BILIRUBIN, TOTAL

(LIQUID)

R1 3x42, R2 3x11 ml

RE – ORDER BRB1060

INTENDED USE

This reagent is intended for the quantitative in vitro determination of total bilirubin in serum. For *in vitro* diagnostic use only.

METHOD HISTORY

Since the introduction of the diazo method for bilirubin determination by Ehrlich in 1883[1], several modifications have been proposed to enhance the reaction. The Malloy and Evelyn method[2] employs methanol to catalyze the azo-coupling reaction of the indirect bilirubin, as well as to keep the azobilirubin on solution. A serious disadvantage of this method lies in the fact that protein may precipitated by the methanol solution to yield falsely lowered results.

In 1938, Jendrassik and Grof[3] . presented an assay that gave reliable results. The method is, however, cumbersome and involves several pipetting steps. The method presented here was developed by Wahlefeld et al[4]. A detergent is used to accelerate the reaction and to avoid protein precipitation. The diazo reagent is 2,5- dichlorophenyldiazonim tetrafluoroborate (DPD) that reacts very rapidly in coupling with bilirubin under acidic conditions. The resulting procedure is simple, yet exhibits good correlation when compared with the method Jendrassik and Grof.

PRINCIPLE

Total bilirubin is coupled with a diazonium salt (DPD) in sstongly acid medium (pH 1-2).

Acid, surfactant

Bilirubin + DPD----------→ Azobilirubin

The intensity of the color of the azobilirubin produced is proportional to the total bilirubin concentration and can be measured photometrically.

REAGENTS

1. Total bilirubin R1 reagent: acid buffer 50 mmol/L , Surfactant.
2. Total bilirubin R2 reagent: acid buffer >30 mmol/L, >2.0 mmol/l DPD and stabilizer.

REAGENT PREPARATION

Reagent provided as ready to use liquids.

REAGENT STORAGE

1. Store the reagents in the refrigerator at 2–8 °C.
2. Do not freeze.
3. Avoid exposure to direct sunlight.

REAGENT DETERIORATION

1. Do not use if the reagents show evidence of contamination (tubidity)
2. The R2 may develop very slight precipitation that does not affect performance and will re-dissolve if the R2 is warmed gently.
3. R2 reagent containing a precipitate that dies nit re-dissolve and results in product discoloration should not be used and discarded.
4. Do not use if reagent fails to achieve assigned assay values of fresh control sera.

PRECAUTIONS

1. Reagents are toxic and corrosive. Do NOT pipette by mouth. Avoid contact with skin and clothing.
2. This reagent is for *in vitro* diagnostics use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

1. Fresh, unhemolyzed serum is recommended.
2. Samples should be analyzed within two hours of collection if kept at room temperature in the dark and within twelve hours of kept refrigerated (2-8°C) and protected from light.[5]
3. Bilirubin in serum is stable for three months when frozen (-20°C) and protected form light.[5]
4. Direct Sunlight may cause up to 50% decrease in bilirubin within one hour.[6]
5. Specimen collection should be carried out in accordance with NCCLS M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

INTERFERENCES

In the presence of hemoglobin lower results can be expected in the assay of bilirubin (5, 6). This effect may be negligible up to a level of hemoglobin of 1000 mg/dL Hemolyzed samples should not be used for this determination.

Young, et al.[7] have 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

Calibrator and controls.

MATERIALS PROVIDED

Total Bilirubin R1 and total Bilirubin R2.

TEST PROCEDURE

See instrument set up parameters.

CALIBRATION

Abs. = Absorbance
Unk.= Unknown
Cal.= Calibration

$$\frac{\text{Abs.Unk.-Abs.Unk.Blank}}{\text{Abs.Cal. - Abs.Cal Blank}} \times \text{Conc.Of Cal(mg/dl)} = \text{Total Bilirubin (mg/dl)}$$

QUALITY CONTROL

The validity of the reaction should be monitored by use of the control sera with known normal and abnormal total bilirubin values. These controls should be run at least with every working shift in which total bilirubin assays are performed. It is recommended that each laboratory establish its own frequency of control determination.

LIMITATIONS OF THE PROCEDURE

1. Samples with values of 30mg/dl must be diluted 1:1 with isotonic saline, re-assayed and the final answer is multiplied by two.
2. Serum hemoglobin levels of up to 1.0 g/dl do not interfere with results.

REAGENT PERFORMANCE

1. Linerarity: 30mg/dl
2. Comparison: Testing performed between this and a similar method yielded a coefficient of correlation of 0.987 with regression equation of y=0.98x+0.02.
3. Percision:

Within Day			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
0.5	0.05	8.8	0.9	0.05	5.6
8.3	0.06	0.7	7.9	0.14	1.8

REFERENCE RANGE

Total: Adults 0.2-1.2 mg/dl

It is recommended that each laboratory establish its own reference range.

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