ALA

Chromatographic – Colorimetric Determination
of δ-Aminolevulinic Acid
in Urine

20 tests **REF KR01-20**

PRINCIPLE

In hemoglobin biosynthesis lead inhibits the activity of ALA dehydrase enzyme, which catalyzes the condensation of two molecules of ALA (δ-aminolevulinic acid) to form a molecule of PBG (porphobilinogen). In lead intoxication only a little part of ALA is used for hemoglobin synthesis, while the most part is emptied in urine. So the increase of urinary ALA is the most certain index to evaluate lead poisoning.
In the screening of lead exposed workers it is the normal use to determine contemporary urinary PBG and ALA. In accordance with recent studies PBG determination has lost its diagnostic value because it has been ascertained that PBG concentration in lead exposed workers is very low or even null like in normal subjects nor exposed to lead. Consequently urinary ALA can be effectively determined without a preventive PBG removal by passing it through a column charged with anionic ion-exchange resin. They use only one column of cationic ion-exchange resin, where ALA is adsorbed. After washing of interfering substances, it is eluted and quantitatively determined by Ehrlich's reaction.

REAGENTS AND COLUMNS

Components of the kit:	**Code KR01-20**
REAGENT 1 Sodium acetate	**2x60 ml**
***REAGENT 2** Acetylacetone	**1x1.5 ml**
REAGENT 3/A DMAB (predosed)	**1 vial**
***REAGENT 3/B** Acetic acid	**1x25 ml**
***REAGENT 3/C** Perchloric acid	**1x5 ml**
REAGENT 4 Standard δ-aminolevulinic acid 0.2 g/L	**1x1 ml**
COLUMNS chromatographic columns	**20**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.
STABILITY: the reagents and the columns, stored at 2-8°C, are stable up to the expiry date shown on the package.

NECESSARY INSTRUMENTS

Bain-marie 100°C.
Spectrophotometer or filter photometer at 553 nm (520 - 570 nm)

PREPARATION OF THE REAGENTS

REAGENT 3 (3/A + 3/B)
Dissolve the contents of a vial of Reagent 3/A into the vial of Reagent 3/B and mix well to complete solubility.
STABILITY: 6 months at 2-8°C.

EHRLICH'S REAGENT (3 + 3/C)
Add 1.9 ml of Reagent 3/C to 10 ml of Reagent 3 and mix till obtaining an homogeneous solution. The solution obtained is sufficient for 5 determinations. If necessary, they can be prepared higher quantities. Each column requires 2 ml of this reagent. STABILITY: **6 hours at room temperature**.

SAMPLE

24-hour urine.
Collect the urine and add concentrated hydrochloric acid till the relative pH is less than 6. Mix well, measure the volume and store at 2-8°C.
STABILITY: δ-amino levulinic acid is stable for at least one month; if stored at 2-8°C and at pH < 6.

PROCEDURE

Wavelength:	553 nm (520 - 570 nm)
Light path:	1 cm
Reading:	against reagent blank
Temperature:	boiling water-bath
Linearity:	up to 6 mg/100 ml
Sensitivity:	0.1 mg/100 ml
C.V. (intra-assay):	2%
C.V. (inter-assay):	3%

PREPARATION OF THE COLUMNS

Take the upper cap off and snap the bottom tip. Let the liquid completely flow out and discard it.

CHROMATOGRAPHIC SEPARATION

. Pipette into the column:

Distilled water	5.0 ml	discard the eluate
Urine	0.5 ml	discard the eluate
Distilled water	10.0 ml	discard the eluate

Place the column over a clean test tube and pipette:

Reagent 1	5.0 ml	collect the ALA ELUATE

COLORIMETRIC REACTION

Accurately mix the collected eluate and pipette into 3 test tubes labeled as it follows:

	Blank reagent	Sample	Standard
ALA ElUATE	---	2.0 ml	---
Reagent 4 standard	---	---	0.02 ml
Reagent 1	2.0 ml	---	1.98 ml
Reagent 2	0.04 ml	0.04 ml	0.04 ml

Shake vigorously and incubate the test tubes in a hot bain-marie for 10 minutes.
Cool under running water, mix well and pipette into 3 new test tubes:

Preincubated solution	1.0 ml	1.0 ml	1.0 ml
Ehrlich's reagent	1.0 ml	1.0 ml	1.0 ml

Mix and incubate at room temperature for 15 minutes. Read the sample (As) and the standard (Astd) absorbencies against the blank reagent, preferably within 5-10 minutes. The developed color reaches its highest intensity within 15 minutes and remains stable for 15 minutes.

CALCULATION

δ-amino levulinic acid (mg/100 ml) = (A sample /A standard) x 2
mg ALA/100 ml x 10 x L of 24-hour urine = mg ALA / 24 hours

REFERENCE VALUES

δ-amino levulinic acid: up to 0.60 mg/100 ml

NOTES

1. Indication of lead intoxication degree:

ALA (mg/100 ml)	Intoxication Degree
up to 0.60	None
0.60 - 1.50	Moderate
1.50 - 3.00	High
3.00 - 6.00	Very High
more than 6.00	Critical

2. The quantity of reagents is enough for 28 tests (20 samples, 4 stardards and 4 blanks)
3. FAR kit (y) to define ALA shows a correlation coefficient of 0.98, in comparison to a direct method

REFERENCE

1. J.R. Davis et S.L. Andelman Arch. Environ Health" 15,53-59 (1967)

IVD CE Issue 02 - MAR 2022

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