

Total Bilirubin

1.0 INTENDED USE

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

2.0 BACKGROUND

2.1 METHOD AND HISTORY

In 1883 Ehrlich introduced the diazo reaction for the detection of bilirubin (10.1). In 1913 Van den Bergh and Snapper (10.2) applied the diazo reaction to serum after deproteinization. In 1916 Van den Bergh and Muller (10.3) discovered the direct and indirect reading of bilirubin in serum. In 1937 Malloy and Evelyn (10.4) adapted the bilirubin procedure to the photoelectric colorimeter. The Biotron Diagnostics Total Bilirubin method uses DMSO based on modification of Walters and Gerard (10.5). The method is sensitive, accurate and easy to perform. It compares very favorably with Malloy and Evelyn (10.4) and Jendrassik and Grot (10.6).

2.2 TEST PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to produce diazotized sulfanilic acid (diazo). Direct and indirect bilirubin couple with diazo to produce azobilirubin in the presence of dimethyl sulfoxide (DMSO). The intensity of the color produced is directly proportional to the amount of total bilirubin concentration present in the sample (at 546 nm.)

2.3 CLINICAL SIGNIFICANCE (10.9)

Total serum bilirubin is elevated in cases of obstructive jaundice, hepatitides, and cirrhosis. Elevations will occur when there is excessive destruction of hemoglobin.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, fasting serum is the preferred specimen.

Fasting avoids lipemic interference. Hemolyzed samples may produce falsely low values.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-200 µl.

Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Specimens must be stored away from direct light as bilirubin is subject to photodegradation. Serum samples may be stored for 2 hours at room temperature, 12 hours when refrigerated and 3 months when frozen. (10.10) Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS (2 X 125 ml)

Reagents necessary for the determination of total bilirubin are included in the kit.

4.1 REAGENT

4.1.1 Total bilirubin reagent contains:

Sulfanilic acid	32 mM
hydrochloric acid	165 mM
dimethyl sulfoxide	7000 mM

4.1.2 Sodium nitrite reagent contains:

Sodium nitrite	≥ 29 mM
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4.1.3 Standard/Control/Calibrator

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

4.3 REAGENT PREPARATION

The working reagent is prepared adding 1 drop of sodium nitrite for each 3 ml of total bilirubin reagent. Mix well before using. Record the data and time of reconstitution.

4.4 REAGENT STORAGE AND STABILITY

When stored at 18°-26°C unopened reagents are stable until the expiration date printed on the label. The working reagent is stable for 8 hours at 18°-26°C or 30 days at 2-8°C when stores tightly capped in an amber bottle.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 550-560 nm.

4.5.2 1 cm cuvettes or a flow cell.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 3 ml and 200 µl.

4.5.5 Timer for a 1 or 5 minute incubation.

4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	550-560 nm
Temperature	18 - 26°C or 37°C

Pathlength	1.0 cm
Mode	endpoint
Reaction time	5 minutes at 18 - 26° C 1 minute at 37° C
Sample volume	200 µl
Reagent volume	3 ml
Total volume	3.2 ml
Sample to reagent ratio	1/15

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 550-560 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The total bilirubin assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

- 5.4.1 Prepare the required volume of working reagent. (See 4.3 Reagent Preparation section.)
- 5.4.2 Into appropriately labeled tubes, add 3 ml of working reagent.
- 5.4.3 Add 0.2 ml (200 µl) of sample to the appropriate tube.
- 5.4.4 Incubate all test tubes at 18 - 26°C (room temperature) for 5 minutes or at 37°C for 1 minute.
- 5.4.5 Determine the absorbances of the all test tubes at 550-560 nm using the working reagent as the reagent blank.

5.5 PROCEDURE NOTE

The color of the final mixture is stable for 30 minutes.

5.6 CALCULATION AND RESULTS

$$\text{Bilirubin (mg/dl)} = \frac{A}{A_c} \times \text{bilirubin value of calibrator}$$

A = absorbance of unknown, A_c = absorbance of calibrator

Example:

$$\text{Bilirubin concentration} = \frac{0.230}{0.275} \times 5.0 \text{ mg/dl} = 4.2 \text{ mg/dl}$$

with A = 0.230, A_c = 0.275 and bilirubin value of calibrator = 5.0 mg/dl.

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.7)

The range of expected values is: 0.2 - 1.2 mg/dl

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.11)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

A comprehensive list of drugs and other substances that affect total bilirubin is given by Young. (10.8)

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The total bilirubin assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run: In this study, 15 replicates of 2 control sera were run.

Mean (mg/dl)	SD (mg/dl)	CV (%)
1.42	± 0.04	2.82
5.84	± 0.06	1.05

Between-Run: In this study, 5 runs were made, each run consisting of 5 replicates of 2 control sera.

Mean (mg/dl)	SD (mg/dl)	CV (%)
1.44	± 0.05	3.40
5.88	± 0.08	1.33

9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing this method and a similar total bilirubin method. The samples range between 0 mg/dl and 23.8 mg/dl.

Number of Samples	Regression Equation $y = \text{Biotron}, x = \text{Comparative}$	Correlation Coefficient
40	$y = .978x + .031$	0.999

9.3 LINEARITY

This procedure is linear through 20 mg/dl beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

10.0 REFERENCES

- 10.1 Ehrlich, P., Klin Med, 45:721, 1883.
- 10.2 Van den Bergh, AAH, and Snapper, J., Dtsch Arch Klin Med, 110:540, 1913.
- 10.3 Van den Bergh, AAH, and Muller, P., Biochem Z, 77:90, 1916.
- 10.4 Malloy, H.T., and Evelyn, K.A., J Biol Chem, 119:481, 1937.
- 10.5 Walters, M., Gerard, H., Clinical Microchem Journal, 15(1970)231.
- 10.6 Jendrassik, L., and Grot, P., Biochem Z, 297:81, 1938.
- 10.7 Henry, R. J., Clinical Chemistry: Principles and Techniques, Harper and Row, N.Y., pp. 1059-60, 1974.
- 10.8 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 13rd ed., Washington DC, AACC Press (1990).
- 10.9 Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia, Pa, 1970, p.212
- 10.10 Martinek, R.G. Clin Chem Acta 13,161(1966).
- 10.11 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5,