SPECTRUM

The Creative Approach to Bioscience

# TOTAL Bilirubin
## *(Single Reagent)*

MODIFIED BERGH & MÜLLER METHOD

REF: 223 001 ( 4 x 25 ml) 100 test
REF: 223 002 ( 2 x 100 ml) 200 test

**SYMBOLS IN PRODUCT LABELLING**

| | |
|---|---|
| EC REP Authorised Representative | Use by/Expiration Date |
| IVD For in-vitro diagnostic use | CAUTION. Consult instructions for use |
| LOT Batch Code/Lot number | Manufactured by |
| REF Catalogue Number | (Xi) - Irritant |
| Consult instructions for use | |
| Temperature Limitation | |

### Intended Use

Spectrum Diagnostics Total Bilirubin single reagent is intended for the In-vitro quantitative, diagnostic determination of bilirubin in human serum or plasma.

### Background

The average level of the bilirubin produced in humans from different sources range between 250 to 300 mg/day, of which 85 % is derived from the heme moiety of the haemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system . The remaining 15 % is produced from erythrocytes destroyed in the bone marrow and from catabolism of other heme containing proteins such as cytochromes and myoglobin .
After it is produced in the peripheral tissues , bilirubin is transported to the liver in association with albumin . In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Disease or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

### Method

MODIFIED BERGH & MÜLLER METHOD (Colorimetric, End Point)

### Assay Principle

The azobilirubin produced by the reaction between bilirubins and the diazonium salt of 3,5 dichlorophenyl tetraflouroborate salt shows maximum absorption at 540 nm. The intensity of the colour produced is proportional to the quantity of bilirubin which has reacted. In the presence of caffeine and surfactants as accelerators,conjugated and free bilirubin participate in the reaction in the same way, so that the level of total bilirubin is determined.

### Reagent (R)

| | |
|---|---|
| 3,5-dichlorophenyl tetrafluoroborate | 0.2 mmol/l |
| Caffeine | 50 mmol/l |
| Surfactants and stabilizers | < 3% |

For further information, refer to the Total Bilirubin reagent material safety data sheet.

### Precautions and Warnings

Do not ingest or inhalate. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

### Reagent Preparation, Storage and Stability

Spectrum Total Bilirubin Reagent is supplied ready to use.

Stability: at +2°C to +8°C up to the expiration date
Once opened, the reagent is stable for 2 months at the specified temperature if contamination is avoided.

### Deterioration

Do not use the Spectrum bilirubin reagents if precipitate forms .Failure to recover control values within the assigned range may be an indication of reagent deterioration .

### Specimen Collection and Preservation

Avoid exposure of the specimen to light. If plasma is used, only heparin and oxalate plasma are suitable. Other anticoagulants should not be used. The average half-life of total bilirubin and direct bilirubin in serum is 17 days and few hours respectively .

### System Parameters

| | |
|---|---|
| Wavelength | 546 nm |
| Optical path | 1 cm |
| Assay type | End-point |
| Direction | Increase |
| Sample : Reagent Ratio | 1 : 20 |
| Temperature | 37 $^{o}$C or 20 – 25 $^{o}$C |
| Incubation time | 5 minutes at 20 – 25 $^{o}$C |
| Zero adjustment | Reagent Blank |
| Reagent Blank Limits | Low 0.00 AU<br>High 0.15 AU |
| Sensitivity | 0.1 mg/dL |
| Linearity | 25 mg/dL |

### Procedure 1 ( with factor )

| | Blank | sample blank | sample |
|---|---|---|---|
| **Samlpe** | ..... | 50 µl | 50 µl |
| **Reagent** | 1 ml | ..... | 1 ml |
| **N.Saline** | ..... | 1 ml | ..... |

Mix and incubate for 5 minutes at 15 -25$^{o}$Cor 3 minutes At 37 $^{o}$C. Measure absorbance of sample ($A_{sample}$) and Sample blank ($A_{sample\ blank}$) against reagent blank.

