LiquiMAX BILIRUBIN - (TOTAL)
(JENDRASSIK & GROF' S METHOD)

ORDERING INFORMATION

Ref. No.	Pack Size
AVBILT - 200	4 x 50 ml
AVBILT - 1000	2 x 500 ml

INTENDED USE:
LiquiMAX BILIRUBIN is an in-vitro diagnostic kit for the quantitative determination of Total Bilirubin in human serum.

PRODUCT FEATURES:
1. Both Monochromatic and Bichromatic estimations .
2. Neonatal bilirubin can be estimated.
3. 5 minutes End Point assay.
4. Linearity 25 mg/dl
5. Estmination with fixed factors : Direct Bilirubin : 24.0
6. Can be used on any colorimeter, spectrophotometer , discrete semi automated and automated analyzers

CLINICAL SIGNIFICANCE:
Red blood cells at the end of their circulating life are broken down in the reticuloendothelial system, mainly the spleen. The resulting heme, once the iron is removed, is then converted to bilirubin. This process accounts for about 80% of the 500 µmol (300 mg) of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red blood cells in the bone marrow.
Once formed, bilirubin is transported to the liver bound to albumin. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid (mono- and diglucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine, where it is metabolized by bacteria to a group of products known collectively as stercobilinogen. Elimination is almost complete and serum levels are normally negligible.
Direct bilirubin is the sum of the conjugated fractions. Direct bilirubin is elevated in conditions causing hepatic obstruction, hepatitis, cirrhosis, several inherited enzyme deficiencies, and inherited defects in canalicular excretion.

PRINCIPLE
Bilirubin reacts with diazotized sulphanilic acid to form an azocompound the colour of which is measured at 546 nm (530-560 nm) and is proportional to the concentration of bilirubin.

STORAGE AND STABILITY
All the reagents are stable up to the expiry date mentioned on the labels when the proper storage conditions are maintained.

PREPARATION OF WORKING SOLUTIONS
All the reagents are ready to use and there is no need to prepare working reagents any where

KIT COMPONENTS
1. Bilirubin Total Reagent R1
2. Bilirubin Total Reagent R2

COMPOSITION

Sulphanilic Acid	24mmol/l
Sodium Nitrite	300 mmol/l
Cone : Hcl	165 mmol/l

REAGENT RECONSTITUTION & STABILITY
Reagent are liquid stable no need for reconstitution.
When the reagent is stored properly at RT & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED
Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION
Discard any turbid reagent if blank reagent absorbance exceeds 0.3 at 546 nm against distilled water.

WARNING & PRECAUTIONS
- Reagent may contain some non reactive and preservative components. It is
- recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN COLLECTION & STORAGE
Serum is the preferred specimen . Plasma with heparin as anticoagulant may be used. Serum or Plasma should be separated as early as possible. (Samples are stable for a day when stored tightly capped at 2°c - 8°c and for a month at-10°c. Avoid exposure of samples to direct light during processing & storage. Gross contamination at any stage makes the samples un suitable for use. The samples should be brought to room temperature prior to use.
Do not use hemolysed /grossly contaminated samples

SYSTEM PARAMETERS
A) Bichromatic
(Analyzers with "Dual Wave Length" option)

Reaction Type	:	End Point		
Reaction Slope	:	Increasing		
Wavelength	:	546 nm & 630 nm		
Flowcell Temp .	:	37°C		
Incubation	:	5 Minutes at RT		
Sample Vol.	:	50µl		
Reagent Vol.	:	1050 µl		
Factor	:	Total Billrubin	:	24.0
Zero setting with	:	Distilled Water		

B) Bichromatic
(Analyzers with sample blanking option)

Reaction Type	:	End Point		
Reaction Slope	:	Increasing		
Wavelength	:	546 nm		
Flowcell Temp .	:	37°C		
Incubation	:	5 Minutes at RT		
Sample Vol.	:	50µl		
Reagent Vol.	:	1050 µl		
Factor	:	Total Billrubin	:	24.0
Zero setting with	:	Sample Blank		

TEST PROCEDURE :

A) BICHROMATIC (For analyzers with dual wave length option)
Dispense into test tubes:

Reagent	Test (T)
Total Bilirubin Reagent	1.00 ml
Sodium Nitrite	50 µl
Sample	50 µl

Mix and incubate for 5 minutes at room temperature. Read Test (T) against distilled Water at 546 & 630nms (Dual Wave Lengths).

Calculation:
Total Bilirubin (mg/di) = Abs of Test x 24.0

B) MONOCHROMATIC
(For colorimeters & analyzers with sample blanking option)

Dispense into test tubes:

Reagent	Sample Bank	Test (T)
Total Bilirubin Reagent	1.00 ml	1.00 ml
Sodium Nitrite	--	50 µl
Distilled Water	50 µl	–
Sample	50 µl	50 µl

Mix and incubate for 5 minutes at room temperture. Read Test (T) against its Sample Blank at 546 nms(530-560)

Calculation:

Total Bilirubin(mg/di) = (Abs of Test - Abs of Sample Blank) x 24.0

QUALITY CONTROL& CALIBRATION

It is recommend to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

EXPECTED VALUE

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

Total Bilirubin (Serum/ Plasma) : 0.00 - 1.1 mg/dl

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 25 mg/dl

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 0.1 mg/dl

3. Interferences

No significant interference was observed from Bilirubin upto 20mg/dl (Both conjugated and unconjugated Bilirubin), Lipema as Triglycerides upto 2000mg/dl,Ascorbic acid upto 30mg/dl.

4. PRECISION

Intra-assay precision	mean	SD	CV
N=20	(mg/dl)	(mg/dl)	(%)
Sample 1	1.2	0.013	1.08
Sample 2	3.6	0.1	2.71
Sample 3	10.2	0.3	2.94

Intra-assay precision	mean	SD	CV
N=20	(mg/dl)	(mg/dl)	(%)
Sample 1	1.21	0.014	1.15
Sample 2	3.62	0.12	3.31
Sample 3	10.3	0.35	3.39

Method comparison:

A comparison of the LiquiMAX BILIRUBIN - (TOTAL) with a commercial obtainable assay (x) gave with 36 samples the following results

y = 0.972x + 1.282; r = 0.999

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

LIMITATION

The method is linear up to 25mg/dL. For higher values, dilute the sample suitably with 0.9%saline and Repeat the assay. Apply proper dilution factor to calculate the final result.

REFERENCES:

1. Jendrassik, L.and Grof.P.Biochem Z.297,81 (1938).

Symbols Used on Pack

REF	Catalogue Number	⚠	Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
Σ	Contains sufficient no. of test		

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