

PROTOPORPHYRIN IX free erythrocyte and ZincPROTOPORPHYRIN
IN WHOLE BLOOD BY FLUORIMETRY – FAST –
CODE Z39110

BIOCHEMISTRY

The determination of ZincProtoporphyrin erythrocyte (ZPP-WB) has been used as an indicator of lead intoxication.

The risk of exposure to lead in the population at large is different from that in occupational settings. In the latter, the main route of absorption is through respiratory inhalation. After absorption, lead is transported to the blood, where it is equally distributed in red blood cells and plasma; then it reaches diverse organs and tissues, and is deposited mainly in bone.

Lead inhibits the biosynthesis of hemoglobin in red blood cells; by blocking ferrochelatase, it obstructs the binding of iron to Protoporphyrin IX, which in turn binds to zinc, forming ZincProtoporphyrin (ZPP). As a result, the concentration of ZPP in the red blood cell increases and that of hemoglobin decreases, leading to normocytic hypochromic anemia and high levels of serum iron.

Protoporphyrin IX is an important precursor of biologically essential prosthetic groups such as heme, cytochrome C and chlorophylls. As a result, a number of organisms are able to synthesize this tetrapyrrole from basic precursors such as glycine and succinyl-CoA, or glutamate. Despite the wide range of organisms that synthesize Protoporphyrin IX, the process is largely preserved by bacteria to mammals, with some distinct exceptions in higher plants.

Porphyrias are a group of hereditary disorders resulting from enzymatic defects in the biosynthetic pathway of heme. Depending on the specific enzyme involved, various porphyrins and their precursors accumulate in different types of samples. Patterns of porphyrin accumulation in erythrocytes and plasma and excretion of heme precursors in urine and faeces allow to detect and differentiate porphyrins.

PPIX analysis should be performed in patients with clinical suspicion of erythropoietic protoporphyria (EPP) or X-linked dominant protoporphyria (XLDPP). The clinical presentation of EPP and XLDPP is identical to the onset of typically infantile symptoms. Skin photosensitivity in sun-exposed areas of the skin generally worsens in the spring and summer months. Common

symptoms may include itching, edema, erythema, burning or burning sensation, and occasionally scarring of the skin in sun-exposed areas. Although genetic in nature, environmental factors aggravate the symptoms, significantly affecting the severity and course of the disease.



This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 005		06/2024
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TECHNICAL FEATURES

Principle of the Method :

This method determines the concentrations of **PROTOPORPHYRIN IX and ZincPROTOPORPHYRIN in Whole Blood (WB)**. The Internal Standard (50 µl) and a Precipitation Reagent (600 µl) are added to 200 µl of WB; after 20 sec. at vortex and 5 minutes in microcentrifuge at 10.000 rpm, 20 µl are injected into HPLC system.

Recovery :

about 98 - 102 %

Sensitivity (LLOD) :

0,2 µg/dl

Minimum Concentration analyzable (LLOQ) :

0,5 µg/dl

Linearity :

0,5 - 10.000 µg/dl

Accuracy intra serie (relative error %) PPIX :

C1	C2
2,5 µg/dl	50 µg/dl
4,79%	1,92%

Accuracy inter serie (relative error %) PPIX :

Ci	Cs
2,5 µg/dl	50 µg/dl
6,44%	1,94%

Reproducibility intra serie (coefficient of variation %) PPIX :

C LLOQ	Cm	Cs
0,5 µg/dl	10 µg/dl	25 µg/dl
3,13%	0,74%	2,51%

Reproducibility inter serie (coefficient of variation %) PPIX :

C LLOQ	Cm	Cs
0,5 µg/dl	10 µg/dl	25 µg/dl
5,98%	8,03%	2,13%

Coefficient of correlation R2 + Dev. Std

PPIX 0,9990 ± 0,0003

Accuracy intra serie (relative error %) ZnPP :

C1	C2
2,5 µg/dl	50 µg/dl
2,29%	1,66%

Accuracy inter serie (relative error %) ZnPP :

Ci	Cs
2,5 µg/dl	50 µg/dl
2,68%	4,48%

Reproducibility intra serie (coefficient of variation %) ZnPP :

C LLOQ	Cm	Cs
0,5 µg/dl	10 µg/dl	25 µg/dl
2,17%	2,34%	2,85%

Reproducibility inter serie (coefficient of variation %) ZnPP :

