AMMONIA FL

NH F060 CH 6 x 10 ml

INTENDED USE

Reagent for quantitative in vitro determination of ammonia in biological fluids.

SUMMARY OF TEST

Ammonia is formed as a by-product of protein catabolism or through renal acid/base balance. Usually, excess ammonia is converted to urea in liver and excreted. However, when the urea excretion system fails to function properly, ammonia is accumulated to toxic levels. Increase of ammonia concentration in blood is a typical marker for liver diseases such as hepatitis and hepatic cirrhosis, and can have toxic effects on central nervous system. Ammonia determination is indispensable in case of hepatic encephatology.

PRINCIPLE OF THE METHOD

Ammonia reacts with α-ketoglutarate in the presence of Glutamate Dehydrogenase (GlDH) and NADH. Absorbance decrease due to NADH consumption, is proportional to ammonia concentration in the sample and can be measured at 340 nm.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

NH3 R1 **F060:** **6 x 8 ml (liquid) blue cap**

NH3 R2 **F060:** **1 x 12 ml (liquid) red cap**

Test composition: Good's buffer 200 mM, NADH ≥ 0.1 mM, α-ketoglutarate ≥ 10 mM, LDH ≥ 5 KU/l, GlDH ≥ 5 kU/l, stabilizers and preservatives.

Standard: **ammonia solution 500 µg/dl - 10 ml**

Store all components at 2-8°C.
Ammonia is volatile! Immediately after use, close carefully the Standard dropper.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Reagent as starter procedure:
Use separate reagents ready to use.
Stability: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: use preferably within 60 days at 2-8°C.

Sample as starter procedure:
This procedure is less recommended, because the mechanisms which reduce the interferences are less effective.

Mix 1 part of reagent R2 with 4 parts of reagent R1.

Stability of working reagent: preferably within 7 days at 2-8°C, away from light sources.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.
Laboratory air, because of smoking or traffic, can be a source of ammonia contamination, as well as water and glassware residues.
Keep the sample tubes well capped, to avoid ammonia evaporation.

SPECIMEN

Plasma, preferably collected with EDTA. Plasma-heparin can be used (not ammonium heparin).
Hemolyzed samples should not be used, because of the high level of ammonia in erythrocytes.
Plasma specimens should be assayed within 30 minutes. If it is not possible, specimens can be stored 2 hours at 2-8°C, or 24 hours at -20°C.

TEST PROCEDURE (reagent as starter)

Wavelenght:	340 nm	
Lightpath:	1 cm	
Temperature:	37°C	
dispense:	standard	sample
reagent R1	1 ml	1 ml
standard	100 µl	-
sample	-	100 µl

Mix, incubate at 37°C for 5 minutes.

dispense:	standard	sample
reagent R2	250 µl	250 µl

Mix, after 60 seconds read the absorbance A_1 against water, by incubating at 37°C. After 4 minutes, read the absorbance A_2.

TEST PROCEDURE (sample as starter)

Wavelength:	340 nm	
Lightpath:	1 cm	
Temperatur:	37°C	
dispense:	standard	sample
working reagent	1 ml	1 ml
standard	80 µl	-
sample	-	80 µl

Mix, after 60 seconds read the absorbance A_1 against water, by incubating at 37°C. After 4 minutes, read the absorbance A_2.

RESULTS CALCULATION

$$\text{ammonia µg/dl} = \frac{A_2\text{-}A_1 \text{ (sample)}}{A_2\text{-}A_1 \text{ (standard)}} \times \text{Standard value}$$

EXPECTED VALUES

adults 20-100 µg/dl

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose a suitable human based control sera has to be used.
Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
The method is linear up to 2000 µg/dl.
If the value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
The limit of detection is 7 µg/dl.

Interferences
No interference was observed by the presence of:

hemoglobin	≤ 300 mg/dl
bilirubin	≤ 36 mg/dl
lipids	≤ 590 mg/dl
ascorbic acid	≤ 34 mg/dl
pyruvic acid	≤ 12.5 mg/dl
ALT	≤ 1500 U/l

Precision

intra-assay (n=10)	mean (µg/dl)	SD (µg/dl)	CV%
sample 1	102	1.78	1.73
sample 2	377	4.22	1.12

inter-assay (n=20)	mean (µg/dl)	SD (µg/dl)	CV%
sample 1	101	3.76	3.71
sample 2	377	7.26	1.93

Methods comparison
A comparison between Chema and a commercially available product gave the following results:

ammonia competitor = x
ammonia Chema = y

Plasma (n=38)

$y = 1.04x - 6.8$ µg/dl $r^2 = 0.999$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

Tietz Textbook of Clinical Chemistry, Fourth Edition, Burtis-Ashwood-Bruns (2006), 1789-91
Clinica Chimica Acta 2018, 478, 37-43

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

CE