

Infinity™

Ammonia Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 1000 µmol/L (1700 µg/dL)
Specimen Type	:	Plasma
Method	:	Kinetic
Reagent Preparation	:	Supplied ready to use.



SYMBOLS IN PRODUCT LABELLING

EC	Authorised Representative		Temperature Limitation
REP	For in vitro diagnostic use		Use by/Expiration Date
IVD	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
LOT	Catalogue number		Manufactured by
REF	Consult instructions for use		
	Prescription Use Only		
Rx ONLY			

INTENDED USE

This reagent is intended for the in vitro quantitative determination of Ammonia (NH₃) in human plasma on automated systems.

CLINICAL SIGNIFICANCE^{1,2,3}

Ammonia, derived from the catabolism of amino acids and from the action of intestinal bacteria on dietary protein, is converted to urea in the liver hepatocytes and so rendered non toxic. Under normal circumstances the concentration of ammonia in the circulation remains low, typically less than 50 µmol/L (85 µg/dL). Studies have shown that excess ammonia can have a toxic effect on the central nervous system and clinical manifestations are typically neurological disturbances.

Elevated levels of ammonia may be either due to:

- (i) Inborn errors of metabolism; or
- (ii) Secondary to other conditions.

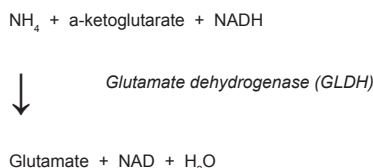
Inborn errors of metabolism are the major cause of elevated ammonia in infants and usually the result of urea cycle enzyme deficiencies. Inherited disorders affecting the metabolism of the dibasic amino acids (lysine and ornithine) and those involving the metabolism of organic acids may also produce elevated levels of circulating ammonia. Elevated ammonia may also be observed in severe liver failure as may occur in Reye's Syndrome, viral hepatitis or cirrhosis.

METHODOLOGY¹

A number of methods have been developed for the estimation of plasma ammonia and these can be broadly classified into either indirect or direct methods.

In the indirect procedures, ammonia is first of all isolated, for example by the addition of alkali or the use of a cation exchange resin, after which it is measured colourimetrically by nesslerization or Berthelot reaction. These procedures are not easily automated or require dedicated equipment.

Direct procedures, such as enzymatic methods, are more widely used in routine laboratories as they do not require the separation of ammonia from the specimen prior to the analytical step. Direct procedures are therefore more easily automated. The Infinity ammonia reagent is a direct enzymatic procedure based on the following reaction sequence:-



The reagent contains LDH in excess, to rapidly reduce endogenous pyruvate so that it does not interfere with the assay system.

The Infinity Ammonia reagent also incorporates a patented stabilization process which renders the reagent stable in the liquid phase.

REAGENT COMPOSITION

Active Ingredient

	Concentration
a-Ketoglutarate	7.5 mmol/L
NADH	>0.2 mmol/L
GLDH (Micro-organism)	>4000 U/L
LDH (Micro-organism)	>30,000 U/L
Tris Buffer	100 mmol/L

pH 8.7 ± 0.1 at 20°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information, consult the Infinity Ammonia Liquid Stable Reagent Material Safety Data Sheet.

CAUTION: This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION

The reagent is supplied ready to use.

STABILITY AND STORAGE

Prior to use:

When stored at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:

When stored capped at 2-8°C, the reagent is stable until expiry. It is recommended that when the reagent is not in use for prolonged periods of time (eg: overnight) the reagent be capped and stored at 2-8°C.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent Absorbance <1.3 AU at 340 nm (1 cm); and /or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING¹

Plasma: Plasma, collected with EDTA or heparin (not ammonium heparin) into an evacuated collection tube is recommended. Ideally, the collection tube should be completely filled with blood and immediately placed on ice. Centrifuge (cold) the sample as soon as possible and separate plasma and store at 2-4°C until analysis.

