510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k183517

B. Purpose for Submission:

New device

C. Measurand:

Ammonia

D. Type of Test:

Quantitative, enzymatic

E. Applicant:

Roche Diagnostics Operations (RDO)

F. Proprietary and Established Names:

Ammonia II

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1065 Ammonia test system

2. Classification:

   Class I, reserved

3. Product code:

   JIF

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Refer to Indications for use below.

2. Indication(s) for use:

   The Ammonia II assay is an enzymatic in vitro test for the quantitative determination of ammonia in human plasma on Roche/Hitachi cobas c systems.

   Ammonia measurements are used in the diagnosis and treatment of severe liver disorders, such as cirrhosis, hepatitis, and Reye's syndrome.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Roche cobas c 501 Analyzer

I. Device Description:

The reagent consists of 2 components R1 and R3. R1 contains N,N-bias (2-hydroxyethy)-glycine buffer: 300 mmol/L, pH 8.3; GLDH(microbial): ≥16.7 µkat/L, a detergent and a preservative. R3 contains GLDH(microbial) ≥5.0 µkat/L, 2-oxoglutarate 78 mmol/L, NADPH ≥ 1.3 mmol in a non-reactive buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   SYNCHRON Systems Ammonia Reagent

2. Predicate 510(k) number(s):

   k003196

3. Comparison with predicate:

Similarities		
Item	Candidate Device Ammonia II (k183517)	Predicate device SYNCHRON Systems Ammonia Reagent (k003196)
Intended use	Quantitative measurement of ammonia in human plasma	Same
Test principle	Biochromatic Rate	Same
Format	Prepackaged for use on an automated system	Same

Differences		
Item	Candidate Device Ammonia II (k183517)	Predicate device SYNCHRON Systems Ammonia Reagent (k003196)
Measuring range	10-1000 µmol/L (17-1703 µg/dL)	9-1000 µmol/L (16 – 1700 µg/dL)
Sample type	K2- and K3-EDTA plasma	Sodium heparin and EDTA plasma

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods, 3rd Edition.

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach, 2nd Edition.

CLSI EP07-A2: Interference Testing in Clinical Chemistry, 2nd Edition.

CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation, 2nd Edition.

L. Test Principle:

The Ammonia II assay is an enzymatic method, with glutamate dehydrogenase. Glutamate dehydrogenase (GLDH) catalyzes the reductive amination of 2-oxoglutarate with NH4+ and NADPH to form glutamate and NADP+. The concentration of the NADP+ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance. Ammonia concentrations can be reported in conventional (µg/dL) or International System (µmol/L) units, the conversion factor is: µmol/L × 1.703 = µg/dL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision measurements were conducted to evaluate repeatability (within-run precision) and the intermediate precision (within-laboratory precision) according to the CLSI EP05-A3 guideline. The testing was performed over 21 days, with two separate runs per day and two replicate measurements per run resulting in a total n of 84. Two levels of control and 5 human plasma samples were evaluated. The results are summarized in the tables below:

Repeatability Summary:

samples	Mean (µmol/L)	SD (µmol/L)	CV (%)
AEC- Control N	66.6	1.40	2.1
AEC-Control A	243	3.45	1.4
Human Plasma 1	26.0	1.26	4.8
Human Plasma 2	57.7	1.63	2.8
Human Plasma 3	110	1.62	1.5
Human Plasma 4	492	4.12	0.8
Human Plasma 5	868	9.54	1.1

Intermediate Precision:

samples	Mean (µmol/L)	SD (µmol/L)	CV (%)
AEC-Control N	67.9	1.61	2.4
AEC-Control P	243	4.26	1.8
Human Plasma 1	26.0	1.29	4.9
Human Plasma 2	57.7	1.72	3.0
Human Plasma 3	110	1.92	1.7
Human Plasma 4	480	6.30	1.3
Human Plasma 5	853	12.4	1.5

b. Linearity/assay reportable range:

Linearity was evaluated in accordance with the CLSI EP-06A guideline. 18 levels were prepared using a human plasma samples pool with ammonia concentration above the upper measuring range. The mean of 3 replicates was used to evaluate linearity at each level using 3 reagent lots. The range of samples tested was 0-1089 µmol/L.

