LIQUID LINE

Bilirrubina Total

AA

DPD method for total bilirubin determination in serum or plasma

SUMMARY

Bilirubin, a compound produced by the degradation of hemoglobin, is captured by the liver for its conjugation and bile excretion. Hepatocellular disorders or biliary obstructions may lead to hyperbilirubinemia.

Erythroblastosis fetalis or hemolytic anemia of the newborn are pathologies due to mother-fetus blood incompatibility, in which an excessive destruction of red blood cells occurs. The result is a severe increase of serum bilirubin, with the consequent risk of pigment diffusion to the central nervous system. Therefore, bilirubin determination in these newborns is extremely important.

PRINCIPLE

Indirect bilirubin, bound to albumin, is released by a surfactant. Total bilirubin reacts with dichlorophenyldiazonium salt (DPD) forming a red azocompound in acid medium.

PROVIDED REAGENTS

A. Reagent A: aqueous solution containing 150 mmol/l hydrochloric acid and surfactant.

B. Reagent B: aqueous solution 1.5 mmol/l dichlorophenyldiazonium salt in 150 mmol/l hydrochloric acid.

NON-PROVIDED REAGENTS

Wiener lab's **Calibrador A plus**.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use. Mix by inversion before use.

Reagent B may develop a slight turbidity which does not affect its performance.

WARNINGS

Reagents are for “in vitro” diagnostic use.

Reagents are corrosive.

H315+H320: Causes skin and eye irritation. H314 Causes severe skin burns and eye damage. P262 Do not get in eyes, on skin, or on clothing. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P280 Wear protective gloves/protective clothing/eye protection/face protection.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date stated on the box.

SAMPLE

Serum or plasma

a) Collection: obtain as usual. Protect from natural or artificial light covering the tube with black paper.

b) Additives: heparin or EDTA.

c) Known interfering substances: no interference has been observed with hemoglobin up to 500 mg/dl (0.5 g/dl) and triglycerides up to 500 mg/dl (5 g/l). Nonetheless, samples with moderate hemolysis or hyperlipemic samples, may lead to erroneous results.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: sample should be preferably fresh. If assay is not performed immediately, serum can be stored up to 48 hours at 2-10ºC.

The action of light is capable of destroying up to a 50% of the bilirubin present in the sample. Consequently, it should be carefully protected from light.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Micropipettes and pipettes to measuring the stated volumes
- Stopwatch
- Autoanalyzer

ASSAY CONDITIONS

- Wavelength: 546 nm (520 - 550 nm)
- Reaction temperature: 25ºC (30ºC or 37ºC)
- Reaction time: 5 minutes 30 seconds
- Sample volume: 80 ul
- Final reaction volume: 1.28 ml

PROCEDURE

In 3 tubes labeled RB (Reagent Blank), SB (Sample/Calibrator/Control Blank) and S (Sample/Calibrator/Control), place:

	RB	SB	S
Reagent A	1 ml	1.2 ml	1 ml
Distilled water	80 ul	-	-

Sample	-	80 ul	80 ul
Mix and exactly incubate for 30 seconds. Then, add:			
Reagent B	0.2 ml	-	0.2 ml

Mix and incubate for 5 minutes. Measure optical density at 546 nm (520 - 550 nm), setting the instrument to zero with the Reagent Blank (RB). Reading 1 (OD_1): SB (Sample Blank) or CB (Calibrator Blank). Reading 2 (OD_2): S (Sample) or C (Calibrator).

STABILITY OF FINAL REACTION

Color is stable for 30 seconds, so absorbance should be read within that period.

CALCULATIONS

Total Bilirubin (mg/l) = $(OD_{2\,S} - OD_{1\,SB}) \times f$

where:

$$f = \frac{X^{*}\ mg/l}{OD_{2\,C} - OD_{1\,CB}}$$

(*) total bilirubin concentration in Wiener lab.'s **Calibrador A plus**

QUALITY CONTROL METHOD

Each time the test is running, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known total bilirubin concentration.

REFERENCE VALUES

Total bilirubin in serum or plasma

- Adults: up to 10 mg/l

- Newborns:

	Term infant	Preterm infant
umbilical cord blood	< 20 mg/l	< 20 mg/l
up to 24 hrs	14 - 87 mg/l	< 80 mg/l
up to 48 hrs	34 - 115 mg/l	< 120 mg/l
from 3rd to 5th day	15 - 120 mg/l	< 160 mg/l

Within a month after birth, total bilirubin concentration decrease reaching the adult's average level.
In preterm infants, levels take longer to reach normality, depending on the hepatic immaturity degree.
It is recommended that each laboratory establishes its own reference values.

SI SYSTEM UNITS CONVERSION

Bilirubin (umol/l) = Bilirubin (mg/l) x 1.71

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
The action of light, both on samples and on standard solutions, is capable of destroying up to 50% of bilirubin in one hour.

PERFORMANCE

a) Reproducibility: CLSI protocol EP15-A was applied. Three concentration levels were tested, in replicates by four, during 5 days. With the obtained data, total and intra-assay precision were calculated.

Intra-assay precision (n = 20)

Level	S.D.	C.V.
8.5 mg/l	± 0.28 mg/l	3.29 %
46.1 mg/l	± 0.31 mg/l	0.67 %
164.5 mg/l	± 1.21 mg/l	0.74 %

Total precision (n = 20)

Level	S.D.	C.V.
8.5 mg/l	± 0.30 mg/l	3.53 %
46.1 mg/l	± 0.77 mg/l	1.67 %
164.5 mg/l	± 3.15 mg/l	1.92 %

b) Linearity: the reaction is linear up to 300 mg/l bilirubin. For higher values, repeat the determination using diluted sample 1:2 or 1:4 with saline solution. Multiply the obtained result by 2 or 4 according to the dilution performed.
c) Detection limit: in spectrophotometers with 1 cm optical length square cuvettes, for a ΔA minimum of 0.001, the minimum detectable concentration change will be of 0.031 mg/dl.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.
For calibration, it must be used Wiener lab.'s **Calibrador A plus**.

WIENER LAB. PROVIDES

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1120008)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009334)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009244)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009605)

REFERENCES

- Burtis, C.A.; Ashwood, E.R. - Tietz Fundamentals of Clin. Chem. 5th Ed.: 966, 2001.
- Weigl, E.; Bach, H.; Krieg, D. - Med. Klin. 70/15:664 (1975).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 5th ed., 2000.
- CLSI: Clinical and Laboratory Standards Institute (ex-NCCLS) - Protocols EP 15A, 2001 / EP 17A, 2004.
- Tietz Textbook of Clinical Chemistry - Saunders Co., 3rd ed. (2001).

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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