

Intended Use

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

Clinical Significance

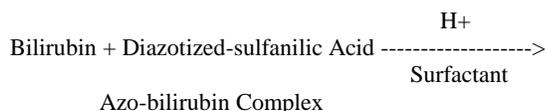
Measurements of Bilirubin in blood are used for diagnosing obstructive jaundice and hepatitis, as well as to monitor the causes and treatment.

Method History

In 1883 Ehrlich reported the reaction of Bilirubin with diazotized sulfanilic acid to form a chromagenic complex. (2) VandenBerg applied this colorimetric reaction to the quantitative determination of Bilirubin in serum. (3). Later, Pearlman, et al (4) and Norvos, et al (5) modified the method of VandenBerg by using surfactants to free the protein bound Bilirubin without protein precipitation. Catachem's Total Bilirubin method for manual or automated applications is based upon the method of VandenBerg as modified by Pearlman (4) and Norvos (5)

Method Principle

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

**Reagent Content**

When reconstituted according to directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

Total Bilirubin Diluent

Each liter contains:

Sulfanilic Acid	14.0 mmol/L
Hydrochloric Acid	0.12 mol/L
Surfactant	
Nonreactive ingredient	

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 Reagents (If used as a single reagent.)

Prepare the Catachem Total Bilirubin Working Reagent by adding the contents of one vial of Sodium Nitrite to one bottle of Total Bilirubin Diluent. Mix well for a minimum of 15 minutes. Use a magnetic stirrer (if available). (No preparation required if used as a dual reagent.)

Reagent Storage And Stability

Store Catachem Total Bilirubin Reagents at 2-8°C. When stored as directed, the reagents are stable until the expiration date stated on the label. When prepared as a single reagent and stored as directed, the Total Bilirubin Working Reagent is stable for 30 days at 2-8°C. With time, some color will appear in the Working Reagent. This does not interfere with reagent performance.

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 Total Bilirubin Method. (6) A comprehensive discussion on these interfering substances is beyond the scope of this product labeling.

Expected Values

The range of expected values determined for this method is 0.1 - 1.3 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.

Procedure

Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

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

Materials Provided

Catachem Total Bilirubin Diluent and Sodium Nitrite

Analytical Parameters

Wavelength	550nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5 minutes
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 1.0 ml of Total Bilirubin Working Reagent into each of three cuvettes marked "Calibrator", "Sample" and "Blank".
2. Pipette 0.05 ml of "Calibrator" and "Sample" into their respective cuvettes. Use 0.05 ml of distilled water for "Blank". Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes 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 Total 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 Total Bilirubin Diluent.

Calculations And Results

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

	<u>Assay OD</u>	<u>Blank OD</u>
Example: Sample	0.320	0.020
Calibrator	0.250	0.015

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

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

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 0-30 mg/dL.

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

Precision Study

T. Bili.	Within-Run		Total Precision	
	Mean	SD	SD	CV
mg/dL	mg/dL	%	mg/dL	%
0.45	0.09	*	0.12	*
13.15	0.11	0.86	0.16	1.20
26.50	0.16	0.61	0.39	1.5

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

Correlation

The use of an automated analyzer and a reference method based on the procedure of VandenBerg and Muller resulted in the following regression statistics:

Range	=	0.3 - 16.7
N	=	108
Y	=	0.982x + 0.13
r	=	0.999
Sy.x	=	0.22

References

1. Tietz NW. (Editor) Fundamentals of Clin Chem, 2nd Ed (1982). WB Saunders Co, Philadelphia.
2. Ehrlich P. Sulfodiazobenzol ein reagens auf bilirubin. Centr Klin Med 4,721-723 (1883).
3. VandenBerg AH and Snapper J. Die farbstoffe des blutserum. Deut Arch Kiln Med 110, 540-561.
4. Pearlman FC, Lee RTY. Detection and measurement of total bilirubin in serum with use of surfactants as solubilizing agents. Clin Chem 20:447 (1974).
5. Norvos JD, Koch TR, Knoblock EC. Improved method for accurate quantitation of total and conjugated bilirubin in serum. Clin Chem 25:1891-1899 (1979).
6. Young DS, Pestaner LC, Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21(5):1D-432D (1975).

REV: BK123002dt