C LLOQ	Cm	Cs
0,5 µg/dl	10 µg/dl	25 µg/dl
2,90%	4,58%	2,70%

Coefficient of correlation R2 + Dev. Std

Znpp 0,9954 ± 0,0039

Acceptable values in urine of not exposed subjects:

5 - 40 µg/dl ZnPP
8 - 34 µmol ZnPP/mol EME
6 - 15 µg/dl Proto IX

References:

-Pediatric reference ranges for zinc protoporphyrin *ClinBiochem* 2003 February; 36(1):21-25
-Establishment of normal values in adults for zinc protoporphyrin (ZPP) using hematofluorometer: correlation with normal blood lead values R.S.Suga, A.J. Fischinger & F.W.Knoch Page 637-642

NOTE: each laboratory must calculate its own experimental ranges, relative to the reference ones

Components of the kit :

All the components are ready-to-use and stable for 3 years at 2 – 8 °C.

Reagent A – Deproteinization Solution, **1 x 70 ml**

Reagent B – Internal Standard Solution, **1 x 5 ml**

Reagent L - Test Solution ZincProtoporphyrin, **1 x 5 ml**

Reagent N - Test Solution Protoporphyrin IX, **1 x 5 ml**

Calibrator lyophil.

Code Z39016 (packed separately – see data sheets)

Reagent M - Mobile Phase, **3 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 µl
Fluorimetric Detector
To underline the ZnPP: $\lambda_{ex}= 417\text{ nm}$,
 $\lambda_{em}= 635\text{ nm}$
To underline the Proto IX: $\lambda_{ex}= 387\text{ nm}$, $\lambda_{em}= 633\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure:

Collect blood in a suitable test tube with anticoagulant EDTA or HEPARIN

Blood Storage:

Stable for 4 weeks at 2 – 8 °C or 4 months at – 20 °C.

PREANALYTICAL PROCEDURE

Pipette in a vial standard:

- 900 µl of **Reagent A** – Deproteinization Sol.
- 50 µl of **Reagent L** – Test Sol. ZincProtoporphyrin
- 50 µl of **Reagent N** - Test Sol. Protoporphyrin IX
- 50 µl of **Reagent B** – Internal Std Sol.

Vortex for 10 sec.

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation.

Pipette in a 1,5 ml tube:

	Reconstituted Calibrator (see data sheets)	Reconstituted Control L1 (see data sheets)	Reconstituted Control L2 (see data sheets)	Samples
Reconstituted Calibrator (see data sheets)	100 µl			
Reconstituted Control L1 (see data sheets)		100 µl		
Reconstituted Control L2 (see data sheets)			100 µl	
Samples				100 µl
Reagent B – Internal Standard	50 µl	50 µl	50 µl	50 µl
Reagent A – Deproteinization Sol.	600 µl	600 µl	600 µl	600 µl

Vortex for 20 sec.

STEP 2 : Centrifuge at 10.000 rpm for 5 min.

STEP 3 : Take 400 µl of supernatant

STEP 4 : Add 200 µl of H₂O HPLC grade

Vortex for 10 sec.

N.B.: at this step, the sample is stable for 3 days at 2-8 °C

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

PROTOPORPHYRIN IX e ZincPROTOPORPHYRIN in WHOLE BLOOD - Warnings

REAGENT L : TEST SOLUTION ZincPROTOPORPHYRIN

ZincPROTOPORPHYRIN	About 162,4 µg / dl
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REAGENT N : TEST SOLUTION PROTOPORPHYRIN IX

PROTOPORPHYRIN IX	About 100,0 µg / dl
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SPECTROFLUORIMETRIC DETECTOR PARAMETERS

λ OF EXCITATION ZnPP	417 nm
λ OF EMISSION ZnPP	635 nm
λ OF EXCITATION PROTO IX	387 nm
λ OF EMISSION PROTO IX	633 nm
GAIN	1 - HIGH
INTEGRATION TIME	4

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL C18 4,6 x 50 mm, 2,7 µm, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column POROSHELL C18 4,6 x 50 mm, 2,7 µm. Disconnect the detector and filter 30 ml of **H₂O : Acetonitrile or Methanol (20 : 80 v/v)** solution and subsequently 30 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v/v)** solution, set flow at 1,0 ml/min. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,4 ml/min. For 15 min. Finally inject the Chemical Standard and verify the quality of the HPLC run.

