BILIRUBIN TOTAL VANADATE

Multi-Purpose Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL265BR	4 x 50ml	Reagent 1	10-25 °C
	2 x 25ml	Reagent 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of Total Bilirubin in serum on automated and semi-automated analysers.

SUMMARY AND EXPLANATION:

Serum Bilirubin is present in 4 different isomers. Unconjugated α, monoconjugated β, diconjugated γ and covalently albumin bound δ. Bilirubin is produced from the degradation of red blood cells. It is extracted and biotransformed in the liver and excreted and the bile and urine. Bilirubin measurements are made primarily for the diagnosis of liver diseases and the detection of haemolytic anaemia.

PRINCIPLE OF THE TEST:

In an acidic solution, Total Bilirubin present in a sample reacts with the detergent of Reagent 1 and the Vanadate of Reagent 2 to be oxidised to Biliverdin. The absorbance of the yellow Bilirubin decreases. The Total Bilirubin concentration in the sample is directly proportional to the decreases in absorbance after addition of reagent 2.

Bilirubin (Yellow) $\xrightarrow{\text{Detergent+Vanadate+Acidic\_pH}}$ Biliverdin (Green)

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear colourless liquid.
Reagent 2: Clear, pale yellow liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
Material Safety Data Sheet is available upon request.

Label Elements:

WARNING

H319 - Causes serious eye irritation.

Precautionary Statements:
P280 – Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes.
Remove contact lenses, if present and easy to do. Continue rinsing.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	Ingredients	Initial Concentrations
Reagent 1	Citrate Buffer pH 2.8	90 mmol/l
	DETERGENT	---
Reagent 2	Phosphate Buffer pH 7.0	4.6 mmol/l
	Vanadate	3.0 mmol/l

REAGENT PREPARATION AND STABILITY:

Reagent 1 and 2 are ready for use.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, components are stable until expiry date stated on the label.
Stability on board the Instrument: 28 days.
R1 and R2 should be stored long term at 10-25˚C. However, short term storage (4 weeks) at 2-8˚C is permitted

TYPE OF SPECIMEN:

Use serum as specimen.

It is recommended to follow CLSI procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sampling container, with proper specimen identification. Serum should be assayed fresh.
Storage: It is essential to store specimens in the dark, at 2-8˚C.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
General Chemistry Calibrator	GL983	General Laboratory Equipment	N/A
General Chemistry Control Level 1	GL922		
General Chemistry Control Level 2	GL932		

Assay procedure:

Wavelength: λ: 450 nm
Temperature: 37°C (25°C or 30°C)
Optical path: 1 cm light path.

	Blank	Calibrator	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	40 µl
Calibrator	----	40 µl	----
Gently mix and Incubate at 37°C Measure the Optical Density (OD1) after 2 minutes.			
Reagent 2	250 µl	250 µl	250 µl
Gently mix and Incubate at 37°C Measure the Optical Density (OD2) after 5 minutes.			

Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At intervals established by the Q.C. Laboratory Programme.

CALCULATION:

$$\text{Concentration of Bilirubin} = \frac{\text{Sample}_{OD2} - \text{Sample}_{OD1}}{\text{Calibrator}_{OD2} - \text{Calibrator}_{OD1}} \times \text{Concentration of Calibrator}$$

(Conversion Factor: Qty in µmol/l = 17.1 x Qty in mg/dl.)

EXPECTED VALUES:

	µmol/l	mg/dl
Serum	5.1 – 18.8	0.3 – 1.1

Each laboratory should establish its own reference range. Total Bilirubin results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 600 µmol/l.
For samples with a higher activity, dilute 1:1 with Saline solution 0.9 g/l % NaCl (9 g/l) and re-assay. Multiply result by 2.

Interfering Substances:
Results of study are as follows

Lipemia: Less than 10% interference up to 2.5g/L
Ascorbic Acid: Less than 10% interference up to 50mg/dl
Haemolysis Less than 5% interference up to 2.5g/L.

Sensitivity:

The lowest detectable level of Total Bilirubin Vanadate was estimated at 0.58µmol/L.

Precision:

Within Run N=10	Mean (µmol/L)	SD	%CV	Within Run N=10	Mean (µmol/L)	SD	%CV
Level 1	28.1	0.4	1.3	Level 1	25.2	0.99	3.9
Level 2	77.4	0.7	0.9	Level 2	77.0	0.91	1.2

Method Comparison:

Using 31 samples, a comparison, between this Total Bilirubin Vanadate test (y) and another commercially available test (x) gave the following results:

Y=0.9834 + 0.8165	R^2= 0.9997	Sample Range:2-189.5 µmol/L

BIBLIOGRAPHY:

1. Burtis CA, Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed. 30-54, 601-606 and 966.

SYMBOLS:

The following symbols are used in the labelling of Glenbio systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	STD	Aqueous Standard
CE	CE Mark - Device complies with the Directives 98/79/EC		
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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