### Calculation

### With factor

$\Delta A$ Sample = $A_{sample} - A_{sample\ blank}$
Total Bilirubin Factor = 28
Serum Total Bilirubin Conc (mg/dl) = $\Delta A$ Sample x 28

Conversion Factor = mg/dl x 17.1 = µmol/l

### Procedure 2 ( with Bilirubin Calibrator )

| | Blank | Calibrator | sample blank | sample |
|---|---|---|---|---|
| **Samlpe** | ..... | ..... | 50 µl | 50 µl |
| **Reagent** | 1 ml | 1 ml | ..... | 1 ml |
| **Calibrator** | ..... | 50 µl | ..... | ..... |
| **N.Saline** | ..... | ..... | 1 ml | ..... |

Mix and incubate for 5 minutes at 15 -25$^{O}$Cor 3 minutes At 37 $^{O}$C. Measure absorbance of sample ($A_{sample}$) Calibrator ( A cal )and Sample blank ($A_{sample\ blank}$) against reagent blank.

### Calculation

$\Delta A\ Sample = A_{sample} - A_{sample\ blank}$

$$T.Bilirubin\ concentration\ (mg/dl) = \frac{(\Delta A\ Sample)}{(A_{cal.})} \times conc.\ of\ cal$$

***Bilirubin calibrator is not included in the kit. Any commercial Bilirubin calibrator is required for the test ( procedure 2)***

### Important Note :

**For severely haemolyzed or lipemic sera serum, correction is required by performing serum blank. Use normal saline as serum blank reagent.**

### Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

### Performance Characteristics

### Precision

Within run (Repeatability)

| | Level 1 | Level 2 |
|---|---|---|
| **n** | 20 | 20 |
| **Mean (mg/dL)** | 0.79 | 4.37 |
| **SD** | 0.016 | 0.18 |
| **CV%** | 2.03 | 4.12 |

Run to run (Reproducibility)

| | Level 1 | Level 2 |
|---|---|---|
| **n** | 20 | 20 |
| **Mean (mg/dL)** | 0.82 | 4.52 |
| **SD** | 0.02 | 0.17 |
| **CV%** | 2.44 | 3.76 |

### Methods Comparison

A comparison of the Spectrum BIL-T (y) with a commercial obtainable assay (x) gave the following result:

$$y = 0.852\ x + 0.22; \qquad r = 0.997$$

### Sensitivity

The sensitivity of the reagent is 0.1 mg/dl. The lower detection limit represents the lowest measurable Bilirubin concentration that can be distinguished from zero.

### Linearity

The reaction is linear up to a total bilirubin concentration of 25 mg/dl. Specimens showing higher concentration should be diluted 1+4 with physiological saline and repeat the assay (result × 5)

### Interfering Substances

Hemolysis: Elevated levels of haemoglobin may interfere.
Lipemia (Intralipid): Elevated levels of triglycerides may interfere.

### Expected Values

Serum: 0.1 to 1.2 mg/dl .

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

***Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.***

### Dynamic Range

0.1 - 25 mg/dL .

### Waste Disposal

This product is made to be used in professional laboratories.
Please consult local regulations for a correct waste disposal.
**S56:** dispose of this material and its container at hazardous or special waste collection point.
**S57:** use appropriate container to avoid environmental contamination.
**S61:** avoid release in environment. refer to special instructions/safety data sheets.

### References


1. Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of clinical chemistry.3 rd ed. Philadelphia:WB Saunders: 1987:729-761.
2. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimetric method. J Biol Chem.1937:119:481-490.
3. Tietz NW, ed. Clinical guide to laboratory tests. 3rd ed.Philadephia: WB saunders; 1995:268-273.


| ORDERING INFORMATION | |
|---|---|
| **CATALOG NO.** | **QUANTITY** |
| 223 001 | 100 Test |
| 223 002 | 200 Test |


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