

411-23695
0521D7

Intended use

Wako Direct Bilirubin L-Type is a liquid stable reagent for the quantitative determination of direct bilirubin in serum and plasma.

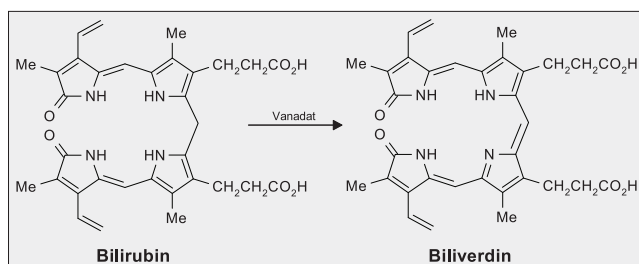
Summary and explanation of the test

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 Direct 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, direct bilirubin in the sample is oxidized to biliverdin. This causes the absorbance of yellow, specific to bilirubin, to decrease. Therefore, the direct 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 2–10°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 2–10 °C. After opening the bottle store at 2–10°C and use within one month.

R2: Vanadate Solution: Use as supplied. This solution is stable until expiration date when stored at 2–35 °C. After opening the bottle store at 2–10°C and use within one month.

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.

This kit (R1) contains components classified as follows according to Regulation (EC) No. 1272/2008. Please note the safety data sheet!

Hazard pictograms:



Signal word: Warning

Hazard statements:
 H319 Causes serious eye irritation.
 H412 Harmful to aquatic life with long lasting effects.

Precautionary statements:

P280 Wear protective gloves/protective clothing/eye protection/face protection.
 P308+P311 IF exposed or concerned: Call a POISON CENTER/doctor.

Supplemental

hazard information: EUH208 Contains hydroxylammonium chloride. May produce an allergic reaction.

Expected values

Direct bilirubin in Serum: 0.0–0.4 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.

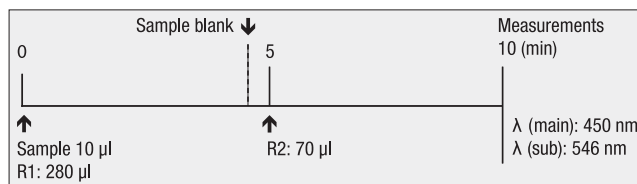
Specimen collection and preservation

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)



Results

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

Limitation of the procedure

Linearity: 0.1–20 mg/dL. When direct bilirubin concentration exceeds 20 mg/dL, dilute the sample 1+1 with saline, repeat the assay and multiply result by 2.

Performance characteristics

Accuracy: 50 serum samples were assayed by described procedure and by a commercially available method (Azobilirubin). Correlation coefficient: $r = 0.993$; $y = 1.003x - 0.070$.

Specificity: When a sample of known concentration is assayed, the measured value is within $\pm 20\%$ of the known concentration. (In the case of a sample of 1 mg/dL direct 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 human sample of 1 mg/dL or more)

Sensitivity: a) When purified water is used as a sample, the absorbance is 0.02 or less.
 b) When a control serum (10 mg/dL direct 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. Jendrossik 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. Akiyama, K. and Makino, I.: *Rinsho-i*, 19 (Suppl.), 242–244 (1993) (Japanese)

Ordering information

Code No.	Product	Package
411-23695	Direct Bilirubin L-Type R1	R1: 4 x 70 mL
413-23895	Direct Bilirubin L-Type R2	R2: 4 x 18 mL
419-73295	Bilirubin Calibrator	CAL: 4 x for 3 mL