Storage: Ammonia samples are stable for 3 hours at 2-4°C or 24 hours at -20°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm (334 nm, 365 nm).
- Analyser specific consumables, eg: sample cups.
- Ammonia Standard(s).
- Normal and Abnormal assayed control material.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	340 nm (334, 365 nm)
Secondary Wavelength	600 nm
Assay Type	Rate/Kinetic
Direction	Decrease
Sample : Reagent Ratio	1 : 11
eg: Sample Vol	30 µL
Reagent Vol	330 µL
First Read Time	30 seconds
Delay Time	90 seconds
Last Read Time	120 seconds
Reagent Blank	Low 1.3 AU
(1cm lightpath, 340 nm)	High 2.5 AU
Linearity	1000 µmol/L
(refer to Linearity section)	(1700 µg/dL)
Sensitivity	0.20 ΔmA per µmol/L
(1cm lightpath, 340 nm)	(0.11 ΔmA per µg/dL)

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Ammonia} = \frac{\Delta\text{Abs/min of Unknown}}{\Delta\text{Abs/min of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of Calibrator	=	0.04 ΔAbs/min
Absorbance of unknown	=	0.10 ΔAbs/min
Value of Calibrator	=	200 µmol/L (340 µg/dL)

$$\text{Ammonia} = \frac{0.10}{0.04} \times 200 = 500 \mu\text{mol/L}$$

$$\text{Ammonia} = \frac{0.10}{0.04} \times 340 = 850 \mu\text{g/dL}$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with Ammonia concentrations greater than 1000 µmol/L (1700 µg/dL) should be diluted with ammonia free water and reassayed. Multiply results by the dilution factor.
3. Unit conversion: µmol/L x 1.7 = µg/dL

CALIBRATION

Calibration is required. An aqueous standard which is traceable to a primary reference standard is recommended. For calibration frequency on automated instruments refer to the instrument manufacturers specifications.

However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control and repeat the test.
- If results are still out of control, recalibrate with fresh standard, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Haemolysed samples should not be used as erythrocytes contain levels of ammonia approximately 3 times that of plasma.¹
2. No interference from pyruvate was observed up to a level of 0.75 mmol/L (0.01 mg/dL).
3. No interference from ALT was observed up to a level of 4000 U/L.
4. Reliable estimations of ammonia can only be achieved if steps are taken to avoid contamination from ammonia. Sources of contamination include, but are not restricted to, cigarette smoking (patient and collection staff), laboratory atmosphere and laboratory glassware.
5. Young DS⁴ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES⁵

6 - 47 µmol/L (10 - 80 µg/dL)

The quoted values were derived from a normal population and should serve as a guide only. It is recommended that each Laboratory verify this range or derives a reference interval for the population that it serves.⁶

PERFORMANCE DATA

The following data was obtained using the Infinity Ammonia Liquid Stable Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.⁷

Within Run:	LEVEL I	LEVEL II
Number of data points	76	76
Mean (µmol/L / µg/dL)	61.1 / 103.9	115.5 / 196.4
SD (µmol/L / µg/dL)	3.6 / 6.1	8.5 / 14.5
CV (%)	6.0	7.4

Total:	LEVEL I	LEVEL II
Number of data points	76	76
Mean (µmol/L / µg/dL)	61.1 / 103.9	115.5 / 196.4
SD (µmol/L / µg/dL)	8.7 / 14.8	9.8 / 16.7
CV (%)	14.3	8.5

ACCURACY

Comparison studies were carried out using another similar commercially available ammonia reagent. Plasma samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	42
Range of sample results	8 -799 µmol/L (13 -1359 µg/dL)
Mean of reference method results	312 µmol/L (530µg/dL)
Mean of Infinity Ammonia results	316 µmol/L (536 µg/dL)
Slope	1.002
Intercept	1.55 µmol/L (2.6 µg/dL)
Correlation coefficient	0.9974

LINEARITY

When run as recommended the assay is linear between 0 and 1000 µmol/L of Ammonia (0 -1700 µg/dL).

Linearity on various automated instruments may vary from this value. The user should consult the specific Infinity instrument application.

SENSITIVITY

When run as recommended the sensitivity of this assay is 0.2 ΔmA per µmol/L or 0.11 ΔmA per µg/dL (1cm light path, 340 nm).

REFERENCES

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2. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994; 32:1485-88.
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4. Young DS. Effects of Drugs on Clinical Laboratory Tests Third Edition 1990; 3: 30-2.
5. Pagana K.D., Pagana T. J., Pagana, T.N., eds., Mosby's Diagnostic and Laboratory Test reference, 13th ed., Elsevier, 2017, p. 45.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
7. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T.



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JL840765-en (R2)

REF

Reorder Information

Catalogue No.

TR60101

Configuration

2 x 28 ml