It is NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of a solution of **H₂O : Acetonitrile (20 : 80 v/v)** set flow at 1,4 ml/min and discharge. Store the column in a solution of **H₂O : Acetonitrile (20 : 80 v/v)**. **It is recommended to wash the column at the end of each analytical session.**

INJECTION NEEDLE WASHING

Flux 15 ml of a solution of **H₂O : Acetonitrile (80 : 20 v/v)** set flow at 1,4 ml/min.

HPLC PARAMETERS

LOOP	20 µl
FLOW	1,4 ml / min.
PRESSURE	About 70 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z39016	Calibrator for Protoporphyrin IX and Zincprotoporphyrin	1 x 2 x 0,5 ml/ 4 x 1 ml
Z39019	Control for Protoporphyrin IX and Zincprotoporphyrin – Levels 1 and 2	1 x 3 x 0,5 ml/ 10 x 1 ml
Z39111	Mobile Phase	4 x 500 ml
Z699975902	Poroshell C 18 (50 x 4,6mm -2,7 um) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs



PROTOPORPHYRIN IX and ZincPROTOPORPHYRIN BY FLUORIMETRY (Reference Chromatograms)

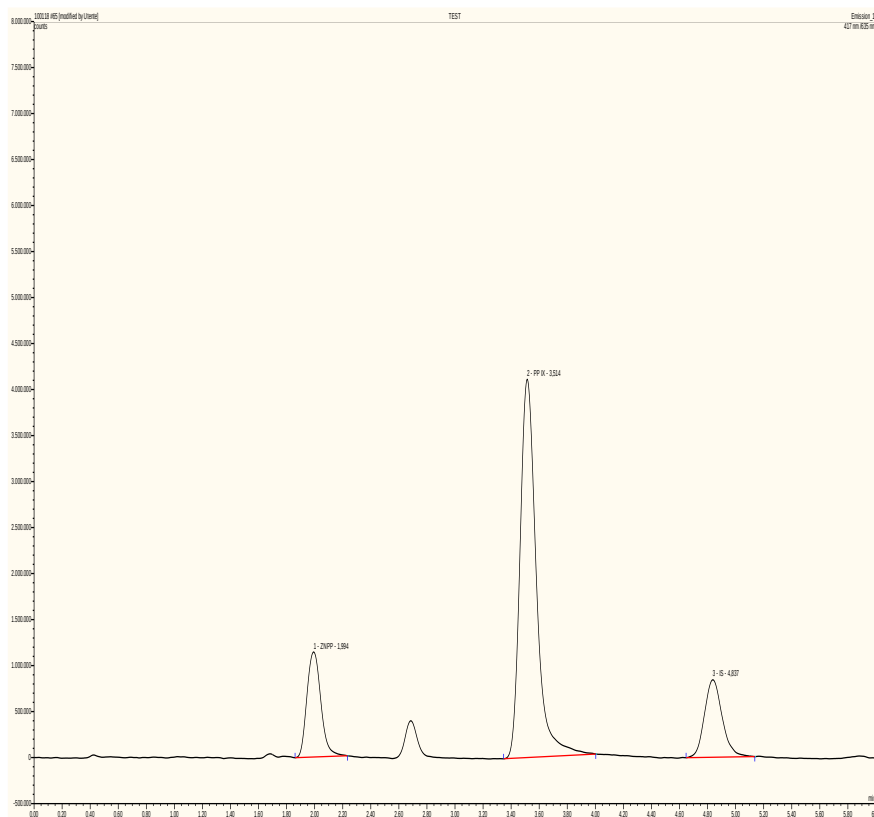


Fig. 1 :	Test Solution		
	R.T. 1.99	ZnPP	
	R.T. 3.51	Proto IX	
	R.T. 4.83	Internal Standard	

