ANA BIO ISP Bilirubin Total

(Jendrassik and Grof Method)

For Miura Instruments

Intended Use

Bilirubin Total is a reagent kit used for the determination of Total Bilirubin based on Jendrassik and Grof method.

Principle

Bilirubin reacts with diazotized Sulphanilic acid to produce azobilirubin (Pink colour). The intensity of the pink colour compound is directly proportional to the total bilirubin concentration in the sample.

TOTAL BILIRUBIN

Bilirubin + Sulphanilic acid + Sodium nitrite ⟶ Azobilirubin (Activator)

Components & Concentration of Reagents

Reagent	Component	Concentration
Reagent 1	Sulphanilic Acid	29 mmol/L
	Cetramide	37 mmol/L
	HCL	67 mmol/L
	Stabilizers, excipients & surface active agents	
Reagent 2	Sodium Nitrite	5.8 mmol/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability

The reagent kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label.

Reagent Preparation

Liquid reagents ready for use. After opening the reagents of R1 and R2 are stable for 30 days if closed, stored at 2° - 8°C, and protect from direct light. Do not mix different batches.

Specimen collection and preservation

Blood should be collected in a clean dry container. Although serum is preferred, plasma can also be used as sample. Following anticoagulants can be used for plasma separation:

- EDTA 2 mg/ml of blood
- HEPARIN 200 IU/ml of blood

Bilirubin is light sensitive. Avoid exposure of serum or plasma to direct light. Bilirubin in serum and plasma is stable for a day at 2° - 8°C or one month at -20°C. Samples should be brought to room temperature before use.

Automation

This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration

For Calibration use the "Multicalibrator"

Calibration Stability

For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit

Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Total Bilirubin
Method Code	TB
Type	Differential-Smpl Blk
Unit	mg/dl
Filter F1	546 nm
Blank in	Not Use
Step	Reaction Volume	U.M.
Volume reagent R1	180	µl
Volume reagent R2	45	µl
Sample Volume	23	µl
First Incubation	36	Sec
Final Incubation	300	Sec.

Normal range

Total Bilirubin : Up to 1 mg/dl

Note: Expected range varies from population to population and each laboratory should establish the normal range for its own population.

Limitations

Dilute the specimen if the Bilirubin value is above 20 mg/dl. Suitable dilution can be done with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct Bilirubin value.

Quality Control

It is recommended that each batch should include a normal and an abnormal commercial reference control serum or a known patient serum. Use of quality control serum, checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents, accuracy of prob aspiration, and serum exposure to light.

Accuracy-Recovery

Bilirubin added to a serum matrix containing known amounts of Bilirubin gave an average recovery of 96.5%.

Interference

The high dilution of the sample with the reagent reduces to a minimum possible interference by lipids. In the case that these are present, the concentration must not exceed 500 mg/dl of Triglycerides. Haemoglobin influences the reaction at concentrations over 250 mg/dl. Ascorbic acid not interfere the reaction up to 2 mg/dl.

In very rare cases, monoclonal gammapathies (multiple myeloma), in particular IgM type (Waldenstrom's macroglobulinemia) can cause unreliable results.
Other compounds may interfere.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	0.81	0.01	1.84	20
High	mg/dl	4.82	0.10	2.11	20
Between run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	0.81	0.01	1.83	60
High	mg/dl	4.82	0.11	2.27	60

Sensitivity

At 546 nm the concentration of 0.02 mg/dl of Total Bilirubin can estimate.

References

1. Jendrassik, L., et al.(Biochem. 2. 297, 81 (1938)
2. Practical Clinical Biochem. Vol 1, 5th edition, H. Varley, page 1012, (1980).

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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