BILIRUBIN, DIRECT

(LIQUID)

R1 3x28, R2 3x7ml

RE – ORDER BRD1050

INTENDED USE:

This reagent is intended for the quantitative in vitro determination of direct bilirubin in serum.

TEST SUMIMARY:

Bilirubin is formed from the heme portion of hemoglobin released by aged or damaged red blood cells. It is then converted in the liver to bilirubin monoglucuronide and bilirubin diglucuronide. The reaction of bilirubin with diazotized sulfanilic acid was first described by Ehrlich (1) and was subsequently used by Van den Bergh (2, 3) to provide the first method for the qualitative and quantitative determination of bilirubin in normal and pathological serum samples. Modifications and improvements of this reaction, in acid and alkaline medium have been described by many authors. This same reaction is employed in the present procedure.

Free bilirubin is not soluble in aqueous solution and requires solubilization by alcohols or other solvents to react. Reactions carried out in these solvents provide measurements of total bilirubin. Mono and diglucuronides of bilirubin are water soluble and measurements performed in aqueous solution measure what in this form is called direct bilirubin.

The assay of bound (direct) bilirubin is performed in an aqueous acid solution of diazotized sulfanilic acid. The intensity of color of the diazo dye formed with bilirubin in aqueous solution is proportional to the concentration of direct bilirubin.

REAGENT COMPOSITION:

The kit is composed of 2 reagents. The reagents have the following composition:

Reactive ingredients:

Bilirubin Direct Reagent

Sulfanilic Acid 32.2 mmol/L

Bilirubin Activator

Sodium Nitrite 109 mmol/L

Non-reactive buffers, stabilizers and fillers:

REAGENT PREPARATION:

Ready to use.

REAGENT STORAGE AND STABILITY:

Store the reagents in the refrigerator (2–8 °C). The separate reagents are stable until the expiration date on the label. The mixed working reagent is stable for 8 hours in amber bottles at room temperature (22–28 °C).

Discard the Bilirubin Activator if it develops a yellow coloration.

PRECAUTIONS:

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE:

Use only clear, unhemolyzed serum. Bilirubin is unstable in the samples and the assay should be completed within 2 hours from collection. If longer delay is unavoidable, refrigerate the sample (4).

Protect samples from direct solar and artificial light. Samples can be frozen at -15 °C or below, in which case bilirubin is stable for two months.

INTERFERING SUBSTANCES:

Hemolyzed samples should not be used for this determination. Young (5) has published a comprehensive list of drugs and substances which may interfere with in vitro diagnostic assays, including that of bilirubin in serum.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 555 nm.

Matched cuvettes.

Constant temperature incubator set at 30 °C or 37 °C. Use the same temperature for assay of standard, controls and samples.

Pipettes to measure reagent and samples.

Distilled or deionized water

Direct Bilirubin Standard or Calibrator. If not available, then factor from Total Bilirubin assay.

MATERIALS PROVIDED:

Bilirubin Direct Reagent

Bilirubin Activator

TEST PROCEDURE:

Bring reagents to selected incubation temperature, 30 °C or 37 °C.

Procedure A:

Using a Direct Bilirubin Standard or Calibrator

Wavelength: 555 nm.

	Standard		Sample	
	Blank	**Test**	Blank	Test
Bilirubin Direct Reagent	3 mL	3 mL	3 mL	3 mL
Bilirubin Activator	–	20 µL	–	20 µL
Standard	0.2 mL	0.2 mL	–	–
Sample	–	–	0.2 mL	0.2 mL

Mix without delay. Incubate for 3 minutes at 30 °C or for 1 minute at 37 °C. Zero the spectrophotometer with distilled or deionized water. Immediately after the selected incubation, read absorbance of each test and its respective blank. Subtract the absorbance of each blank from the absorbance of the respective test. This is the corrected absorbance (A_c) for each standard and sample.

Procedure B:

Using a Factor from the Total Bilirubin Assay

Wavelength: 555 nm.

	Sample	
	Blank	Test
Bilirubin Direct Reagent	3 mL	3 mL
Bilirubin Activator	–	20 µL
Sample	0.2 mL	0.2 mL

Use Total Bilirubin Reagent (Order No. BRT1060)

	Standard	
	Blank	Test
Bilirubin Total Reagent	3 mL	3 mL
Bilirubin Activator	–	20 µL
Standard	0.2 mL	0.2 mL

Mix without delay. Incubate for 3 minutes at 30 °C or for 1 minute at 37 °C. Zero the spectrophotometer with distilled or deionized water. Immediately after the selected incubation, read absorbance of each test and its respective blank. Subtract the absorbance of each blank from the absorbance of the respective test. This is the corrected absorbance (A_c) for each standard and sample.

CALIBRATION:

The assay requires the use of a Direct Bilirubin Standard or Calibrator. If a standard or calibrator is not available, then a factor, based upon the absorbance of the standard, as measured with the Total Bilirubin Assay, may be used.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Commercially available control material with established values for direct bilirubin concentrations may be used.

CALCULATIONS:

Procedure A:

$$\frac{A_c \text{ Sample}}{A_c \text{ Standard}} \times \text{Conc. of Std.} = \text{mg/dL of direct (conjugate) bilirubin in sample.}$$

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Sample Calculation:
A_c of Sample = 0.408
A_c of Standard = 0.322
Concentration of Standard = 4.6 mg/dL

$\frac{0.408}{0.322} \times 4.6$ mg/dL = 5.8 mg/dL direct (conjugate) bilirubin in sample.

Procedure B:

A_c Sample x Factor = mg/dL of direct (conjugate) bilirubin in sample

Where: Factor = $\frac{\text{Concentration of Standard}}{A_c \text{ Standard}}$

Sample Calculation:
A_c of Sample = 0.408
A_c of Standard = 0.350
Concentration of Standard = 5.0 mg/dL
Factor = $\frac{5.0}{0.350} = 14.29$
0.408 x 14.29 = 5.8 mg/dL direct (conjugate) bilirubin in sample

LIMITATIONS OF THE PROCEDURE:

Samples with bilirubin concentrations higher than 20 mg/dL should be diluted with an equal volume of water and assayed again; multiply results by 2.

REAGENT PERFORMANCE:

Linearity: The assay is linear to 20 mg/dL.

Correlation: Employing a commercial reagent (Sclavo) as reference, results were obtained in 39 serum samples ranging in concentration from 0.08 to 9.76 mg/dL; the correlation coefficient was 0.998 and the regression equation was:
y = 0.989x + 0.001.

Precision:

Within Run:

Mean (mg/dL)	5.44	0.306
SD	0.004	0.011
CV	0.078	3.75
N	10	10

REFERENCE RANGE:

The following table is extracted from reference (6):
Suggested Reference Range: **0-0.5 mg/dL**
Conjugated bilirubin: 0-0.2
Total bilirubin: 0.2-1
It is recommended that each laboratory establish its own reference range.

REFERENCES:

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