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BILIRUBIN DIRECT

STABLE LIQUID REAGENT

CE

Cat. No.: 4274D
2x150 ml
(2x125 ml + 1x50 ml)

Reagent kit for the quantitative determination of 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 <5,1µmol/l (<0,3mg/dl)
total bilirubin <17µmol/l (<1,0mg/dl)

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

Reagents

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 in serum is light sensitive and it is recommended that serum be stored in the dark.
The reagenst is not suitable for bilirubin determination of infants.

Procedure

All reagents are ready for use.
Avoid direct exposure to light!

Preparation of working reagent
Mixing ratio of RD1 and R2: 125 ml/25 ml
Reagents can only be applied with previous mixing!

Stability of working reagent

20-25°C 1 day
2-8°C 5 days

If the optical density 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
Measure: end point
Read against: reagent blank

Pipette into cuvette

	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

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

A = Absorbance, 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.

Linearity
The test is linear up to 100 µmol/l (5,88 mg/dl) bilirubin concentration (37°C).

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 (0,03 mg/dl) Bilirubin concentration at 540 nm.

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 Bilirubin Direct reagent.
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).

Specificity
Lipid 400 mg/dl, glucose 55.5 mmol/l (1000 mg/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

IVD For in vitro diagnostic use
Use by (last day of the month)
Temperature limitation
LOT Batch Code
REF Code

Bibliography
Hijmans Van den Bergh A. A., Muller P.: Biochem, 77; 90, (1916). Walters M. I., Gerarde R. W.: Microchem., 15; 231 (1970).