Ammonia (plasma, blood)

1 Name and description of analyte

1.1 Name of analyte
Ammonia

1.2 Alternative names
None

1.3 NLMC code

1.4 Description of analyte
Ammonia has the formula NH_3. At physiological pH, 97% is present in the blood in its ionised form, ammonium (NH_4^+).

1.5 Function of analyte
Ammonia is produced from the deamination of amino acids in the liver, muscle and the kidneys, and by the action of bacteria in the colon and small intestine. Ammonia is neurotoxic and is detoxified in the body by conversion to urea via the urea cycle in the liver, thus maintaining a low concentration of ammonia in the circulation. It can also be incorporated into glutamate to form glutamine, an important metabolic fuel for some tissues, and a source of amino groups in purines and pyrimidines.

2 Sample requirements and precautions

2.1 Medium in which measured
Ammonia is routinely measured in plasma from a venous (or arterial) blood sample. It can also be measured in whole blood, erythrocytes, saliva, sweat and urine. Only measurements in blood and plasma are considered in this article

2.2 Precautions re sampling, handling etc.
A free-flowing venous (or arterial) blood sample should be collected into a specimen tube (preferably pre-chilled) containing either lithium heparin or EDTA as an anticoagulant and which has been determined to be free of ammonia contamination. Ideally, the patient should be non-stressed, as difficult venepuncture can cause a spurious increase in [ammonia].

The sample should be transported on ice to the laboratory, separated within 15 minutes of collection and analysed immediately. These precautions are necessary as the [ammonia] of standing blood increases spontaneously, due to generation and release of ammonia from red blood cells and, to a lesser extent, the deamination of amino acids by enzymes in the circulation, such as γ-glutamyltransferase. Once separated, plasma [ammonia] is stable for 4 h at 4 °C and 24 h at -20 °C. Unless in an emergency, the sample should be collected in a fasted state (or at least 4–6 hours after a meal), not following physical exercise, and smoking should be avoided for at least 9 h before the sample is collected. Clotted samples, samples collected via indwelling catheters and capillary samples

should not be used, as any significant haemolysis of the sample will cause a spuriously elevated [ammonia].
Note that due to the high risk of an elevated [ammonia] caused by mishandling of the sample or delay in its reaching the laboratory, it is advisable that the analysis should be repeated on a subsequent sample to confirm the result. This is especially true if the initial measurement was performed on a point-of-care device; such results must always be confirmed by measurement in the laboratory.

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Ammonia measurements are used to diagnose hyperammonaemia, which often presents with non-specific (mainly neurological) symptoms. Hyperammonaemia can be caused by a wide variety of conditions, which are discussed further in section 7.1.

3.2 Limitations

The measured [ammonia] can only be used as a guide to the cause of hyperammonaemia; further investigation is required, as detailed in section 7.1.1. Point-of-care analysis using free-flowing blood can only be used to exclude hyperammonaemia. These analysers often have a low upper limit of measurement (<300 μmol/L) and therefore may underestimate a high blood [ammonia].

4 Analytical considerations

4.1 Analytical methods

Ammonia can be measured by both indirect and direct methods.
1. Use of a micro-diffusion apparatus is an indirect method, which is often used in point-of-care and dry-slide analysers. Free ammonia is liberated from the sample by alkalisation, and is allowed to pass through a semi-permeable membrane. The ammonia is measured by the change of colour of an ammonium indicator, for example the Vitros® (Ortho Diagnostics Ltd) ammonia measurement utilises bromophenol blue. Bromocresol green can also be used. The change in absorbance is measured at 605 nm by reflectance spectroscopy.
2. The enzymatic method is a direct method and is the most commonly used method in the UK. It utilises the reaction of glutamate dehydrogenase, in which ammonium in the sample reacts with α-oxoglutarate and reduced nicotinic adenine dinucleotide (phosphate) (NAD(P)H) to form glutamate and $NAD(P)^+$ and water. Absorbance spectroscopy can then be used to measure the decrease in [NADH] or [NADPH].

$$NH_3 + \alpha\text{-oxoglutarate} + NAD(P)H \rightarrow \text{glutamate} + NAD(P) + H^+$$

3. The ammonium electrode is a direct method that uses an NH_4^+-selective membrane, which is typically based on a mixture of the antibiotics nonactin and monoactin.

4.2 Reference method

Not established.

4.3 Reference material
Not determined.

4.4 Interfering substances
1. Haemolysis causes a positive interference.
2. In the enzymatic assay, NADH is used as a coenzyme; other NADH consuming systems such as pyruvate are present in blood, for example pyruvate, which may give a positive interference. To prevent interference from pyruvate, lactate dehydrogenase can be added to the assay mixture to reduce the pyruvate present. There are fewer NADPH consuming systems present in blood, so fewer potential sources of interference.
3. Alanine aminotransferase (ALT) has been reported to interfere positively with ammonia measurements using the Infinity™ method, and may lead to underestimation of [ammonia] in patients with acute liver disease.

