**Intended use**

Wako Direct Bilirubin L-Type is a liquid stably 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 function. The methods most widely used for determination of serum bilirubin are the diazo coupling methods and the bilirubin oxidase enzymatic method. However, these methods have disadvantages such as interferences by protein 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 constant serum substances, and is convenient ready-to-use liquid type reagent.

**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**

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.

**Expected values**

Direct bilirubin in serum: 0.0–0.4 mg/dL.

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

**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 analyzers. 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**

Fresly 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 do 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 = 0.953; y = 1.003x - 0.070.

Specificity: When a sample of known concentration is assayed, the measured value is within ±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 0.02 or less.

**Quality control**

A quality control program is recommended for all clinical laboratories.

**References**


