

Ammonia II (GLDH - UV - Test)

REF: 333 001 100 tests
 R1a : 5 x 18 ml
 R1b : 1 x 11 ml
 R2 : 1 x 12 ml

Intended Use

Spectrum Diagnostics ammonia II reagent is intended for the in-vitro quantitative, diagnostic determination of ammonia in human plasma on both automated and manual systems.

Background

Ammonia enters the body in nitrogen-containing foods via the gastrointestinal tract and is excreted largely as urea in urine and as bacterial protein in feces. Ammonia, the end product of nitrogen metabolism is absorbed into the portal venous blood and, after passing through the liver enters the systemic circulation. Normally about half the ammonia is extracted from the body by the skeletal muscle and about 16 % by the liver and brain. Clinically, the extraction of ammonia by individual organs has different implications. The hepatic conversion of ammonia to urea represents the primary mechanism of eliminating ammonia from the body. Conversely, the excessive uptake of ammonia by the brain results in ammonia intoxication, increased intracranial pressure and hepatic encephalopathy. Hyperammonemia in infants may be due to inherited deficiencies of the urea cycle enzymes or acquired through acute (as in Reye's syndrome) or chronic (as in cirrhosis) liver disease.

Method

Kinetic enzymatic method with glutamate dehydrogenase.

Assay Principle

α - ketoglutarate reacts with ammonium ions in presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate and NAD^+



The concentration of The NADP^+ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance at 340 nm.

Reagents

Standard ammonia (ST)
 521 $\mu\text{g/dL}$ 307 $\mu\text{mol/L}$

Reagent R1a Buffer
 Tris buffer (pH 8.0) 50 mmol/L
 GLDH > 6 kU/L
 LDH > 2 kU/L
 EDTA 5.3 mmol/L

Reagent R1b
 Coenzyme (NADH) 0.18 mmol/L

Reagent R2
 α - ketoglutarate 18 mmol/L

For further information, refer to the Ammonia reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in-vitro diagnostic use		Use by/Expiration Date
	Batch Code/Lot number		CAUTION. Consult instructions for use
	Catalogue Number		Manufactured by
	Consult instructions for use		

Reagent preparation

Prepare R1 Mixture as following:

REF: 333 001: add 2 ml from R1b to one bottle of R1a; mix gently
 Or prepare the working solution according to the number of tests required by mixing 9 volumes of reagent 1a (R1a) and 1 volume of reagent 1b (R1b); eg. 900 ml R1a + 100 μl R1b.

Reagent Storage and Stability

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 $^{\circ}\text{C}$.
 R1 Mixture is stable for 1 month at 2- 8 $^{\circ}\text{C}$.

Deterioration

Do not use Ammonia II reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

Specimen Collection and Preservation

EDTA is the only acceptable anticoagulant because it reduces red cell ammonia production. Other anticoagulants produce spontaneously high results. Collect blood from stasis-free vein of fasting patient. Smoking should be avoided prior to sample. Tubes should be filled completely and kept tightly stoppered at all times. Place immediately on ice and centrifuge, preferable at 4 $^{\circ}\text{C}$. Perform analysis within 30 minutes of venipuncture.

Note: avoid contamination of samples by ammonia from smoking or traffic in laboratory or patient's room, glassware, or water. One known source of spontaneous ammonia formation is an increased α -glutamyl-transferase activity leading to decomposition of glutamine.
Stability: 15 minutes. at 15 - 25 $^{\circ}\text{C}$; 2 hours at 4 - 8 $^{\circ}\text{C}$;
 3 weeks at -20 $^{\circ}\text{C}$

System Parameters

Wavelength	340 nm
Optical path	1 cm
Assay type	Fixed Rate
Direction	Decrease
First read time	2 seconds
Delay time	300 seconds
last read time	5 Minutes
Temperature	37 $^{\circ}\text{C}$
Zero adjustment	Reagent blank
Reagent Blank Limits	Low 1.00 AU High 2.0 AU
Sensitivity	9 $\mu\text{g/dL}$ (5.3 $\mu\text{mol/L}$)
Linearity	1700 $\mu\text{g/dL}$ (1000 $\mu\text{mol/L}$)

Procedure

	Standard	Specimen	Reagent blank
R1 mixture	1ml	1ml	1ml
Standard	100 μl	-----	-----
Specimen	-----	100 μl	-----
Dist. H ₂ O	-----	-----	100 μl
Incubate for 3 minutes at 37 $^{\circ}\text{C}$, Then add			
R2	100 μl	100 μl	100 μl

Mix, and immediately read the absorbance A1 of the standard or specimen against reagent blank. Exactly 5 minutes later, read absorbance A2 of standard or specimen.

Calculation

$$A1 - A2 = \Delta A_{\text{specimen}} \text{ or } \Delta A_{\text{standard}}$$

Concentration of ammonia in Plasma:

$$\text{Ammonia } (\mu\text{g/dl}) = \frac{\Delta A_{\text{specimen}}}{\Delta A_{\text{standard}}} \times 521$$

Quality Control

Normal & abnormal commercial control of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean ($\mu\text{g/dL}$)	1.8	3.5
SD	0.04	0.06
CV%	2.3	1.3

run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean ($\mu\text{g/dL}$)	1.8	3.5
SD	0.07	0.14
CV%	3.4	4.1

Methods Comparison

A comparison between Spectrum Diagnostics Ammonia reagent and a commercial reagent of the same methodology was performed on 20 human plasma. A correlation of 0.976 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 9.0 $\mu\text{g/dL}$.

Linearity

The reaction is linear up to ammonia concentration of 1700 $\mu\text{g/dL}$.

Interfering Substances plasma

Haemolysis

Avoid haemolyzed specimen since RBCs contain three times the ammonia content of plasma.

Icterus

Bilirubin levels higher than 30 mg/dL increase the ammonia concentration significantly.

α -globulin

Elevated α -globulin levels (more than 3 g/dL) may increase the apparent ammonia concentration values.

Lipemia

Lipemic samples should be centrifuged and the analysis performed on the supernatant.

Anticoagulants

Fluoride, citrate, and heparin must not be used.

Drugs

Sodium cefoxitin causes artificially high ammonia values at the tested drug level.

Expected Values

EDTA plasma

Adults

Females 19- 87 $\mu\text{g/dL}$ (11-51 $\mu\text{mol/L}$)

Males 27-102 $\mu\text{g/dL}$ (16-60 $\mu\text{mol/L}$)

Children < 81.5 $\mu\text{g/dL}$ (< 48 $\mu\text{mol/L}$)

Neonates (1- 6 days) < 228 $\mu\text{g/dL}$ (< 134 $\mu\text{mol/L}$)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

9 – 1700 $\mu\text{g/dL}$.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References

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ORDERING INFORMATION

CATALOG NO.	QUANTITY
333 001	100 Tests



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