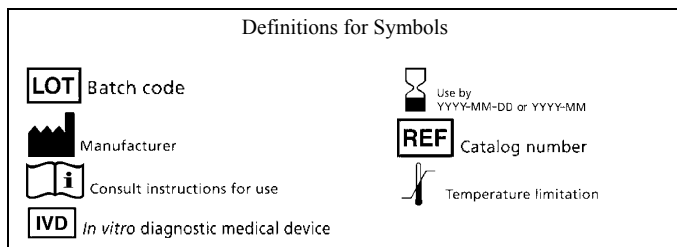


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SEKISUI

DIAGNOSTICS

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TOTAL BILIRUBIN-SL-X ASSAY

CATALOGUE NUMBER:	284-10	SIZE:	R1: 1 x 100 mL,	R2: 1 x 25 mL
	284-30		R1: 3 x 100 mL,	R2: 1 x 75 mL
	284-50A	R1: 1 x 1000 mL		
	284-50B	R2: 1 x 300 mL		

INTENDED USE

For the IN VITRO quantitative measurement of bilirubin (total) in serum.

TEST SUMMARY

Bilirubin is a bile pigment normally found in serum as a result of red cell destruction. It is a product of hemoglobin breakdown by the reticuloendothelial system and exists in two forms. Unconjugated (indirect) bilirubin is transported to the liver bound by albumin where it becomes conjugated (direct) with glucuronic acid and excreted.

The elevation of total serum bilirubin may occur due to hemolytic processes, liver disease, or a disorder of the biliary tract.

Traditional methods of measuring bilirubin are based on the reaction of bilirubin with a diazo reagent to form the colored compound: azo-bilirubin. The diazo reaction can be accelerated by the addition of various chemicals. For example, Malloy-Evelyn⁽¹⁾ used ethanol, Jendrassik-Grof⁽²⁾ used caffeine, and Walters-Gerarde⁽³⁾ used DMSO. Modifications of these methods included the addition of surfactants as solubilizing agents.⁽⁴⁾

In this method, a 2,4-dichlorophenyldiazonium salt is used as the diazo reagent and the reaction is facilitated by the use of a surfactant.

TEST PRINCIPLE

Surfactant

Total Bilirubin + 2,4-dichlorophenyldiazonium salt → Azobilirubin

Bilirubin (conjugated and unconjugated) couples with the diazo reagent in the presence of a surfactant to form azobilirubin. The increase in absorbance at 546 nm is directly proportional to the total bilirubin concentration.

REAGENTS

TBili-SL-X Accelerator Reagent (R1): A solution containing 154 mmol/L NaCl, 190 mmol/L HCl, surfactants, and preservatives.

TBili-SL-X Diazo Reagent (R2): A solution containing 417 mmol/L HCl, 5 mmol/L 2,4 dichlorophenyldiazonium salt and a surfactant.

WARNINGS & PRECAUTIONS FOR USE

S24/25: Avoid contact with skin and eyes.
See Material Safety Data Sheet for additional information.

REAGENT, PREPARATION, STORAGE & STABILITY

The reagents are provided in a ready to use format.

Supplied reagents are stable at 2-8°C until expiry date.

Stability claims are based on real time studies.

REAGENT DETERIORATION

The reagents should be clear. Turbidity would indicate deterioration.

DISPOSAL

Reagents must be disposed of in accordance with all Federal, Provincial, State, and local regulations.

SPECIMEN

Fresh, clear, unhemolysed serum.

SAMPLE STORAGE

The specimen must be protected from light. Specimens for analysis should be stored at 2-8°C and are stable for 3 days. Specimens may be stored frozen at minus 70°C for up to 3 months.⁽⁵⁾

ANALYTICAL SPECIFICITY

Cross contamination studies have not been performed on automated instruments. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Interferences from hemoglobin and lipemia were evaluated for this total bilirubin method on a Roche/Hitachi® analyzer using a significance criterion of > 10% variance from control.

Concentration		Substance Tested	Concentration of Interferent Where Interference is Insignificant	
Conv. Units	SI Units			
1.35 mg/dL	23.1 µmol/L	Hemoglobin	400 mg/dL	62 µmol/L
1.75 mg/dL	29.7 µmol/L	Intralipid	1000 mg/dL	3000 mg/dL (33.9 mmol/L) Simulated Triglycerides

The information presented is based on results from Sekisui Diagnostic studies and is current at the date of publication.

A summary of the influence of drugs on assays may be found by consulting Young, D.S.⁽⁶⁾

ANALYTICAL PROCEDURE

MATERIALS PROVIDED

Sekisui Diagnostics' Total Bilirubin-SL-X reagents.

MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Automated analyzer capable of accurately measuring absorbance at appropriate wavelength as per instrument application.
2. Calibration material.
3. Quality Control materials.

TEST CONDITION

For the data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:32:8 and a wavelength reading of 546 nm. For assistance with applications on automated analyzers within Canada and the U.S., please contact Sekisui Diagnostics Technical Services at (800)565-0265. Outside Canada and the U.S., please contact your local distributor.

CALIBRATION

Calibration material should be used to calibrate the procedure. The frequency of calibration, if necessary, on automated systems is dependant on system and parameters used.

QUALITY CONTROL

A normal and abnormal concentration control should be analyzed as required in accordance with local, state, and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

CALCULATIONS

The analyzer automatically calculates the total bilirubin concentration of each sample.

TEST LIMITATIONS

A sample with a total bilirubin concentration exceeding the linearity limit should be diluted with 0.9% saline and reassaying incorporating the dilution factor in the calculation of the value.

REFERENCE INTERVALS⁽⁵⁾

0.2-1.0 mg/dL (3.4-17.1 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

PERFORMANCE CHARACTERISTICS

Data presented was collected on a Roche/Hitachi® analyzer unless otherwise stated.

RESULTS

Bilirubin (Total) concentration is reported as mg/dL (µmol/L).

REPORTABLE RANGE (CLSI EP6)⁽⁷⁾

The linearity of the procedure described is 20.00 mg/dL (342.0 µmol/L). The lower limit of detection is 0.04 mg/dL (0.7 µmol/L). This data results in a reportable range of 0.04-20.00 mg/dL (0.7-342.0 µmol/L).

PRECISION STUDIES (CLSI EP5)⁽⁷⁾

Day to day data was collected on two control sera using two lots of reagent in 40 runs conducted over 20 days.

Concentration		Day -To-Day SD		Day-To-Day CV %
mg/dL	µmol/L	mg/dL	µmol/L	
0.69	11.7	0.02	0.3	3.4
6.95	118.2	0.16	2.7	2.3

Within run precision data was collected on two control sera, using a single lot of reagent, each run 20 times in a single assay.

Concentration		Within-Run SD		Within-Run CV %
mg/dL	µmol/L	mg/dL	µmol/L	
0.67	11.4	0.01	0.2	1.7
6.76	114.9	0.06	1.0	0.8

ACCURACY (CLSI EP9)⁽⁷⁾

The performance of this method (y) was compared with the performance of a similar method (x) on a Roche/Hitachi® analyzer. Forty samples ranging from 0.18 mg/dL to 19.76 mg/dL (3.1 µmol/L to 335.9 µmol/L) gave a correlation coefficient of 0.9990. Linear regression analysis gave the following equation:

$$\text{This method} = 1.02 (\text{reference method}) + (0.03 \text{ mg/dL}) 0.5 \text{ µmol/L.}$$

These performance characteristics were generated in Sekisui Diagnostics' laboratories using automated procedures unless otherwise stated.