Ammonia – Liquizyme
(9 + 1)

REF: 218 001 (9 x 10 ml) 90 test
REF: 218 002 (4 x 10 ml) 40 test

Intended Use
Spectrum Diagnostics liquizyme ammonia 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
\[ \text{L-glutamate} + \text{NADPH} \rightarrow \text{NH}_4^+ + \text{H}_2\text{O} + \text{NADP}^+ \]

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 µg/dL

Reagent 1 (R1 Buffer)
Bicine buffer (pH 8.5)
100 mmol/L

Reagent 2 (R2 Enzyme)
GLDH (microbial)
500 KU/L

Reagent 3 (R3 Coenzyme)
NADPH
0.2 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.

Both reagents (R1) and (R3) contain sodium azide which may react with copper or lead plumbing.

Reagent preparation
Prepare working solution as following:
REF: 218 001: add 1 ml from R3 to one bottle of R1; mix gently
REF: 218 002: add 1 ml from R3 to one bottle of R1; mix gently
Or prepare the working solution according to the number of tests required by mixing 9 volumes of reagent 1 (R1) and 1 volume of reagent 3 (R3) eg. 800 ml R1 +100 µl R3.

Reagent Storage and Stability
All reagents are stable until expiration date stated on label when stored refrigerated at 2 – 8 ºC. Working solution is stable for 2 weeks at 2– 8 ºC.

Deterioration
Do not use liquizyme Ammonia 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 stopped at all times. Place immediately on ice and centrifuge, preferably at 4ºC. 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 \(\text{N\^2-glutamyl-transferase} \) activity leading to decomposition of glutamine.

Stability: 15 minutes. at 15 – 25 ºC; 2 hours at 4 – 8 ºC;
3 weeks at -20 ºC

System Parameters
Wavelength 340 nm
Optical path 1 cm
Assay type Fixed Rate
Direction Decrease
Sample : Reagent Ratio e.g.: Reagent volume 1 : 10
Sample volume 100 µl
First read time 30 seconds
Delay time 150 seconds
last read time 180 seconds
Temperature 37 ºC
Zero adjustment Against Air
Reagent Blank Limits Low 1.00 AU
High 2.0 AU
Sensitivity 0 µg/dL (5.3 µmol/L)
Linearity 1700 µg/dL (10000 µmol/L)

Procedure

<table>
<thead>
<tr>
<th>Standard</th>
<th>Specimen</th>
<th>Specimen blank</th>
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</thead>
<tbody>
<tr>
<td>Working solution 1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>R2 (Enzyme)</td>
<td>10 µl</td>
<td>10 µl</td>
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</tbody>
</table>

Incubate for 3 minutes at 37 ºC.

<table>
<thead>
<tr>
<th>Standard</th>
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<th>Specimen blank</th>
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<tbody>
<tr>
<td>100 µl</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
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</tbody>
</table>
Mix, and after 30 seconds, read the absorbance A1 of the standard or specimen or specimen blank. Exactly 2.5 minutes later, read absorbance A2 of standard or specimen or specimen blank.

*Note: It is recommended to add the required amount of R2(Enzyme) to the Working Soln. according to the number of tests to be done (e.g. for 5 tests add 50 µl R2 (Enzyme) to 5 ml Working solution). Incubate at 37 °C for 3 minutes. then add 100 µl of the serum or standard to each 1 ml and complete the procedure as above.

**Calculation**

A1 – A2 = Aspecimen or Astandard or Aspecimen blank.

Aspecimen Final = Aspecimen – Aspecimen blank.

Concentration of ammonia in serum:

\[ \text{Ammonia (µg/dL)} = \frac{\text{Aspecimen Final}}{\text{Astandard}} \times 521 \]

**Quality Control**

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

**Performance Characteristics**

**Precision**

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µg/dL)</td>
<td>1.8</td>
<td>3.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>CV%</td>
<td>2.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

run to run (Reproducibility)

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<td>0.14</td>
</tr>
<tr>
<td>CV%</td>
<td>3.4</td>
<td>4.1</td>
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**Methods Comparison**

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

**Sensitivity**

When run as recommended, the minimum detection limit of this assay is 9.0 µg/dL.

**Linearity**

The reaction is linear up to ammonia concentration of 1700 µg/dL.

**Interfering Substances**

**plasma**

Haemolysed Avoid haemolysed 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.

**Expected Values**

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<table>
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<tr>
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<tbody>
<tr>
<td>EDTA plasma</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>19-87 µg/dL (11-51 µmol/L)</td>
</tr>
<tr>
<td>Males</td>
<td>27-102 µg/dL (16-60 µmol/L)</td>
</tr>
<tr>
<td>Children</td>
<td>&lt; 81.5 µg/dL (&lt; 48 µmol/L)</td>
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<tr>
<td>Neonates (1-6 days)</td>
<td>&lt; 228 µg/dL (&lt; 134 µmol/L)</td>
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</tbody>
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**Analytical Range**

9 – 1700 µg/dL.

**Waste Disposal**

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

**References**


**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.

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
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