

Direct Bilirubin

1.0 INTENDED USE

This reagent is intended for the quantitative determination of direct 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 and Direct Bilirubin methods use 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 bilirubin reacts with diazo in the absence of DMSO. The intensity of the color produced is directly proportional to the amount of direct bilirubin concentration present in the sample.

3.3 CLINICAL SIGNIFICANCE

An elevation in body bilirubin because of increased formation or retention of the pigment may cause a jaundiced condition characterized by high levels of serum bilirubin and a yellowish pigmentation of the skin. Jaundice may be classified as prehepatic, hepatic or posthepatic depending upon the principal cause of the condition. (10.10) Determination of both total serum bilirubin and direct bilirubin (water-soluble bilirubin derivatives such as mono and diglucuronides) may help in the differential diagnosis of jaundice.

3.0 SPECIMEN COLLECTION AND HANDLING

Serum is the required sample for Biotron Diagnostics Direct Bilirubin Test.

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed, 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 5-100 μ l. Call King's technical service department at 1-800-262-8655 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. (10.9) 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 within 4 immediately, store the sample properly using the guidelines above.

4.0 MATERIALS (2 X 125 ml)
(4 X 125 ml)

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

4.1 REAGENT

- 4.1.1 Direct bilirubin reagent contains:
sulfanilic acid 32 mM
hydrochloric acid 165 mM
- 4.1.2 Sodium nitrite reagent contains:
Sodium nitrite ≥ 29 mM

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 by combining 3 ml of direct bilirubin reagent and 1 drop of sodium nitrite reagent. Mix well before using.

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 24 hours at room temperature when stored in an amber bottle (or 10 days when refrigerated at 2-8° C.)

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 555 nm.
4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 555 nm.
4.5.3 Test tubes capable of holding 2 ml.
4.5.4 Pipettes capable of delivering 1 ml, 100 μ l.
4.5.5 Timer for 1 or 5 minute incubation.
4.5.6 Calibrator /Control
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	555 nm
Temperature	18 - 26° C or 37° C
Pathlength	1.0 cm
Mode	endpoint
Reaction time	5 minutes at 18 - 26° C, or 1 minute at 37° C
Sample volume	100 μ l
Reagent volume	1.0 ml
Total volume	1.1 ml
Sample to reagent ratio	1/10

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 555 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 direct 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.
5.4.2. Label test tubes "Calibrator Test" and "Patient Test."
5.4.3. Label test tubes "Calibrator Blank" and "Patient Blank."
5.4.4. Add 1.0 ml of working reagent to the test tubes labeled "Calibrator Test" and "Patient Test."
5.4.5. Add 1.0 ml of direct bilirubin reagent to the test tubes labeled "Calibrator Blank" and "Patient Blank."
5.4.6. At timed intervals add 0.1 ml (100 μ l) of patient sample to the "Patient Test" and "Patient Blank" tubes, add 0.1 ml (100 μ l) of calibrator sample to the "Calibrator Test" and "Calibrator Blank" tubes. Mix well.
5.4.7. Incubate all test tubes at 18 - 26° C (room temperature) for 5 minutes or at 37° C for 1 minute.
5.4.8. Determine the absorbance of all test tubes at 555 nm using distilled water as the reagent blank.

5.5 PROCEDURE NOTE

- 5.5.1 Several drugs which may cause elevated bilirubin levels are acetaminophen, chlordiazepoxide, novobiocin and acetoexamide.
- 5.5.2 For pediatric samples with bilirubin above 3.0 mg/dl, the sample should be diluted with an equal volume of deionized water. Reassay the sample and multiply the results by two.

5.6 CALCULATION AND RESULTS

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

A = absorbance of "patient test"
 A_b = absorbance of "patient blank"
 A_c = absorbance of "calibrator test"
 A_{cb} = absorbance of "calibrator blank"

Example:

$$\text{Bilirubin concentration} = \frac{0.05 - 0.01}{0.25 - 0.01} \times 5.0 \text{ mg/dl} = 0.83 \text{ mg/dl}$$

A = 0.05, A_b = 0.01, A_c = 0.25, A_{cb} = 0.01
 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: 0.0 - 0.5 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

Due to the photodegradatory nature of bilirubin, it is important that all samples and standards be protected from direct light. 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 direct 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, 30 replicates of 2 control sera were run.

Mean (mg/dl)	SD (mg/dl)	CV (%)
0.96	± .007	0.69
1.26	± .016	1.3

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 (%)
0.95	± 0.007	0.77
1.20	± 0.030	2.5

9.2 CORRELATION

A correlation study was done on the Gilford Stasar III (registered trademark of Gilford Instruments) comparing this method and a similar direct bilirubin method.

Number of Samples	Regression Equation $y = \text{Biotron}, x = \text{Comparative}$	Correlation Coefficient
25	$y = 1.04x - 0.03$	0.992

9.3 RECOVERY

In this study, known bilirubin protein standards of varying concentration are added to a pool serum. With this method, recovery is in the range of 94-103%.

9.4 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

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