








## REFERENCES

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Definitions for Symbols	
 Batch code	 Use by YYYY-MM-DD or YYYY-MM
 Manufacturer	 Catalog number
 Consult instructions for use	 Temperature limitation
 <i>In vitro</i> diagnostic medical device	

## TRADEMARKS

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**SEKISUI**  
DIAGNOSTICS

## Sekisui Diagnostics P.E.I. Inc.

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## AMMONIA ASSAY

CATALOGUE NUMBER: 200-02

SIZE: 12 x 20 mL

### INTENDED USE

For the IN VITRO quantitative measurement of ammonia in plasma.

### TEST SUMMARY

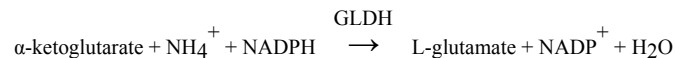
Circulating ammonia in normal individuals is relatively low, despite the fact that ammonia is continuously produced from dietary and amino acid metabolism. Blood ammonia measurements have been used in the diagnosis of coma associated with hepatic dysfunction caused by cirrhosis and neoplasms. The measurement of ammonia is very useful in the diagnosis and prognosis of Reye's Syndrome.

The assay of blood ammonia has always been a tedious and time consuming process. Ammonia assays have generally been based on two approaches: the diffusion of ammonia from an alkaline medium with trapping in acid<sup>(1)</sup> or separation of ammonia from a sample using ion exchange resin.<sup>(2)</sup>

The Sekisui assay is an enzymatic method<sup>(3)</sup> which requires no sample preparation and uses no corrosive reagents.

### TEST PRINCIPLE

Ammonia reacts with  $\alpha$ -ketoglutarate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP. The reaction is catalyzed by glutamate dehydrogenase. The decrease in absorbance at 340 nm due to the oxidation of NADPH is proportional to the ammonia concentration.<sup>(3,4)</sup>



The use of NADPH as the cofactor in place of NADH minimizes the interference by pyruvate and lactate dehydrogenase thus reducing the incubation time of the reaction.<sup>(5)</sup>

### REAGENTS

Ammonia Reagent: An aqueous solution (after reconstitution) containing a buffer, 2.2. mM ADP, 3.5 mM  $\alpha$ -ketoglutarate and 0.2 mM NADPH.

Ammonia GLDH Reagent: An aqueous solution containing a buffer, 1200 U/mL GLDH (mammalian), and a preservative.

Ammonia Calibrator: 1 x 15 mL of an aqueous solution of ammonium sulfate, (ammonia) 2.0  $\mu\text{g/mL}$  (117  $\mu\text{mol/L}$ ).

Ammonia Calibrator: 1 x 15 mL of an aqueous solution of ammonium sulfate, (ammonia) 5.0  $\mu\text{g/mL}$  (294  $\mu\text{mol/L}$ ).

### WARNINGS & PRECAUTIONS FOR USE

S24/25: Avoid contact with skin and eyes.  
Avoid ingestion.  
See Material Safety Data Sheet for additional information.

### REAGENT PREPARATION, STORAGE, AND STABILITY

Add 20 mL of deionized water to the required number of Ammonia reagent vials. GLDH reagent preparation is dependent upon instrument specific parameters.

The reagents included are stable at 2-8°C until the expiry date stated on the labels.

The reconstituted reagent is stable at 2-8°C for 2 weeks and at 18-26°C for 24 hours.

Stability claims are based on real time studies.

### REAGENT DETERIORATION

The reagent solutions should be clear. Turbidity would indicate deterioration.

### DISPOSAL

Reagents must be disposed of in accordance with all Federal, Provincial, State and local regulations.

### **SPECIMEN**

Fresh, clear, unhemolysed plasma. The accuracy of ammonia measurement is extremely dependent on sample collection. Serum is not an acceptable specimen.

Collect blood from a stasis-free vein into an EDTA evacuated tube. Release the residual vacuum immediately, place the sample on ice, and deliver to the lab as quickly as possible. Separate the plasma from the sample without delay. Do not use hemolyzed samples. The ammonia analysis should be performed within 30 minutes.

