TRUEchemie Bilirubin Total & Direct Test Kit (Diazo)

for the quantitative determination of Bilirubin Direct and Total in human serum

IVD

INTENDED USE

The TRUEchemie Bilirubin Total & Direct test kit (Diazo) is used for the quantitative determination of direct and total bilirubin in human serum.

INTRODUCTION

Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Normally it is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the bloodstream where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide which are water-soluble metabolites. The metabolites will react with aqueous diazo reagent and are commonly referred to as "direct bilirubin". Elevation of total serum bilirubin may occur due to

1. excessive hemolysis or destruction of the red blood cells e.g. hemolytic disease of the newborn,
2. liver diseases e.g. hepatitis and cirrhosis
3. obstruction of the biliary tract e.g., gallstones.

There is information in the literature indicating elevated levels of direct bilirubin in patients with liver or biliary tract disease, even though, total bilirubin levels are normal. Therefore, the greatest diagnostic value of direct bilirubin assays stem from their ability to indicate occult liver disease. Most chemical methods for the determination of total bilirubin are based on the reaction between diazotized sulfanilic acid and bilirubin to produce azobilirubin, which absorbs maximally at 546 nm.

PRINCIPLE

Sulfanilic acid reacts with sodium nitrite to form diazotized Sulfanilic acid. In the presence of accelerator (cetrimide), conjugated and unconjugated bilirubin reacts with diazotized Sulfanilic acid to form azobilirubin (Bilirubin Total 4+1). In the absence of accelerator, only conjugated bilirubin reacts (Bilirubin Direct 4+1). The increase of absorbance at 546 nm is proportional to bilirubin concentration

Sulfanilic acid + $NaNO_2$ ---------> Diazotized Sulfanilic acid

Bilirubin + Diazotized Sulfanilic acid ----------> Azobilirubin

PACK SIZE

	BILIRUBIN TOTAL	BILIRUBIN DIRECT	BILIRUBIN (T&D)
Kit size	**4 x 50 ml**	**4 x 50 ml**	**4 x 50 ml**
Cat no.	**ADX161**	**ADX171**	**ADX181**
Kit contents			
1) Bilirubin Total Reagent (R1)	4 x 45 ml	--	2 x 45 ml
2) Bilirubin Total Reagent (R2)	4 x 5 ml	--	2 x 5 ml
3) Bilirubin Direct Reagent (R1)	--	4 x 45 ml	2 x 45 ml
4) Bilirubin Direct Reagent (R2)	--	4 x 5 ml	2 x 5 ml

REAGENT COMPOSITION

Bilirubin Total Reagent (R1):

Sulfanilic acid	:	32 mmol/L
Cetrimide	:	34 mmol/L
Hydrochloric acid	:	165 mmol/L
Stabilizers		

Bilirubin Total Reagent (R2):

Sodium nitrite	:	60 mmol/L

Bilirubin Direct Reagent (R1):

Sulfanilic acid	:	32 mmol/L
Hydrochloric acid	:	165 mmol/L
Stabilizers		

Bilirubin Direct Reagent (R2):

Sodium nitrite	:	26 mmol/L

REAGENT PREPARATION

Ready to use reagents

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The disposal of the residues has to be done as per local legal regulations.

REAGENT STORAGE AND STABILITY

The components of the kit, stored at 15 - 30°C, will remain stable until the expiry date stated on the label.

SPECIMEN COLLECTION AND STORAGE

Serum free of hemolysis. Heparinized plasma.

Bilirubin in serum is stable for 2 days at 2 - 8°C if serum is protect ed from light.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 546 nm

TEST PROCEDURE

BILIRUBIN TOTAL TEST PROCEDURE:

Wavelength : 546 nm

Temperature : 37°C

Prewarm the reagent to reaction temperature.

	Sample Blank (µL)	Sample (µL)
Bilirubin Total Reagent (R1)	1000	800
Bilirubin Total Reagent (R2)	--	200
Sample	100	100

Mix & incubate for 2 minutes at 37°C and read the absorbance of all the sample at 546 nm against respective serum blank imme diately.

