

Bilirubin (Total) Reagent

Catalog #: 43708

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the quantitative *in vitro* determination of total bilirubin in serum.

CLINICAL SIGNIFICANCE

Serum total bilirubin is elevated in cirrhosis, hepatitis, and in obstructive and hemolytic jaundice.¹ Differentiation between direct and indirect bilirubin is important in determining the specific cause of an increase in total bilirubin.

METHOD HISTORY

The most common method for the clinical determination of bilirubin is the coupling of serum bilirubin with diazotized sulfanilic acid (p-diazobenzenesulfonic acid) to produce an azobilirubin dye. The reaction was first described by Ehrlich² in 1884 and was used by van den Bergh and Snapper³ to demonstrate the presence of bilirubin in normal serum. Van den Bergh⁴ further observed that there were two types of serum bilirubin which can be distinguished using the diazo reaction. The direct form reacted with diazo without the presence of alcohol (accelerator). This is bilirubin which has been conjugated with glucuronic acid by the liver and is then water-soluble. The indirect form of bilirubin is unconjugated and exists in serum tightly bound to albumin. This bilirubin-albumin complex is not water-soluble, and therefore requires an accelerator, or solubilizing agent, to remove the bilirubin from the albumin for it to react with the diazotized sulfanilic acid. The total bilirubin in serum is the sum of the direct and indirect forms. Many substances have been used as accelerators for the reaction of unconjugated bilirubin with diazo reagent. Malloy and Evelyn⁵ first introduced methanol in 1937. Jendrassik and Grof⁶ introduced the use of caffeine and sodium benzoate in 1938. Subsequently, there have been many modifications to these two methods.^{7,8,9}

The SDI total bilirubin method is based on a modification of the Pearlmans and Lee¹⁰ method in which a surfactant is used as a solubiliser. Sodium nitrite is added to sulfanilic acid to form diazotized sulfanilic acid. Bilirubin in the sample reacts with the diazotized sulfanilic acid to produce azobilirubin which absorbs strongly at 550 nm. The absorbance measured at 550 nm is directly proportional to the total bilirubin concentration in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
Sulfanilic acid	4.9 mM
HCL	104 mM
Sodium nitrite	145 mM

Precautions:

1. Reagents are toxic and corrosive. Do not pipette by mouth. Avoid contact with skin and clothing.
2. This reagent is for *in vitro* diagnostic use only.

REAGENT PREPARATION

To prepare a working total bilirubin reagent mix 1 part of Sodium Nitrite reagent with 50 parts of Total Bilirubin reagent.

Example: Add 0.2ml sodium nitrite to 10ml total bilirubin reagent.

REAGENT STORAGE

1. Packaged reagents may be stored at 2 - 25°C.
2. Combined working reagent can be stored for up to 21 days when stored at 2-8°C.
3. Do not freeze reagents.
4. Avoid exposure to direct sunlight.

REAGENT DETERIORATION

Do not use the reagent if:

1. Reagent has an absorbance greater than 0.100 when measured against water at 550 nm.
2. Turbid.
3. The reagent fails to meet stated parameters of performance.

NOTE: Working reagent will normally develop a light yellow/orange color upon standing.

SPECIMEN COLLECTION AND STORAGE

1. Fresh, unhemolyzed serum is recommended.
2. Samples should be analyzed within two hours of collection if kept at room temperature in the dark and within twelve hours if kept refrigerated (2-8°C) and protected from light.¹⁰
3. Bilirubin in serum is stable for three months when stored frozen (-20°C) and protected from light.¹¹
4. Direct sunlight may cause up to a 50% decrease in bilirubin within one hour.¹

INTERFERENCES

Studies to determine the level of interference for hemoglobin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (\pm 10%) from hemoglobin up to 400 mg/dL.

Lipemia:

No significant interference (\pm 10%) from lipemia up to 202 mg/dL measured as triglycerides.

Young DS¹² has published a comprehensive list of drugs and substances which may interfere with the Direct Bilirubin assay.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. An SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters

Total Bilirubin	
TEMPERATURE:	37°C
WAVELENGTH:	550 nm
ASSAY TYPE:	End Point
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 20
e.g. Sample Vol.	0.05mL (50mL)
Reagent Vol.	1.0 mL
INCUBATION TIME:	10 min

Procedure Notes:

1. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.
2. Bilirubin is extremely light sensitive. All samples should be stored protected from light sources.

Calculations:

(A = Absorbance)

$$\frac{A_{\text{patient}}}{A_{\text{standard}}} \times \text{Concentration of standard} = \text{Total Bilirubin (mg/dL)}$$

Example:	
A (patient)	= 0.350
A (standard)	= 0.240
Concentration of standard	= 5.0 mg/dL.

$$\frac{0.340}{0.240} \times 5.0 = 7.1 \text{ mg/dL Total Bilirubin}$$

Limitations:

Samples with values exceeding 20 mg/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

CALIBRATION

Use a Total Bilirubin standard or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Total Bilirubin values.

EXPECTED VALUES¹

Adults and infants over 1 month old 0.2 – 1.5 mg/dL.

It is recommended each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 0 to 20.0 mg/dL

Method Comparison:

Number of sample pairs:	45
Range of samples:	0.20 – 25.2 (mg/dL)
Correlation Coefficient:	0.999
Slope:	0.975
Intercept:	0.017 (mg/dL)

Precision:

Within Run	Level 1	Level 2	Level 3
n=40			
Mean (mg/dL)	0.55	1.20	8.06
S.D. (mg/dL)	0.04	0.03	0.12
C.V. (%)	6.4	2.3	1.5

Total

	Level 1	Level 2	Level 3
n=40 (10 days / 2 runs per day / 2 replicates per run)			
Mean (mg/dL)	0.55	1.20	8.06
S.D. (mg/dL)	0.04	0.04	0.16
C.V. (%)	6.8	3.0	2.0

Sensitivity:

A calibration factor of approximately 42.7 was obtained, which is equivalent to a sensitivity of 0.0234 D Abs per mg/dL.

REFERENCES

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