### SAMPLE STORAGE

Samples may be stored tightly sealed for up to 2 hours, provided the sample is kept on ice or refrigerated.

### **ANALYTICAL SPECIFICITY**

Cross contamination studies have not been performed on automated instruments. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Heavy metals will interfere in the reaction by inhibiting GLDH. Care must be taken to close the calibrator cover tightly to avoid ammonia contamination from the environment.

Interferences from icterus, lipemia and hemolysis were evaluated for this ammonia method on a Beckman

Concentration of Analyte		Substance Tested	Concentration of Interferent Where Interference is Insignificant	
Conventional Units	SI Units		Conventional Units	SI Units
1.99 µg/mL	117 µmol/L	Hemoglobin	200 mg/dL	31 µmol/L
1.86 µg/mL	109 µmol/L	Bilirubin	8 mg/dL	137 µmol/L

CX<sup>®</sup> 5 analyzer using a significance criterion of >10% variance from control.

Lipemia produces significant interference with this method; therefore, lipemic samples should not be used.

The information presented above is based on results from Sekisui Diagnostics studies and is current at the date of publication.

A summary of the influence of drugs and other substances on clinical laboratory tests may be found by consulting Young, D.S.<sup>(6)</sup>

### **ANALYTICAL PROCEDURE**

### MATERIALS PROVIDED

Sekisui Diagnostics' Ammonia reagents and calibration materials.

### MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Automated analyzer capable of accurately measuring absorbance at appropriate wavelength as per instrument application.
2. Deionized water for reconstitution
3. Quality Control materials

### TEST CONDITION

For data presented in this insert, studies using this reagent were performed on an automated analyzer using an endpoint test mode, with a sample to reagent ratio of 1:12 and a primary wavelength reading of 340 nm and a secondary wavelength reading of 380 nm. For assistance with applications on automated analyzers within Canada and the U.S., please contact Sekisui Diagnostics Technical Services at (800)565-0265. Outside Canada and the U.S., please contact your local distributor.

### CALIBRATION

Ammonia calibrators are included and should be used to calibrate the procedure. The frequency of calibration, if necessary, using an automated system is dependent on the system and the parameters used.

### QUALITY CONTROL

A normal and abnormal concentration control should be analyzed as required in accordance with local, state and federal guidelines. The results should fall within the acceptable range as established by the laboratory.

### CALCULATIONS

The analyzer automatically calculates the ammonia concentration of each sample.

### TEST LIMITATIONS

A sample with an ammonia concentration exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

### **REFERENCE INTERVALS<sup>(7)</sup>**

0.17-0.80 µg/mL (10-47 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish its own expected range.

### **PERFORMANCE CHARACTERISTICS**

Data presented was collected in Sekisui Diagnostics laboratories using automated procedures.

### RESULTS

Ammonia concentration is reported as µg/mL (µmol/L).

### REPORTABLE RANGE

The linearity of the procedure described is 20 µg/mL (1175 µmol/L).

### PRECISION STUDIES

Total precision data was collected on two aqueous standards assayed twelve times within one day.

Concentration		Total SD		Total CV %	Concentration		Within Run SD		Within Run CV %
µg/mL	µmol/L	µg/mL	µmol/L		µg/mL	µmol/L	µg/mL	µmol/L	
1.87	110	0.06	3.5	3.2	2.03	119	0.04	2.3	1.9
9.78	574	0.11	6.5	1.1	9.73	571	0.05	2.9	0.5

Within run precision data was collected on two aqueous standards each run 20 times in a single assay.

### ACCURACY

The performance of this method (y) was compared to the performance of a similar method (x). Forty-nine patient samples ranging from 0.0 – 15.5 µg/mL (0.7-910 µmol/L) gave a correlation coefficient of 0.999. Linear regression analysis gave the following equation:

$$\text{This method} = 1.12 (\text{reference method}) + 0.7 \mu\text{g/mL} (4.1 \mu\text{mol/L}).$$