

BILIRUBIN TOTAL & DIRECT-TAB (Page-1 BILIRUBIN DIRECT)

4 x 50 mL
51003004

INTENDED USE

This reagent is intended for *in vitro* quantitative determination of Bilirubin in serum or plasma .

- Diazo method
- Linear up to 20 mg/dL
- Fast incubation 5 minutes at room temperature.
- Sample volume only 50 µL.
- Without sample blank procedure also included.

CLINICAL SIGNIFICANCE

Bilirubin is formed by the break down of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred to a direct bilirubin.

Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Direct Bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

REAGENT COMPOSITION

DIRECT BILIRUBIN REAGENT	2 x 50 mL
Sulfanilic acid	28.9 mmol/L
Hydrochloric acid	165 mmol/L
Preservatives and stabilizers	

DIRECT BILIRUBIN ACTIVATOR	1 x 4 mL
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BILIRUBIN CALIBRATOR

Not provided along with the Kit, Agappe Multicalibrator Product Code : 51610001 is recommended.

STORAGE AND STABILITY

The sealed reagents are stable up to the expiry date stated on the label, when stored at RT. The standard & activator should be stored at 2 - 8°C

LINEARITY

This reagent is linear up to 20 mg/dL.

If the concentration is greater than linearity (20 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

NORMAL RANGE

It is recommended that each laboratory establish its own reference values.

The following value may be used as guide line.

Direct Bilirubin up to 0.4 mg/dL

PREPARATION AND STABILITY OF WORKING REAGENT

Reagents are ready to use.

PRECAUTION

To avoid contamination, use clean laboratory wares.

Avoid direct exposure of reagent to light.

SAMPLE

Serum / plasma (free of haemolysis)

GENERAL SYSTEM PARAMETER

Mode of Reaction	End point
Slope of reaction	Increasing
Wavelength	546 nm
Temperature	30°C
Factor (Direct)	16.0
Blank	Sample blank
Linearity	20 mg/dL
Reaction time	5 min
Sample volume	50 µL
Reagent volume	1000 µL
Activator	20 µL
Cuvette	1 cm light path

LABORATORY PROCEDURE

	Sample Blank	Test
Direct bilirubin reagent	1000 µL	1000 µL
Activator Direct	-	20 µL
Serum	50 µL	50 µL

Mix well and incubate for 5 minutes at room temperature. Measure the absorbance of test against respective Blank at 546 nm.

CALCULATION

With factor :

Direct Bilirubin = OD of test – OD of sample blank x16

With calibrator :

OD of test –OD of sample blank

Bilirubin Concentration = $\frac{\text{OD of test} - \text{OD of sample blank}}{\text{OD of calibrator} - \text{OD of Calibrator blank}} \times \text{Concentration of Calib.}$

Alternative Method – without sample blank

GENERAL SYSTEM PARAMETER

Mode of Reaction	End point
Slope of reaction	Increasing
Wavelength I	546 nm
Wavelength II	630 nm
Temperature	30°C
Factor (Direct)	20.0
Blank	Reagent
Linearity	20 mg/dL
Reaction time	5 min
Sample volume	50 µL
Reagent volume	1000 µL
Activator	20 µL
Cuvette	1 cm light path

LABORATORY PROCEDURE

	Reagent Blank	Test
Direct Bilirubin Reagent	1000 µL	1000 µL
Activator Direct	20 µL	20 µL
Serum / Calibrator	-	50 µL

Mix well and incubate for exactly 5 minutes. Measure the absorbance of calibrator and test against reagent blank at 546/630 nm.

CALCULATION

With factor :

Direct Bilirubin = OD of test – OD of reagent blank x 20

With calibrator :

OD of test –OD of reagent of blank

Bilirubin Concentration = $\frac{\text{OD of test} - \text{OD of reagent of blank}}{\text{OD of calibrator} - \text{OD of Reagent blank}} \times \text{Concentration of Calib.}$

BIBLIOGRAPHY

1. Water, M., Gerard, H.; MICROCHEM JM 15, 231(1980)
2. Annino J. S.; C.C. Principles and procedure,1960
3. A.A. A.C.C.; Clin. Chem. 8 : 405,196

SYMBOLS USED ON THE LABELS: IN VITRO DIAGNOSTIC USE SEE PACKAGE INSERT FOR PROCEDURE LOT NUMBER MANUFACTURER'S ADDRESS MANUFACTURING DATE EXPIRY DATE TEMPERATURE LIMIT



AGAPPE DIAGNOSTICS SWITZERLAND GmbH.

