

Intended Use

The reagents are used for the quantitative determination of bilirubin in serum or plasma. For in-vitro diagnostic use only.

Introduction

Red blood cells at the end of their circulating life are broken down in reticulo-endothelial systems, mainly in the spleen. The resulting haem, once the iron is removed, is then converted to bilirubin (yellow orange bile pigment). Water insoluble bilirubin also called indirect or un-conjugated bilirubin is then released into the blood stream where it binds tightly to albumin and is transported to the liver. In the liver un-conjugated bilirubin binds with glucuronic acid (mono and di glucuronides) to form conjugated bilirubin (direct bilirubin) by the enzyme glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine. Total bilirubin is the sum of unconjugated bilirubin and conjugated fractions. Bilirubin is elevated in the hemolysis or increased breakdown of red blood cells in hepatitis, cirrhosis and obstruction of the biliary tract e.g. gallstone.

Method

Diazo method- End Point.

Principle

Bilirubin is coupled with diazotized sulphanilic acid in acidic medium to form the pink colored azobilirubin. The intensity of the color produced is directly proportional to bilirubin concentration present in the sample. Direct bilirubin or conjugated bilirubin is water soluble and it directly reacts in acidic medium. However, for indirect bilirubin or un-conjugated bilirubin a surfactant is used for dissolving it in water and then the dissolved material reacts similar to direct bilirubin.

Reagent Composition

Total bilirubin Reagent (Reagent 1):

Sulphanilic acid	4 mmol/l
Surfactant	0.6 mmol/l

1

Direct Bilirubin Reagent (Reagent 2):

Sulphanilic acid	4 mmol/l
Hydrochloric acid	33 mmol/l

Reagent 3:

Sodium nitrite	144 mmol/l
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Precautions

Following precautions should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Do not swallow or inhale vapor, it may cause irritation to mouth, throat and stomach.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened Reagents are stable till expiry mentioned on the label when stored at 2-8°C.

Reagent Preparation

Working solution for Total Bilirubin

Mix 50 volumes of Reagent R1 with 1 volume of Reagent R3 to prepare the working solution. Example: For the preparation of 1.020 ml or 0.510 ml of working solutions, add the reagents as given below:

	Volume of working soln.	Reagent 1	Reagent 3
Total Bilirubin	1.020 ml	1.0 ml	20µl
	0.510 ml	0.5 ml	10µl

Working solution for Direct bilirubin

Mix 50 volumes of Reagent 2 with 1 volume of Reagent R3 to prepare the working solution. Example: For the preparation of working solutions of 1.020 ml or 0.510 ml, add the reagents as given below:

	Volume of working soln.	Reagent 2	Reagent 3
Direct Bilirubin	1.020 ml	1 ml	20 µl
	0.510 ml	0.5 ml	10µl

2

Reagent Deterioration

Reagent solutions should be clear. The reagents should be discarded if:

1. Sodium nitrite reagent has a yellow discoloration or
2. Appearance of turbidity and/or precipitation in the reagents.

Specimen Collection and Storage

Unhaemolyzed serum or heparinized plasma can be used for the testing. Sample should be protected from bright light as bilirubin is photolabile. Sample may be stored refrigerated for 3 days.

General Assay Parameters

Parameter	Total Bilirubin	Direct Bilirubin
Mode	Differential	Differential
Wavelength 1 (nm)	546	546
Wavelength 2 (nm)	670	670
Blank with	Air or Water	Air or Water
Sample Volume (µl)	25/50	25/50
Working Reagent (µl)	500/1000	500/1000
Incubation time (min.)	10	10
Incub. Temp. (°C)	37	37
Normal Low (mg/dl)	0.1	0
Normal High (mg/dl)	1	0.2
Linearity (mg/dl)	Upto 25	Upto 25
Factor	23	13
Units	mg/dl	mg/dl

PROCEDURE

Pipette into test tubes:

Tests	Working solution	Working solution
Total bilirubin	500 µl	-
Direct bilirubin	-	500 µl
Sample	25 µl	25 µl

3

Mix well and incubate for 10 min. at 37°C for total & direct bilirubin. Read the absorbance at 546/670 nm.

Calculation

Bilirubin concentration in the sample can be calculated using the following formula:

Total bilirubin = Absorbance of Test Sample X Factor (23)

Direct bilirubin = Absorbance of Test Sample X Factor (13)

If the bilirubin concentration in the sample exceeds 25 mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Serum

Total bilirubin: upto 1.0 mg/dl
Direct bilirubin: upto 0.2 mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample:reagent, ratio remains same.
2. Temperature has to be maintained constant throughout the reaction as the rate of color development is highly temperature sensitive.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for bilirubin.

Performance

Linearity Limit: 25 mg/dl

Precision:

Within run

Particulars	TOTAL BILIRUBIN		DIRECT BILIRUBIN	
	Cont.1	Cont. 2	Cont. 1	Cont. 2
CONTROL	20	20	20	20
Sample Nos.	20	20	20	20
Mean (mg/dl)	0.81	4.82	0.61	2.83
S.D.	0.01	0.10	0.01	0.03
C.V. %	1.84	2.11	2.08	1.18

Between run

Particulars	TOTAL BILIRUBIN		DIRECT BILIRUBIN	
	Cont.1	Cont. 2	Cont. 1	Cont. 2
CONTROL	60	60	60	60
Sample Nos.	60	60	60	60
Mean (mg/dl)	0.81	4.83	0.61	2.75
S.D.	0.01	0.11	0.02	0.06
C.V. %	1.83	2.27	2.39	2.00

References

1. "Bilirubin's Chemical Formula". http://www3.nsta.org/main/news/stories/college_science.php?news_story_ID=48991. Retrieved on 2007-08-14.
2. Golonka, D et al.. "Digestive Disorders Health Center: Bilirubin". WebMD. 3. <http://www.webmd.com/digestive-disorders/Bilirubin-15434?page=3>. Retrieved on 2008-11-19.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests, Third Edition. AACC Press, 1990. [http://www.sdbiomed.com/pdf/CA480_%20Bilirubin_\(Direct\).pdf](http://www.sdbiomed.com/pdf/CA480_%20Bilirubin_(Direct).pdf)

Pack Presentation

Product Code/ Catalogue No.	KGBIL102.4.1	KGBIL102.4.2
Pack Size *	2x50ml	4x50ml
Reagent 1	1x50ml	2X50ml
Reagent 2	1X50ml	2X50ml
Reagent 3	1X2ml	1X5ml











* Pack size may vary on market demand.

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Symbols

Following symbols are used in the labeling of KEE GAD kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions
	In Vitro Diagnostics		Storage temperature
	Expiry Date		Content
	Product Name		Manufactured By



Manufactured by:
KEE GAD Biogen Pvt. Ltd.
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