# Total Bilirubin Assay Kit (TBil)

**Method:** Vanadate Oxidation

| Cat .No. | Size | Instrument |
|---|---|---|
| GB9020G | R1: 4×90 ml<br>R2: 2×45 ml | For Hitachi 717<br>& ShimadzuCL7200/8000 |
| GS9021G | R1: 6×60 ml<br>R2: 2×45 ml | For Hitachi917<br>& OlympusAU640/400/600 |
| GH9021G | R1: 2×50 ml<br>R2: 1×25 ml | For Hitachi902 |
| GX9021G | R1: 2×80 ml<br>R2: 2×20 ml | For SYNCHRON CX4-5-7-9 /LX20/DXC600-800 |
| GT9021G | R1: 2×50 ml<br>R2: 1×25 ml | For TOSHIBA |

## INTENDED USE

For the *in vitro* quantitative determination of total bilirubin in serum.

## CLINICAL SIGNIFICANCE[1,2,3]

Bilirubin, a product of red blood cell destruction, is a bile pigment normally found in the blood. The average life expectancy of red blood cells is 120 days. Approximately 6 gm of hemoglobin is released per day due to their disintegration. Reticuloendothelial cells from the spleen, liver, and bone marrow phagocytize aged red cells and convert the released hemoglobin to bilirubin. Serum albumin links to bilirubin and transports it to the liver where it is metabolized. Elevated serum bilirubin can indicate impairment of liver excretory function, excessive hemolysis, or biliary tract obstruction. Hyperbilirubinemia can also be associated with obstructive jaundice, hemolytic and hepatic jaundice, infectious hepatitis, and pernicious anemia.

## ASSAY PRINCIPLE

When a sample is mixed with the reagent containing the detergent and the vanadate, at around pH 3, total bilirubin in the sample is oxidized to biliverdin. This causes the absorbance of yellow, specific to bilirubin, to decrease. Therefore, the total bilirubin concentration in the sample can be obtained by measuring the absorbances before and after the vanadate oxidation.

bilirubin $\xrightarrow{\text{vanadic acid}}$ biliverdin

## SAMPLE COLLECTION AND PREPARATION

Serum samples. Should be tested within 2 hours after the collection.

Serum samples are stable for 12 hours at 2-8 ℃, or for 3 months at -20 ℃. Be taken to avoid hemolysis and dark save.

## REAGENT COMPOSITION

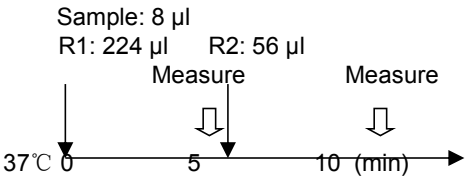
| Contents | Concentration of Solutions |
|---|---|
| **Reagent 1 (R1)** | |
| citric acid buffer solution | 0.1 mol/L |
| surfactants | |
| **Reagent 2 (R2)** | |
| Phosphate buffer solution | 0.01 mol/L |
| Sodium metavanadate | 4 mmol//L |

## STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-35℃.
The reagents are stable for 1 month on-board the analyser after opening .

## ASSAY PROCEDURE

**Test Procedure for Analyzers** (HITACHI 917)
Assay Mode: 2 Point End, 16 - 34
Wave Length (main/sub): 450 nm/546 nm




1. Mix 8 µl sample with 224 µl R1 and incubate at 37℃ for 5 minutes, then read initial absorbance $A_1$ at 450 nm.
2. Add 56 µl R2 into cuvette, mix and incubate for 5 minutes at 37℃, read final absorbance $A_2$.
3. Calculate the absorbance change $\Delta A=A_2-A_1$.

## MATERIALS REQUIRED BUT NOT PROVIDED

Recommend that this assay should be calibrated using Randox Calibration Serum Level 3 or Level 2. Randox Assayed Multi-sera Level 2 (Cat .No:HN1530) and Level 3 (Cat .No: HE1532).

## CALCULATION

$$\text{Concentration} = \frac{A_{sample} - A_{blank}}{A_{calibrator} - A_{blank}} \times \text{Calibrator value}$$

## CALIBRATION

Recommend that this assay should be calibrated using Randox Calibration Serum Level 3 or Level 2.

## QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

## NORMAL VALUE[4]

3-20 µmol/L (0.17-1.17 mg/dl).
It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

## UNIT CONVERSION

mg/dl × 17.1 = µmol/L

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CE For professional use

Revised 01DEC11 version 0901

## SPECIFIC PERFORMANCE CHARACTERISTICS

### LINEARITY
The method is linear up to 800 µmol/l. If the sample above this concentration should be diluted with 0.9% NaCl and reassay. Multiply the result by dilution factor.

### PRECISION
The CV of the test should be less than 5%.

| **Intra assay precision** | | |
|---|---|---|
| N=20 | Level1 | Level 2 |
| Mean (µmol/L) | 83.11 | 25.8 |
| SD | 0.647 | 0.448 |
| CV | 1.019% | 1.738% |
| **Inter assay precision** | | |
| N=5 | Level1 | Level 2 |
| Mean (µmol/L) | 25.67 | 83.47 |
| SD | 0.209 | 0.721 |
| CV | 0.813% | 0.863% |

### SENSITIVITY
The minimum detectable level that can be distinguished from zero has been determined as 1.01 µmol/L.

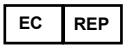
### INTERFERENCE
The following analytes were tested up to the levels indicated and found not to interfere:
Introlipid : 1000 mg/dl
Heparin Na: 60 U/ml
Ascorbic Acid: 50 mg/dl
Hemoglobin: 50 mg/dl

### CORRELATION
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:
Y=1.0273X-0.3805, $R^2$=0.9997; 74 patient samples were analyzed .

### SAFETY PRECAUTIONS AND WARNINGS
1. For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. The regents contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

### REFERENCES
1. Tietz, N.W., Fundamentals of Clinical Chemistry, p. 1028, W.B. Saunders Co., 1976.
2. Annino, J.S., Clinical Chemistry Principles and Procedures, 2nd ed., Little, Brown and Company, Boston, 1960, p. 203.
3. Bilissis, P.K., and Spear, R.J., Clin. Chem., 9, 552 (1963).
4. Shull, B., Clin. Chem., 26, 22 (1980).

### INDEX OF SYMBOLS

| | |
|---|---|
|  | Manufacture |
|  | Catalogue Number |
|  | Lot number |
|  | Date of manufacture |
|  | Use by(Expiration date) |
|  | For In-Vitro Diagnostic use only |
|  | Stored at 2-8℃ |
|  | Attention:See instruction for use |
|  | Authorized Representative in the European Company |

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