

Quantitative determination of Direct & Total Bilirubin in serum / plasma. Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
TDBIL 100	2 X 50 mL
TDBIL 200	2 X 100 mL
TDBIL 1000	2 X 500 mL

CLINICAL SIGNIFICANCE

Bilirubin is a breakdown product of hemoglobin, insoluble in water. It is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma. Causes of hyperbilirubinemia:

Total bilirubin: Increase hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, and drugs.

Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage.

Method

Photometric Test method

PRINCIPLE

Total bilirubin and Direct bilirubin in the sample reacts with diazotized sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both direct and indirect bilirubin couple with diazo in the presence of Accelerator. The terms "direct" and "total" refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilising (accelerating) reagents. The "direct" and "indirect" bilirubin is only approximately equivalent to the conjugated and unconjugated fractions.

REAGENT

- Reagent 1 : Bilirubin Total Reagent.
- Reagent 2 : Bilirubin Direct Reagent.
- Reagent 3 : Total Nitrite Reagent.
- Reagent 4 : Direct Nitrite Reagent.

REAGENT PREPARATION

Working Reagent: Direct Reagent – Mix Total/Direct reagent and Total/Direct nitrite in the ratio of 1:50 i.e for making 5 ml working reagent add 100 µl of respective nitrite reagent to 5.0 ml of Total/Direct reagent.

REAGENT STORAGE AND STABILITY

When stored at 2-8°C reagent is stable until the expiration date stated on the bottle and kit box label

Working Reagent

This working reagent is stable for 7 days at 2-8°C. The reagent may develop slight yellow colour upon storage but this does not affect the performance.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum or heparin plasma

It is very important to store the sample protected from light!

Stability: 7 days at 4 – 8°C

6 months at –20°C in case of immediate freezing.

Freeze only once! Discard contaminated specimens!

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28°C, prior to analysis.

AUTOMATED PARAMETERS

	Direct Bilirubin	Total Bilirubin
Wavelength I	546 nm	546 nm
Wavelength II	630 nm	630 nm
Cuvette Light Path	1 cm	1 cm
Reaction Type	End Point	End Point
Reaction Temperature	37°C	37°C
Measurement	Against Reagent blank	
Sample Volume	50 µl	50 µl
Reagent Volume	1000 µl	1000 µl
Incubation	5 mins.	5 mins.
Factor	20.00	24.00
Low Normal	0.0 mg/dl	0.0 mg/dl
High Normal	0.5 mg/dl	1.2 mg/dl
Linearity	25 mg/dl	25 mg/dl

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

Working reagent	1000 µl
Sample	50 µl

- Mix & Incubate for 05 min. at 37°C or 10 min. at R.T. Measure absorbance at 546/630 nm.

SAMPLE DILUTIONS

- This method is linear upto a concentration of 25 mg/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

Total Bilirubin (mg/dl) = Abs of test X 24
Direct Bilirubin (mg/dl) = Abs of test X 20

CALBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

This method has been standardized against the manual Jendrassik-Gróf test.

The assigned calibrator values for Total & Direct Bilirubin have been made traceable to the NIST SRM 916 reference material.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS WITHIN RUN

Sample	Mean Concentration	SD	CV %
Direct Bilirubin			
Norm	0.843	0.028	3.30%
Path	3.117	0.0394	3.02%

Total Bilirubin			
Norm	1.14	0.04	3.69%
Path	4.04	0.12	2.95%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Direct Bilirubin			
Norm	0.0837	0.020	2.38%
Path	3.18	0.067	2.16%
Total Bilirubin			
Norm	1.10	0.03	2.72%
Path	4.04	0.11	2.72%

LINEARITY

The method is linear upto a concentration of 25mg/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for Bilirubin direct is 0.2 mg/dL and Total Bilirubin is 1.2 mg/dL.

METHOD COMPARISON

A comparison of Accucare Bilirubin direct with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

Adults	
Direct	Up to 0.5 mg/dL = 3.4 mmol/L
Total	Up to 1.2 mg/dL = 17 mmol/L

Newborns (Total bilirubin) :		
Age	premature	full-term
Up to 24 h	1.0-8.0 mg/dL = 17-137 mmol/L	2.0-6.0mg/dL= 34-103 mmol/L
Up to 48 h	6.0-12.0 mg/dL = 103-205 mmol/L	6.0-10 mg/dL = 103-171 mmol/L
3-5 days	10-14 mg/dL = 171-239 mmol/L	4.0-8.0mg/dL= 68-137 mmol/L

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.







INTERFERENCE

- Hemoglobin: No interference found upto 150 mg/dL.
- Lipemia: No interference found upto 500 mg/dL.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

- Pearlman FC and Lee RTY. Detection and measurement of bilirubin in serum, with use of surfactants as solubilizing agents. Clin Chem 1974; 20: 447-453.

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For <i>in vitro</i> Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit



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