4.5 Sources of error
Incorrect sample handling, as detailed in section 2.2, may cause a spuriously elevated [ammonia].

5 Reference intervals and variance

5.1.1 Reference interval (adults): 11–32 μmol/L
5.1.2 Reference intervals (others): premature neonates, <150 μmol/L; term neonates, <100 μmol/L; infants <40 μmol/L.
5.1.3 Extent of variation
The major sources of variation in ammonia measurement are likely to be due to pre-analytical factors. The sample handling and collection precautions needed for ammonia measurement make setting up studies into biological variation difficult.
5.1.3.1 Interindividual CV: not determined
5.1.3.2 Intraindividual CV: not determined
5.1.3.3 Index of individuality: not determined
5.1.3.4 CV of method:
Data from the WEQAS Ammonia Scheme suggests that the glutamate dehydrogenase method has a CV of 7–15% for an [ammonia] of 42–490 μmol/L (December 2011 report).
5.1.3.5 Critical difference: not determined
5.1.4 Sources of variation:
[Ammonia] is increased by physical exercise and following a meal.

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
1. Ammonia should be measured in any neonate with neurological symptoms of unknown cause.
2. Mild disturbances of ammonia metabolism may not present until later life or during intercurrent illness, so it should also be measured in patients with symptoms suggestive of hyperammonaemia (vomiting, faddy eating, behavioural changes, slow developmental progress and neurological deficits).

3. Measurement of plasma ammonia for diagnosis and monitoring of hepatic encephalopathy is controversial and not recommended.

6.2. Confounding factor
Poor sample handling (see 2.2)

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

- Inherited defects of the urea cycle
- Other metabolic disorders e.g. organic acidurias and disorders of fatty acid oxidation
- Liver failure or impairment
- Urinary tract infection
- Gastrointestinal bacterial overgrowth
- Drugs e.g. valporate, chemotherapy
- Total parenteral nutrition
- Intercurrent illness in babies e.g. sepsis, asphyxia
- Reye's syndrome
- Transient hyperammonaemia of the newborn (normally seen within 1st 24h of life)
- Artefactual increases (see section 2.2 above).

7.1.2 Investigation

1. Repeat ammonia measurement ensuring appropriate sample handling and timely transit to the laboratory
2. The concentration gives a guide to the underlying cause:

[Ammonia] (μmol/L)	Examples of underlying causes
>1500	Transient hyperammonaemia of the newborn
>600	Urea cycle disorders Propionic acidaemia
200-600	Organic acidurias Fatty acid oxidation disorders
<200	Acquired e.g. illness, sepsis, liver dysfunction

3. First line investigations:
   Blood gases, urea and electrolytes, liver function tests, clotting studies, glucose, lactate and calcium. Urine ketones.
4. The results of the first line investigations may support an acquired or a metabolic cause, which then require further specialist investigations. Confirmation of a urea cycle defect is by analysis of amino acids in plasma and urine, organic acids and orotic acid in urine.

7.2 Low values
Not clinically significant

7.3 Notes
Symptomatic hyperammonaemia is a medical emergency. Patients are likely to require prompt investigation and treatment, often with haemodialysis. Delay in treatment can cause irreversible brain damage.

8. Performance

8.1 Sensitivity, specificity for individual conditions
Hyperammonaemia is a common feature of urea cycle disorders but values for sensitivity and specificity are unknown.

9 Systematic reviews and guidelines

9.1 Systematic reviews
None identified

9.2 Guidelines
1. UK National Metabolic Biochemistry Network. Guidelines for the Investigation of Hyperammonaemia. http://www.metbio.net/docs/MetBio-Guideline-AMUP100834-21-07-2010.pdf
2. National Academy of Clinical Biochemistry. Laboratory Guidelines for Screening, Diagnosis and Monitoring of Hepatic Injury. http://www.aacc.org/members/nacb/Archive/LMPG/HepaticInjury/Pages/default.aspx# *This states that measurement of plasma ammonia for diagnosis and monitoring of hepatic encephalopathy is controversial and not routinely recommended.*

9.3 Recommendations
1. Barsotti RJ,. Measurement of ammonia in blood. J Pediatr 2001;138:S11-S20. *This article summarises methods used for the measurement of ammonia and their advantages and disadvantages.*
2. Häberle J. Clinical Practice: the management of hyperammonaemia. Eur J Pediatr 2011;170: 21-34. *This article summarises diagnosis and treatment of hyperammonaemia.*

10. Links

10.1 Related analytes:
None

10.2 Related tests:
Urea

Author: Louise Hawke

Date Completed: 2.2012
Date Revised: