

Intended use

Wako Total Bilirubin L-Type is a liquid stable reagent for the quantitative determination of total bilirubin in serum.

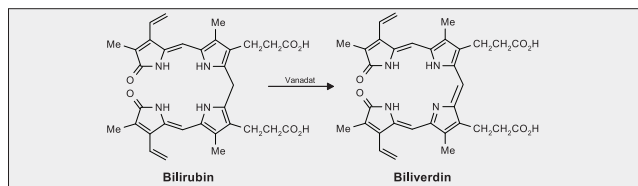
Summary and explanation of the test

Serum bilirubin measurement is widely used as a screening test for liver functions. The methods most widely used for determination of serum bilirubin are the diazo coupling method^{2,3} and the bilirubin oxidase enzymatic method⁴. However, these methods have disadvantages such as interferences by coexistent serum substances and unsatisfactory stability of reagents after preparation. Wako Total Bilirubin L-Type is based on a chemical oxidation method, utilizing vanadate as an oxidizing agent, shows good correlation with conventional methods, practically no interference by coexistent serum substances, and is convenient ready-to-use liquid type reagent⁵.

Principle of the method

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.

Reactions



Reagents

Contents and storage conditions

R1: Buffer Solution	4 bottles	store at 2–35 °C
R2: Vanadate Solution	4 bottles	store at 2–35 °C

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R1: Buffer Solution	Citrate Buffer (pH 2.9) Detergent	0.1 mol/L
R2: Vanadate Solution	Phosphate buffer (pH 7.0) Sodium metavanadate	10 mmol/L 4 mmol/L

R1: Buffer Solution: Use as supplied. This solution is stable until expiration date when stored at 2–35 °C.
R2: Vanadate Solution: Use as supplied. This solution is stable until expiration date when stored at 2–35 °C.

Warnings and precautions

- For *in vitro* diagnostic use.
- For professional use.
- Not to be used internally in humans or animals.
- Do not mix the reagents from one test unit with those of another test unit which has a different lot number.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not use the reagents for any other purpose than described herein.
- After opening the reagent, it is not recommended to store it for a long period of time. When the opened reagent is stored, cap the bottle and keep it at the specified temperature.
- When discarding the reagents, dispose of them according to local or national regulations.

Labelling according to Regulation (EC) No. 1272/2008

- The product is classified and labelled according to the CLP regulation.
- The product does not contain a SVHC according to REACH, Article 57 >0,1 %

Hazard-determining components of labelling

- Cetylpyridinium chloride monohydrate

Hazard pictograms



Hazard statements

- Harmful if inhaled.
- Causes serious eye irritation.
- Very toxic to aquatic life.

Precautionary statements

- Avoid breathing dust/fume/gas/mist/vapours/spray.
- Wash thoroughly after handling.
- Avoid release to the environment.
- Wear eye protection/face protection.
- IF INHALED: Remove person to fresh air and keep comfortable for breathing.
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- Call a POISON CENTER/doctor if you feel unwell.
- Dispose of contents/container in accordance with local/regional/national/international regulations.

Expected values

Total bilirubin in Serum: 0.2–1.0 mg/dL⁶.

Since expected values are affected by age, sex, diet, geographical location, and other factors, each laboratory should establish its own expected values for this procedure.

Physical or chemical indications of instability

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

Instruments

The reagent is designed to be used on commercially available automated analyser. Refer to the operating manual for a description of instrument operation and specifications. A validation by the user in practice at the customer's site in the form of measurements of adequate control or patient sera in sufficient number is indispensable.

Specimen collection and preservation

Freshly prepared serum should be used in this assay procedure. When stored, the serum must be frozen (-20 °C) under conditions with no light exposure since serum bilirubin degrades to biliverdin by light.

Ascorbic acid up to 50 mg/dL does not interfere with the measurement. Hemoglobin concentrations up to 500 mg/dL does not have a significant effect on the measurement.

Standard procedure

37 °C (Hitachi® 911)

0	Sample blank ↓	5	Measurements 10 (min)
↑		↑	
Sample 10 µl		R2: 70 µl	λ (main): 450 nm
R1: 280 µl			λ (sub): 546 nm

Results

The final results are automatically calculated and printed in concentration units (mg/dL).

Limitation of the procedure

Linearity: 0.1–40 mg/dL. When total bilirubin concentration exceeds 40 mg/dL, dilute the sample 1+1 with saline, repeat the assay and multiply result by 2.

Performance characteristics

- Accuracy:** 44 serum samples were assayed by described procedure and by a commercially available method (Azobilirubin). Correlation coefficient: $r = 0.994$; $y = 0.957x - 0.051$.
- Specificity:** When a sample of known concentration is assayed, the measured value is within $\pm 15\%$ of the known concentration. (In the case of a sample of 1 mg/dL total bilirubin or more)
- Precision:** When a sample is assayed 5 times or more in a run, CV is within 5%. (In the case of a sample of 2 mg/dL or more)
- Sensitivity:** a) When purified water is used as a sample, the absorbance is 0.05 or less.
b) When a control serum (10 mg/dL total bilirubin) is used as a sample, the absorbance is 0.12–0.45.

Quality control

A quality control program is recommended for all clinical laboratories.

References

1. Malloy H.T., Evelyn K.L.: The determination of bilirubin with the photoelectric colorimetry. *J. Biol. Chem.*, 199: 481–490, (1937)
2. Jendrassik L., Cleghorn R.A.: Photometrische Bilirubinbestimmung. *Biochem. Z.* 289: 1–14, (1937)
3. Michaelsson M.: Bilirubin determination in serum and urine. *Scand. J. Clin. Lab. Invest.*, 12 (Suppl 56): 1–80, (1937)
4. Murao S., Tanaka N.: A new enzyme „bilirubin oxidase“ produced by *Myrothecium varrucaria* MT-1. *Agric. Biol. Chem.* 45: 2383–2384, (1981)
5. Tokuda K. and Tanimoto K.: New method of measuring serum bilirubin using vanadic acid. *Jpn. J. Clin. Chem.*, 22 (2), 116–122 (1993)
6. Carl A. Burtis, Edward R. Ashwood: *TIEZ TEXTBOOK of Clinical Chemistry*, second edition, P1468 (SAUNDERS)

Bestellinformation

Code No.	Product	Package
417-23295	Total Bilirubin L-Type R1	R1: 4 x 70 mL
419-23495	Total Bilirubin L-Type R2	R2: 4 x 18 mL
419-73295	Bilirubin Calibrator	CAL: 4 x for 3 mL