LIQUID LINE

Bilirrubina Directa

AA

DPD method for direct bilirubin determination in serum or plasma

SUMMARY

Bilirubin is a product resulting from the degradation of the hemo group by the phagocytic mononuclear system. It has two forms, conjugated and non-conjugated. Conjugation with glucuronic acid takes place inside the hepatocytes. The conjugated bilirubin is subsequently excreted in the bile. Direct bilirubin is measured in the investigation of a pre-hepatic, hepatic or post-hepatic jaundice. Increased levels of direct bilirubin are observed in hepatocellular diseases such as hepatitis and post-hepatic cholestasis cases.

PRINCIPLE

Direct bilirubin reacts with dichlorophenyldiazonium salt (DPD) yealding a red azocompound in acid medium.

PROVIDED REAGENTS

A. Reagent A: aqueous solution containing 17 mmol/l hydrochloric acid.
B. Reagent B: aqueous solution containing 0.4 mmol/l dichlorophenyldiazonium salt in 17 mmol/l hydrochloric acid.

NON-PROVIDED REAGENTS

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

INSTRUCTIONS FOR USE

Reagent A: ready to use.
Reagent B: ready to use. The Reagent B may develop a slight brownish color shade which does not affect its performance.

WARNINGS

Reagents are for “in vitro” diagnostic use.
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 at 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: heparine.
c) Known interfering substances:
- Samples with hemolysis produce falsely decreased values.
- No interferences have been observed with triglycerides up to 5 g/l (500 mg/dl). However, hyperlipemic samples produce overvaluation of the results.

See Young, D.S. under 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: 6 minutes
- 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 60 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).

CALCULATIONS

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

where:

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

(*) direct 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 direct bilirubin concentration.

REFERENCE VALUES

Direct bilirubin in serum or plasma:

Adults: up to 2 mg/l

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

SI SYSTEM UNITS CONVERSION

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

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

The action of light, both on sample and on the standard solutions, is capable of destroying up to 50% of the bilirubin in one hour.

PERFORMANCE

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

Intra-assay precision (n = 20)

Level	S.D.	C.V.
6.9 mg/l	± 0.075 mg/l	1.09 %
24.3 mg/l	± 0.255 mg/l	1.05 %

Total precision (n = 20)

Level	S.D.	C.V.
6.9 mg/l	± 0.15 mg/l	2.23 %
24.3 mg/l	± 0.249 mg/l	1.03 %

b) Linearity: the reaction is linear up to 120 mg/l (12 mg/dl) direct bilirubin. For higher values, repeat determination using 1:2 or 1:4 diluted sample with saline solution and multiply the obtained result by 2 or 4 accordingly.

c) Detection limit: it depends on the photometer used and the wavelength. In spectrophotometer with 1 cm optical length square cuvettes, for a ΔA minimum of 0.001, the minimum detectable concentration change will be of 0.012 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. 1120007)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009335)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009246)

240 ml: 4 x 50 ml Reagent A
2 x 20 ml Reagent B
(Cat. 1009604)

REFERENCES

- Burtis, CA; Ashwood, ER - Tietz Fundamentals of Clin. Chem. 5th Ed.: 605, 2001.
- Burtis, CA; Ashwood, ER - Tietz Textbook of Clin. Chem. 3rd Ed.:1170, 1996.
- 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

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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