Bilirrubina Directa

AA

DPD method for direct 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 is a pathology produced by mother-fetus blood incompatibility, in which an excessive destruction of red blood cells occurs. This results in 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

Direct bilirubin reacts with dichlorophenyldiazonium salt (DPD) forming a red azocompound in an acid solution.

PROVIDED REAGENTS

A. Reagent A: vials containing dichlorophenyldiazonium salt.
B. Reagent B: aqueous solution containing 90 mmol/l sulfamic acid.
C. Reagent C (Reagent for Sample Blank): aqueous solution containing 90 mmol/l sulfamic acid.

Final concentrations

sulfamic acid	90 mmol/l
sodium chloride	6.6 g/l
dichlorophenyldiazonium	0.1 mmol/l

NON-PROVIDED REAGENTS

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

INSTRUCTIONS FOR USE

Reagent B and C: ready to use.
Working Reagent: reconstitute each vial of Reagent A with 10 ml of Reagent B. Cap and shake until complete dissolution. Homogenize and date.

WARNINGS

Reagents are for “in vitro” diagnostic use.
Reagent B and Reagent C are irritant. H315+H320: Causes skin and eye irritation. 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.

INSTABILITY OR DETERIORATION OF REAGENTS

Absorbance readings of the Blank Reagent, higher than 0.100 O.D. (at 546 nm) indicate its deterioration. Discard in such case.
Turbidity indicates reagents deterioration. Discard in such case.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box.
Working Reagent: stable for 21 days in refrigerator (2-10ºC).

SAMPLE

Serum or plasma
a) Collection: obtain as usual. Protect from natural or artificial light covering the tube with black paper.
b) Additives: if plasma is used, use heparin as collection.
c) Known interfering substances: no interference have been observed by hemoglobin up to 180 mg/dl, triglycerides up to 550 mg/dl (5,50 g/l) or heparin up to 20 U/ml.
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 in refrigerator (2-10ºC) and whole blood no more than 24 hours in refrigerator (2-10ºC) or 12 hours at room temperature (< 25ºC).
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 or photocolorimeter
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometric cuvettes
- Stopwatch

ASSAY CONDITIONS

- Wavelength: 546 nm in spectrophotometer or 520-550 nm in photocolorimeter with green filter.
- Reaction temperature: room temperature (< 25ºC)
- Reaction time: 10 minutes
- Sample volume: 40 ul
- Final reaction volume: 0.54 ml

PROCEDURE

In 4 photocolorimeter tubes labeled B_C (Calibrator Blank),

C (Calibrator), B_U (Unknown Blank) and U (Unknown), place:

	B	C	B_U	U
Reagent C	0.5 ml	-	0.5 ml	-
Reagent B	-	0.4 ml	-	0.4 ml
Calibrator	40 ul	40 ul	-	-
Sample	-	-	40 ul	40 ul
Working Reagent	-	0.1 ml	-	0.1 ml

Mix and incubate for 10 minutes at room temperature (< 25ºC). Read in spectrophotometer at 546 nm or in photocolorimeter with green filter (520-550 nm), setting the instrument to zero with the Reagent Blank (B_R). Volumes may be proportionally increased.

Note: process a Reagent Blank (B_R) for each series of determinations mixing 0.4 ml Reagent B + 40 ul distilled water + 0.1 ml Working Reagent.

STABILITY OF FINAL REACTION
Absorbance should be read at exactly 10 minutes.

CALCULATIONS
Direct Bilirubin (mg/l) = $(U - B_u) \times f$

$$f = \frac{X^* \text{ mg/l}}{C - B_C}$$

*direct bilirubin concentration in Wiener lab.'s **Calibrador A plus**

Example:
$B_C = 0.008$
$C = 0.135$
$B_U = 0.009$
$U = 0.029$
$X = 23.9$ mg/l

$$f = \frac{23.9 \text{ mg/l}}{0.135 - 0.008} = 188.2 \text{ mg/l}$$

The calculated colorimetric factor (f) may also be employed, using Wiener lab's **Bilirrubina Standard** with Wiener lab.'s **Bilirrubina Total AA** (DPD method) technique.

QUALITY CONTROL METHOD
Each time the test is performed, 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.

UNITS CONVERSION
Bilirubin (mg/l) = Bilirubin (mg/dl) x 10
Bilirubin (mg/dl) x 17.1 = Bilirubin (umol/l)

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE.
The action of light, both on sample and on the standard solutions, are capable of destroying up to 50% of the bilirubin in one hour.

PERFORMANCE
The assays were performed in an Express Plus analyzer(*). If using by manual procedure, user must validate that similar performance to that stated below is obtained.
a) Reproducibility: CLSI's (ex NCCLS) protocol EP15-A was applied. The following results were obtained:

Intra-assay precision

Level	S.D.	C.V.
1.4mg/l	± 0.05 mg/l	4.03 %
18.0 mg/l	± 0.24 mg/l	1.33 %

Total precision

Level	S.D.	C.V.
1.4 mg/l	± 0.06 mg/l	5.09 %
18.0 mg/l	± 0.36 mg/l	2.00 %

b) Linearity: applying the EP6-P protocol, a linearity of 140 mg/l was obtained. For higher values, dilute the sample 1:2 or 1:4 with saline solution, repeat the determination and multiply the obtained result by 2 or 4 accordingly.
c) Analytical sensitivity: 1.1 mg/l.

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**.
The kit provides the Reagent for the Sample Blank (Reagent C) which is only required in some autoanalyzers. In other analyzers this Reagent is not necessary. For further information consult your analyzer's application manual for Wiener lab. reagents.

WIENER LAB. PROVIDES
- 200 ml (Cat. 1009306): - 4 vials Reagent A
  - 4 x 10 ml Reagent B
  - 4 x 40 ml Reagent C
- 200 ml (Cat. 1120006): - 4 vials Reagent A
  - 4 x 50 ml Reagent B
  - 2 x 100 ml Reagent C

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SYMBOLS

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