

Reagent kit for the quantitative determination of total and direct bilirubin in serum. Diazo-sulfanilic acid method.

Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of dimethylsulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. In the absence of dimethylsulfoxide, only the direct bilirubin reacts to give azobilirubin.

Reference value

Serum: direct bilirubin <5.1 μmol/l (<0,3mg/dl)
total bilirubin <17 μmol/l (<1,0mg/dl)

It is recommended that each laboratory should assign its own normal range.

Reagents

A) Total Bilirubin

1. Reagent (RT1)

Sulfanilic acid 3.2 mmol/l
Hydrochloric acid 165mmol/l
Dimethylsulfoxide 7 mmol/l

2. Reagent (R2)

Sodium nitrite 8.6 mmol/l

B) Direct Bilirubin

1. Reagent (RD1)

Sulfanilic acid 3.2 mmol/l
Hydrochloric acid 165mmol/l

2. Reagent (R2)

Sodium nitrite 8.6 mmol/l

Sample

Serum free of haemolysis.

Bilirubin is light sensitive and it is recommended that serum be stored in the dark.

The reagent is not suitable for bilirubin determination of infants.

PROCEDURE

All reagents are ready for use.

Avoid direct exposure to light!

Preparation of working reagent

A: Total bilirubin

Mixing ratio of RT1 and R2: 125 ml/25ml

B: Direct bilirubin

Mixing ratio of RD1 and R2: 125 ml/25ml

Reagents can only be applied with previous mixing!

Stability of the working reagent

20-25°C 1 day

2-8°C 5 days

If the absorbance of working reagent is higher than 0.02 at 546 nm the reagent can not be used.

Assay Conditions

Wavelength: 555 nm (540-560 nm)

Secondary wavelength: 600 nm

Temperature: 37 °C

Cuvette: 1 cm light path

Method: end point

Read against: reagent blank

Pipette into cuvette

A) Total bilirubin:

	Sample	Calibrator
Working reagent	1.0 ml	1.0 ml
Sample	75 μl	-
Calibrator	-	75 μl

A) Direct bilirubin:

	Sample	Calibrator
Working reagent	1,0 ml	1,0 ml
Sample	75 μl	-
Calibrator	-	75 μl

Mix and read the optical density (A) after exactly 3 minutes incubation.

Calibration: (37°C, Diazo with Sulfanilic Acid)

S1:Distilled water

S2:Randox Calibration Serum Level I

S3:Randox Calibration Serum Level II

Calibration is recommended:

- after reagent lot change,

- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{Sample}}{A_{Calibrator}} \times C_{Calibrator} = C_{Sample}$$

A = optical density; C = concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 600 analyzer (37°C).

Linearity

The test is linear up to 340 μmol/l (20.0 mg/dl) bilirubin concentration.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.575 μmol/l (0.034 mg/dl) direct Bilirubin and 1.42 μmol/l (0.08 mg/dl) total Bilirubin concentration at 540 nm.

Direct bilirubin

Precision

	Reproducibility		
	Average concentration (μmol/l)	SD	CV%
sample I	13.8	0.282	2.04
sample II	37.8	1.053	2.79

Correlation

Comparative studies were done to compare our reagent with another commercial direct Bilirubin assay.

The results from these studies are detailed below.

Correlation coefficient: r=0.9957

Linear regression: y (μmol/l)= 1.05x-0.52

(x= other commercial reagent, y= own reagent).

Total bilirubin

Precision

	Reproducibility		
	Average concentration (μmol/l)	SD	CV%
sample I	18.9	0.756	4.00
sample II	82.3	2.902	3.53

Correlation

Comparative studies were done to compare our reagent with another commercial total Bilirubin assay.

The results from these studies are detailed below.

Correlation coefficient: r=0.9998

Linear regression: y (μmol/l)= 0.988x-0.978

(x= other commercial reagent, y= own reagent).

Specificity

Lipid 400 mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50 mg/dl) don't interfere with the assay at the given levels.

Note

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only!

The following symbols are used on labels

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

Bibliography

Hijmans Van den Bergh A. A., Muller P.: *Biochem*, 77; 90, (1916).

Walters M. L., Gerarde R. W.: *Microchem.*, 15; 231 (1970).