CALCULATION:

$$\text{Calculation} = \frac{\text{Sample OD – Sample Blank OD}}{\text{Calibrator / Standard OD}} \times \text{Calibrator / Standard Conc.}$$

(or)

Factor for Bilirubin Total : 14

BILIRUBIN DIRECT TEST PROCEDURE:

Wavelength : 546 nm

Temperature : 37°C

Prewarm the reagent to reaction temperature.

	Sample Blank (µL)	Sample (µL)
Bilirubin Direct Reagent (R1)	1000	800
Bilirubin Direct Reagent (R2)	--	200
Sample	100	100

Mix & incubate for 5 minutes at 37 °C and read the absorbance of all the samples at 546 nm against a serum blank immediately.

CALCULATION:

$$\text{Calculation} = \frac{\text{Sample OD – Sample Blank OD}}{\text{Calibrator / Standard OD}} \times \text{Calibrator / Standard Conc.}$$

(or)

Factor for Bilirubin Direct : 14

QUALITY CONTROLS

Control Sera are recommended to monitor the performance of manual and automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUES

Normal values Total Bilirubin – upto 1.0 mg/dL

Normal values Direct Bilirubin – upto 0.2 mg/dL

It is recommended that each laboratory establishes its own normal range

PERFORMANCE CHARACTERISTICS

Linearity: The assay is linear up to 25 mg/dL of Bilirubin Total & Direct under the described assay conditions. 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. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION (Bilirubin Total):

Intra-assay precision within run (n=10)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	0.9	0.0	2.0
Control Level - 2	4.3	0.0	0.8

Inter-assay precision run to run (n=12)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	0.9	0.0	2.9
Control Level - 2	4.2	0.0	0.5

The reagent was tested for 12 days, using two different Bilirubin Total concentrations. The coefficient of variation was <5%.

PRECISION (Bilirubin Direct):

Intra-assay precision within run (n=10)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	0.3	0.0	3.7
Control Level - 2	1.1	0.0	1.3

Inter-assay precision run to run (n=12)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Control Level - 1	0.3	0.0	3.6
Control Level - 2	1.0	0.0	1.8

The reagent was tested for 12 days, using two different Bilirubin Direct concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Bilirubin Total & Direct reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.982 for Bilirubin total & 0.995 for Bilirubin Direct , the regression equation is y=1.166x-0.108 (Bilirubin Total) & y=1.010x+0.009 (Bilirubin Direct). The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis causes decreased bilirubin values.A list of drugs and other interfering substances with bilirubin has been reported by Young et. al.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEM PARAMETERS

	BILIRUBIN TOTAL	BILIRUBIN DIRECT
Mode	End point	End point
Factor	14	14
Wave length	546 nm	546 nm
Units	mg/dL	mg/dL
Flow cell temp.	37°C	37°C
Blank	Sample blank	Sample blank
BIL reagent-1 volume	800 µL	800 µL
BIL reagent-2 volume	200 µL	200 µL
Sample volume	100 µL	100 µL
Incubation	2 min. at 37°C	5 min. at 37°C
Low normal	0.00	0.00
High normal	1.0	0.20
Linearity	25	25
Reaction Slope	Increasing	Increasing

REFERENCES

1. Tietz, N.W.: Fundamentals of Clinical Chemistry. W. B. Saunders Co., Philadelphia,p. 1028 (1976).
2. Gambino. S.R., et al., JAMA 201:1047 (1967).
3. Walters, M. Gerand, H., Microchem J. 15; 231 (1970).
4. Michaelsson, M.: Scand, J., J. Clin. Lab. Invest. (Suppl. 49) 13, 1 (1961).
5. Young, D.S., et. al.: Clin. Chem. 21, l0 (1975).
6. Gambino, S.R., et. al.: Bilirubin Assay (Revised), Commission on Continuing Education. Am. Soc. of Clin. Path., Chicago (1968).
7. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	Catalogue number	Caution
In vitro diagnostic medical device	Batch code	Non-sterile
Temperature limit 15-30°C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry

Do not use if package is damaged

Keep away from sunlight

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PI-ADX18 Rev. D
Effective date: 28.12.2023
English version