ADS/V.01/260314



ISO 9001 : 2008
ISO 13485 : 2003

BILIRUBIN TOTAL & DIRECT-TAB (Page-2 BILIRUBIN TOTAL-TAB)

**4 x 50 mL
51003004**

INTENDED USE

This reagent is intended for *in vitro* quantitative determination of Bilirubin in serum or plasma.

- Modified TAB method
- Linear up to 25 mg/dL
- Fast incubation 5 minutes at room temperature
- Sample volume only 50 µL
- Without sample blank procedure also included

CLINICAL SIGNIFICANCE

Bilirubin is formed by the break down of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred as direct bilirubin.

Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total Bilirubin reacts with diazotized sulfanilic acid in the presence of TAB form azobilirubin.

REAGENT COMPOSITION

TOTAL BILIRUBIN REAGENT	2 x 50 mL
Sulfanilic acid	28.9 mmol/L
TAB	9 mmol/L
Preservatives and Stabilizers	

TOTAL BILIRUBIN ACTIVATOR **1 x 4 mL**

BILIRUBIN CALIBRATOR

Not provided along with the Kit, Agappe Multicalibrator Product Code : 51610001 is recommended.

STORAGE AND STABILITY

The sealed reagents are stable up to the expiry date stated on the label, when stored at RT. The Calibrator & activator should be stored at 2 - 8°C.

LINEARITY

This reagent is linear up to 25 mg/dL.

If the concentration is greater than linearity (25 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

NORMAL RANGE

It is recommended that each laboratory establish its own reference values.

The following value may be used as guide line.

Total Bilirubin - up to 1.2 mg/dL

PREPARATION AND STABILITY OF REAGENT

Reagents are ready to use.

PRECAUTION

To avoid contamination, use clean laboratory wares.
Avoid direct exposure of reagent to light.

SAMPLE

Serum/Plasma (free of haemolysis)

GENERAL SYSTEM PARAMETER

Mode of Reaction	End point
Slope of reaction	Increasing
Wavelength	546 nm
Temperature	30°C
Factor (Total)	25
Blank	Sample blank
Linearity	25 mg/dL
Reaction time	5 min
Sample volume	50 µL
Reagent volume	1000 µL
Activator	20 µL
Cuvette	1 cm light path

LABORATORY PROCEDURE

	Sample Blank	Test
Total Bilirubin Reagent	1000 µL	1000 µL
Activator Total	-	20 µL
Serum / Calibrator	50 µL	50 µL

Mix well and incubate for 5 minutes at room temperature. Measure the absorbance of calibrator and test against respective Blank at 546 nm.

CALCULATION

With factor :

Total Bilirubin = OD of test – OD of sample blank x 25

With calibrator :

OD of test – OD of sample blank

Bilirubin Concentration = ----- x Conc. of Calib.

OD of calibrator – OD of calibrator blank

Alternative Method – without sample blank

GENERAL SYSTEM PARAMETER

Mode of Reaction	End point
Slope of reaction	Increasing
Wavelength I	546 nm
Wavelength II	630 nm
Temperature	30°C
Factor (Total)	29
Blank	Reagent blank
Linearity	25 mg/dL
Reaction time	5 min
Sample volume	50 µL
Reagent volume	1000 µL
Activator	20 µL
Cuvette	1 cm light path

LABORATORY PROCEDURE

	Reagent Blank	Test
Total bilirubin reagent	1000 µL	1000 µL
Activator Total	20 µL	20 µL
Serum / Calibrator	-	50 µL

Mix well and incubate for exactly 5 minutes. Measure the absorbance of calibrator and test against reagent blank at 546/630 nm.

CALCULATION

With factor :

Total Bilirubin = OD of test – OD of reagent blank x 29.00

With calibrator :

OD of test – OD of reagent of blank

Bilirubin concentration = ----- x Conc. of Calib.

OD of calibrator – OD of Reagent Blank

BIBLIOGRAPHY

1. Walter, M., Gerard, H.; MICROCHEM JM 15, 231.(1980)
2. Annino J. S.; C.C. Principles and procedure, 1960
3. A.A. A.C.C.; Clin. Chem. 8 : 405,196

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