

Intended Use

For **IN VITRO quantitative** determination of Direct Bilirubin in serum or plasma using manual or automated applications.

Clinical Significance

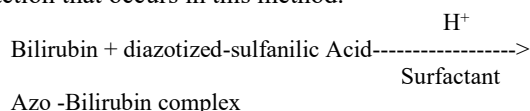
Measurements of Bilirubin in blood are used for diagnosing obstructive jaundice and hepatitis, as well as to monitor the causes and treatment. (1)

Method History

In 1883 Ehrlich reported the reaction of Bilirubin with diazotized sulfanilic acid to form a chromogenic complex. (2) VandenBerg applied this colorimetric reaction to the quantitative determination of Bilirubin in serum. (3) The Catachem Direct Bilirubin method for manual or automated applications is based upon the method of VandenBerg.

Method Principle

The serum sample is mixed with diazotized sulfanilic acid to form the azo-Bilirubin complex. The increase in absorbance is monitored at 550nm. The reaction scheme illustrates the reaction that occurs in this method.

**Reagent Content**

The concentrations of the active ingredients in the reagent will be approximately as follows:

Direct Bilirubin Sample Diluent

Each liter contains:	
Sulfanilic Acid	14.0 mmol/L
Hydrochloric Acid	120.0 mmol/L
Nonreactive ingredients	

Sodium Nitrite Diluent

Each liter contains:	
Sulfanilic Acid	26.3 mmol/L
Hydrochloric Acid	713 mmol/L
Nonreactive Ingredients	

Sodium Nitrite

Each liter contains:	
Buffer	
Sodium Nitrite	87.5 mmol/L
Preservative	

Precautions

Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent

Prepare the Catachem Direct Bilirubin Working Reagent by adding the contents of one vial of Sodium Nitrite to one bottle of Direct Bilirubin Nitrite Diluent. Mix well for a minimum of 15 minutes. Use a magnetic stirrer (if available).

Reagent Storage And Stability

Store Catachem Direct Bilirubin Reagents at 2-26°C. When stored as directed, the reagents are stable until the expiration date stated on the label. When prepared and stored as directed, the Direct Bilirubin Working Reagent is stable for 14 days at 2-8°C.

Specimen Collection And Preparation

Test sera should be fresh, clear, and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

Quality Control

To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances

Various substances have been reported to interfere with the Direct Bilirubin method. (4-6) A comprehensive discussion on these interfering substances is beyond the scope of this product labeling.

Expected Values

The range of expected values for human samples determined for this method is 0.0 - 0.3 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 and the species under test.

Procedure

Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Matched cuvettes	1 cm light path
Timer	to time incubation time
Pipette	2.8 ml for reagent
Pipette	0.2 ml for sample

Materials Provided

Direct Bilirubin Sample Diluent, Sodium Nitrite Diluent, and Sodium Nitrite.

Analytical Parameters

Wavelength	550nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	1 minute
Reagent Volume	2.8 ml
Sample Volume	0.2 ml
Total Volume	3.0 ml
Sample-to-Reagent Ratio	1:15

Note: To eliminate interferences of lipemic and other endogenous interfering substances and to maximize accuracy in the assay procedure, all samples should be blank corrected.

Assay Procedure

1. Pipette 2.8 ml of Direct Bilirubin Working Reagent into each of three cuvettes marked "Calibrator", "Sample", and "Blank".
2. Pipette 0.2 ml of Calibrator or Sample into their respective cuvettes. Use 0.2 ml of water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for exactly 1 minute at room temperature.
4. Set spectrophotometer wavelength at 550nm and zero the instrument with Blank.
5. Read the Calibrator and Sample absorbencies.
6. Calculate the Direct Bilirubin concentration (mg/dL) in the sample(s), as shown in calculations and results.

Blank Procedure

Follow the same procedure as for the assay by substituting the Working Reagent with the Direct Bilirubin Diluent.

Calculations And Results

$$\text{DBILI} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (mg/dL)}$$

	Assay OD	Blank OD
Example: Sample	0.320	0.020
Calibrator	0.250	0.015

$$\text{Calibrator} = 5.0 \text{ mg/dL}$$

$$\begin{aligned} \text{DBILI (mg/dL)} &= \frac{0.320 - 0.020}{0.250 - 0.015} \times 5 \text{ mg/dL} \\ &= 6.4 \text{ mg/dL} \end{aligned}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.040 - 0.051 absorbance units per mg/dL.

Linear Range: In this method there is no significant nonlinearity over the range of 0-15 mg/dL.

Precision: Within-run and day-to-day precision is summarized below.

Precision Study

D Bilirubin mg/dL	Total Precision SD	Total CV %
0.47	0.05	*
6.46	0.10	1.6
12.53	0.13	1.0

D Bilirubin mg/dL	Within-run Precision SD	Within-run CV %
0.47	0.04	*
6.46	0.08	1.20
12.53	0.05	0.43

* CV% values are not meaningful when average approaches zero.

Correlation

Using a reference method based on the procedure of Van den Berg and Muller, linear regression analysis produced the following results:

Range	=	0.1 - 10.8
N	=	30
Y	=	1.000x + 0.02
r	=	0.999
Sy.x	=	0.14

References

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3. Van den Bergh A.H. and Snapper J. Die farbstoffe des blutserums. Deut Arch Klin Med 110, 540-561.
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5. Martin E.W. Hazards of Medication (Alexander SF, Farage D.J. and Hassan W.E. Jr. eds). Philadelphia PA and Toronto, Canada. J.B. Lippincott Co. (1971) pp 169-189.
6. Constantino N.V., Kabat H.F. Drug-induced modifications of laboratory test values. Revised 1973. Am J Hosp Pharm 30:24-71 (1973).

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