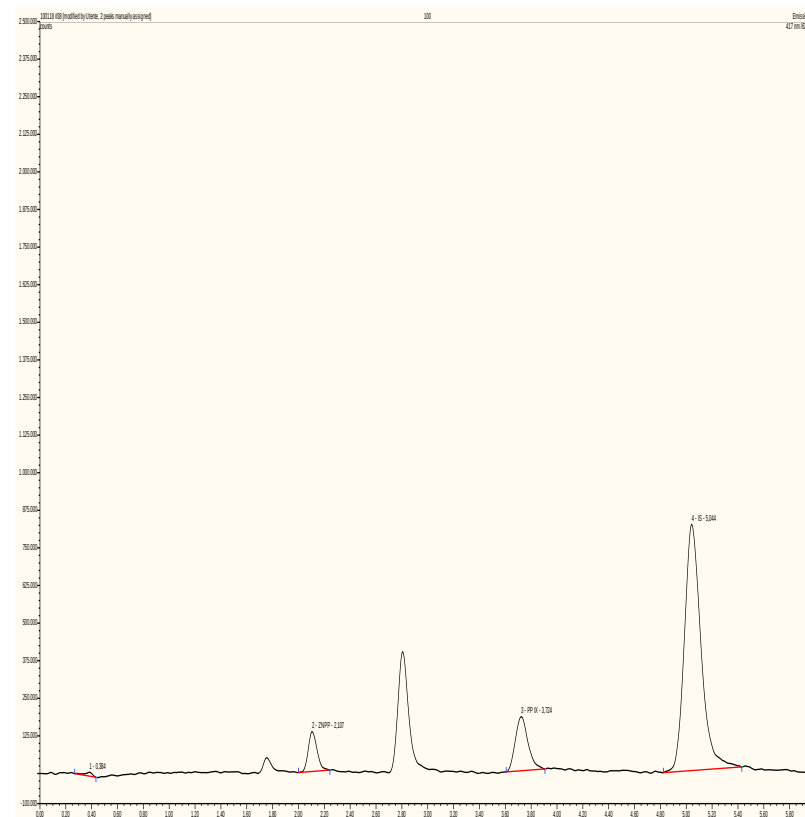


Fig. 2 :	Calibrator in whole blood		
	R.T. 2.10	ZnPP	100,00 µg/dl
	R.T. 3.72	Proto IX	100,00 µg/dl
	R.T. 5.04	Internal Standard	

PROTOPORPHYRIN IX and ZincPROTOPORPHYRIN BY FLUORIMETRY (Reference Chromatograms)

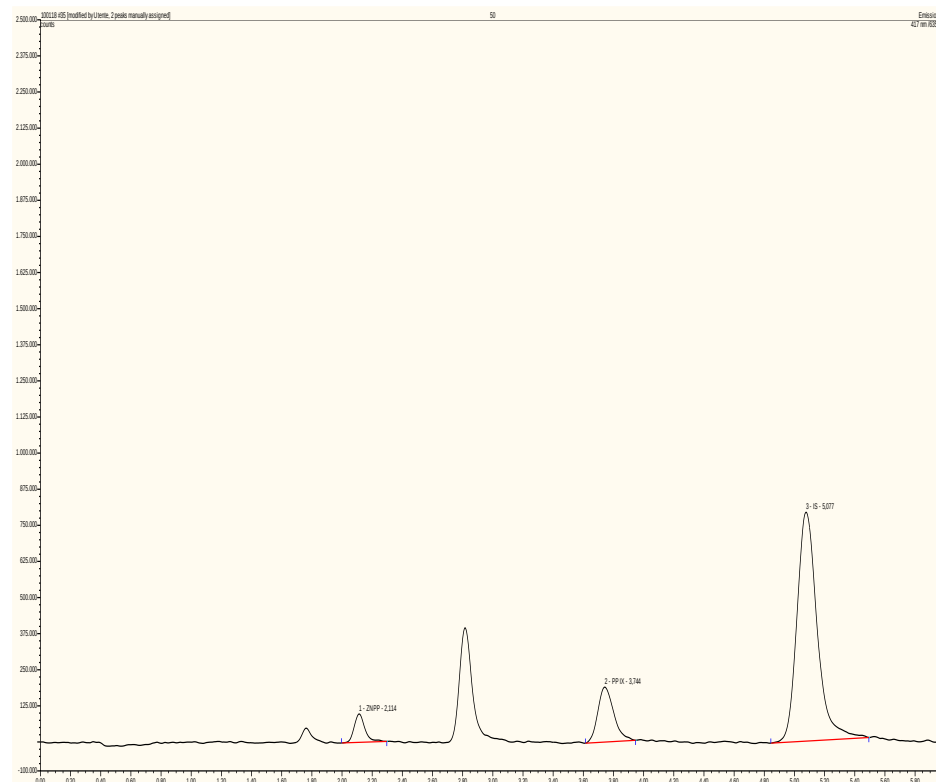


Fig. 3 :	Sample in whole blood		
	R.T. 2.11	ZnPP	5,00 µg/dl
	R.T. 3.74	PP IX	5,00 µg/dl
	R.T. 5.07	Internal Standard	