AMMONIA

Chromatographic – Colorimetric Determination
of Ammonia
in Plasma and Urine

20 tests **REF KR03-20**

INTENDED USE

Kit for quantitative *in vitro* determination of Ammonia in plasma and urine.

PRINCIPLE

Ammonium ion is adsorbed on a cationic resin balanced with a proper buffer. After washing of interfering substances, it is eluted and quantitatively defined by Berthelot reaction.

DIAGNOSTIC IMPLICATIONS

Increased levels are associated with Reye syndrome, other forms of liver cell damage and impending hepatic coma.

REAGENTS AND MATERIALS

Kit components:	REF KR03-20
REAGENT 1 Sodium hydrate	**1 x 35 ml**
WARNING: store tightly closed.	
***REAGENT 2** Phenol-nitroprussiate	**1 x 1 ml**
***REAGENT 3** Sodium hypochlorite-sodium hydrate	**1 x 2 ml**
REAGENT 4 Standard ammoniac nitrogen 1.5 mg/L	**1 x 4 ml**
COLUMNS Chromatographic	**20**
RESIN Resin for water treatment. To obtain ammonium ion free water.	

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and materials are stable up to the expiration date on the label.

PREPARATION OF WORKING REAGENT

AMMONIUM ION FREE WATER

To obtain ammonium ion free water, add de-ionized or distilled water to the vial containing the resin. Shake for 20 seconds, then leave the resin stand until complete sedimentation. The resin retains any eventual trace of ammonia present in the water.
It can be used several times.

REAGENT 2

Dilute the Reagent 2 to 50 ml with ammonium ion free water and mix accurately before use.
STABILITY: at least 6 months at 2-8°C.

REAGENT 3

Dilute the Reagent 3 to 50 ml with ammonium ion free water and mix accurately before use.
STABILITY: at least 6 months at 2-8°C.

SAMPLE

Plasma

Collect the blood in a centrifuge tube containing heparin as anticoagulant. Centrifuge and separate plasma by placing it in an ice bath before use. The test should be performed immediately or within a maximum 2 hour delay; otherwise freeze the plasma. Do not use hemolyzed blood.

Urine

Collect the 24-hour urine in a container with 2-3 ml of concentrated hydrochloric acid. Mix well and store at 2-8°C. Dilute 1 volume of urine with 99 volumes of de-ionized water before the test.

MANUAL ASSAY PROCEDURE

Wavelength:	640 nm (630-650 nm)
Optical path:	1 cm
Reading:	against blank reagent
Temperature:	37°C
Method:	colorimetric endpoint
Linearity:	750 µg/100 ml
Sensitivity:	7.5 µg/100 ml
Recovery:	95 ± 5 %
C.V. (intra-assay):	1%
C.V. (inter-assay):	2%

PREPARATION OF THE COLUMN

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

Gently pipette into the column:

Sample	0.5 ml	discard the eluate
Ammonium ion free water	10.0 ml	discard the eluate
Reagent 1	1.5 ml	collect the eluate
Ammonium ion free water	1.0 ml	collect the eluate

Reagent 1 and ammonium ion free water eluates are to be collected in the same tube for total 2.5 ml. Mix gently.
Pipette into different clean pipettes labeled as it follows:

	Sample	Standard	Blank Reagent
Eluate	2.0 ml	---	---
Reagent 4	---	0.4 ml	---
Ammonium ion free water	---	1.6 ml	2.0 ml
Reagent 2	1.0 ml	1.0 ml	1.0 ml
Reagent 3	1.0 ml	1.0 ml	1.0 ml

Mix carefully and incubate for exactly 10 minutes at 37°C. Read the sample (As) and the standard (Astd) absorbencies at 640 nm.

CALCULATION

Plasma
Ammoniac nitrogen (µg/100 ml) = (As/Astd) x 150
Urine
Ammoniac nitrogen (mg/L) = (As/Astd) x 150

REFERENCE VALUES (expressed in ammoniac nitrogen)

Venous plasma adult	45 - 80	µg/100 ml
Venous plasma newborn babies	90 - 150	µg/100 ml
Venous plasma immature babies	> 150	µg/100 ml
Urine adults	0.6 - 0.73	g/24 hours

NOTES

1. To obtain more reliable and repeatable results, use very clean and ammonium ion free glassware. Moreover do not touch with fingers any parts which come in contact with the sample and reagents.
2. Perform the test only in an ammonia steam free environment.
3. Sometimes water may contain some chloramines, which cannot be removed with distillation nor with de-ionization.
In this case, reconstitute a "Blank Column" using 1 ml of water instead of the sample. Continue the determination like for the sample and then read against blank. If the obtained absorbance is different from the blank reagent, subtract this value from the samples absorbencies.

REFERENCE

1. S.G. Dienst et B. Morris, "J. Lab. Clin. Med." 64,495-500 (1964).

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