Linear regression of one representative lot produced the following result:

$y = 1.003x - 2.19 \quad r^2 = 0.9999$

The results of the linearity study support the claimed measuring range of 10-1000 µmol/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

This method has been standardized against an internal gravimetrically prepared primary standard.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the Ammonia II assay were evaluated in accordance with the CLSI EP17-A2 guideline.

LoB study was performed with one analyte-free sample tested over 3 days using three reagent lots in 10-fold determination for a total 60 measurements per lot, on one cobas c 501 analyzer. Data analysis was based on determination of the 95th percentile of the 60 measured values. The LoB was determined to be 1.80 µmol/L.

The LoD study was performed with five low analyte samples which were tested over 3 days using 3 reagents lots in two-fold determination in 6 runs on one cobas c 501 analyzer for a total of 60 measurements per lot. The LoD was determined as the lowest amount of analytes that can be detected with a 95% probability. The LoD was determined to be 3.46 µmol/L.

The LoQ study a low-level sample set was prepared by diluting 7 human samples. These 7 human plasma samples were tested for 5 days, one run per day in 5 replicates per sample, with three reagent lots on one cobas 501 analyzer instrument. LoQ was determined to be 9.36 µmol/L based on total precision (≤20).

The LoB, LoD and LoQ results are summarized below:

LoB	LoD	LoQ
1.80 µmol/L	3.46 µmol/L	9.36 µmol/L

e. Analytical specificity:

Interference was evaluated according to the CLSI EP07-A2 guideline. The sponsor defined interference as bias exceeding ±5 µmol/L for samples with ammonia levels ≤50 µmol/L or 10% for samples with ammonia levels >50 mmol/L, where bias is calculated as the difference in results between the control sample (without interferent) and the test sample (contains the interferent).

The concentration in the table below represents the highest concentration of the potential interferent tested that did not cause interference.

Substance	Test concentration
Acetaminophen	200 mg/L
Acetylsalicylic acid	1000 mg/L
Albumin	77.2 g/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Bilirubin (conjugated)	64 mg/dL
Bilirubin (unconjugated)	68 mg/dL
Cefoxitin	2500 mg/L
Cyanocobalamin	0.001 mg/L
Cyclosporine	5 mg/L
Doxycycline	50 mg/L
Hemolysis	114 mg/dL
Heparin	5000 U
Ibuprofen	500 mg/L
IgG	71.4 g/L
Levodopa	20 mg/L
Lipemia	764 mg/dL
Methyldopa + 1.5	20 mg/L
Metronidazole	200 mg/L
N-Acetylcysteine	1660 mg/L
Naproxen	500 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Sulfapyridin	300 mg/L
Sulfasalazin	300 mg/L
Temozolomid	13 mg/L
Tetracycline	15 mg/L
Theophylline	100 mg/L

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

112 human plasma samples were tested in singlicate with the SYNCHRON Systems Ammonia Reagent on Beckmann Synchron DxC 800 (predicate device) and the Ammonia II reagent on cobas c 501 (candidate device). The samples tested had concentrations between 10.8 and 996 µmol/L.

The results obtained by linear regression analysis are summarized below:

y = 1.001x – 1.90 µmol/L, r = 1.000

b. Matrix comparison:

To demonstrate equivalence between K2-EDTA and K3-EDTA anticoagulants, 52 matched plasma samples were obtained and tested using the Ammonia II reagent on the cobas c 501. The linear regression is summarized below:

y = 1.005x – 1.39 µmol/L, r = 1.000

The study results support the sponsor's claim that human K2-EDTA and K3-EDTA plasma are acceptable sample types to be used with this assay.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The following are the reference ranges from published literature:

Females 11-51 µmol/L (18.7-86.9 µg/dL)

Males 16-60 µmol/L (27.2-102 µg/dL)

The sponsor recommends that each laboratory should investigate the transferability of the expected values to its own patient population and, if necessary, establish their own reference ranges.

Reference: Da Fonseca-Wollheim F. Deamidation of glutamine by increased plasma γ-glutamyl transferase is a source of rapid ammonia formation in blood and plasma specimens. Clin Chem 1990;36:1479-1482.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.