500 mg/l and 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	0.97	0.005	0.50
level 2	4.20	0.016	0.37

Reproducibility (day to day) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	0.99	0.030	3.01
level 2	4.06	0.117	2.88

- **Method comparison**

A comparison between CORMAY reagent (y) and another commercially available assay (x) using 72 samples gave following results:

y = 0.9247 x + 0.0431 mg/dl;

R = 0.9999 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Tokuda K. Tanimoto K. New method of measuring serum bilirubin using vanadic acid. Jpn J Clin. Chem. 1993:22(2);116-122.
2. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders; 1999: 1803.
3. Tietz NW. Fundamentals of Clinical Chemistry, 4th ed. Edited by Burtis CA. and Ashwood ER. WB Saunders Company; 1996: 547.

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