510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K260613

B Applicant

Sequitur Health Corp.

C Proprietary and Established Names

AMI™
AMI™ Plasma Cartridge

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JID	Class I, reserved	21 CFR 862.1065, Ammonia test system	Chemistry (75)
JJE	Class I	21 CFR 862. 2160 - Discrete photometric chemistry analyzer for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Ammonia

C Type of Test:

Quantitative photometric

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The AMI™ Plasma Cartridge is a photometric in vitro test for the quantitative determination of ammonia in human plasma on the AMI™ analyzer. Ammonia measurements are used in the diagnosis and treatment of severe liver disorders, such as cirrhosis, hepatitis, and Reye's syndrome.

The AMI™ is an analyzer for in vitro diagnostic use in clinical laboratories, utilizing photometric technology for analyte detection.

C Special Conditions for Use Statement(s):

Rx - For prescription use only

This device is intended for clinical laboratory use only and not for point-of-care (near-patient) testing.

D Special Instrument Requirements:

AMI™

IV Device/System Characteristics:

A Device Description:

The AMI™ is an instrument which consists of a cartridge reader and computerized display with a QR code reader. The AMI™ measures color changes in a dry chemical AMI™ Cartridge through reflected light when you apply a fixed volume of sample to an AMI™ Cartridge.

Each single-use AMI™ Plasma Cartridge contains:
Reactive Ingredients per cm2 - Bromophenol blue ≥ 2.3 μg
Other ingredients: buffer

B Principle of Operation:

The AMI™ measures ammonia by detecting color changes in a single-use AMI™ Plasma Cartridge using reflected light. Capillary action moves the applied plasma through four layers: a cell-trapping layer, a buffer layer that converts ammonium to ammonia gas, a gas-permeable barrier layer that blocks liquids, and a reagent layer with color-changing dye. The system measures the resulting color change, which is proportional to ammonia concentration.

The chemical reactions that occur in an AMI™ Cartridge are:
(1) Plasma + buffer → NH3
(2) NH3 + bromophenol blue (ammonia indicator) → blue dye

The AMI™ measures 604nm light reflected from the Cartridge window every second. To calculate the amount of ammonia in an unknown sample, the AMI™ solves the software-resident equation for the unknown value using the Cartridge lot-specific calibration information. The AMI™ calculates the concentration of ammonia in µmol/L.

C Instrument Description Information:

1. Instrument Name:

   AMI™

2. Specimen Identification:

   The AMI™ allows manual entry of a sample identifier on the touchscreen.

3. Specimen Sampling and Handling:

   Specimen sampling and handling procedures are documented in the AMI™ Plasma Cartridge Instructions for Use.

4. Calibration:

   The AMI™ Plasma Cartridges are pre-calibrated during manufacturing and automatically transferred via QR code scanning of the reagent cartridge for each test.

5. Quality Control:

   The sponsor recommends AMI™ Quality control solutions for use with the AMI™. The labeling also recommends that quality control testing should be performed in accordance with state, local, and federal regulations.

V Substantial Equivalence Information:

A Predicate Device Name(s):

KODAK EKTACHEM Clinical Chemistry Slides (NH_3); KODAK EKTACHEM 400 Analyzer

B Predicate 510(k) Number(s):
K820723
K811919

C Comparison with Predicate(s):

Device & Predicate Device(s):	K260613	K820723
Device Trade Name	AMI™ Plasma Cartridges	KODAK EKTACHEM Clinical Chemistry Slides (NH_3)
General Device Characteristic Similarities		
Intended Use/Indications For Use	Quantitative measurement of ammonia	Same
General Device Characteristic Differences		
Instrument Platform	AMI™	KODAK EKTACHEM 400 Analyzer

VI Standards/Guidance Documents Referenced:

- Clinical and Laboratory Standards Institute (CLSI) EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
- CLSI EP06-Ed2: Evaluation of the Linearity of Quantitative Measurement Procedures; Approved Guideline.
- CLSI EP07-Ed3: Interference Testing in Clinical Chemistry.
- CLSI EP09c-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition.
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- IEC 61010-1 Edition 3.1 2017-01 consolidated version: Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General Requirements (including: Corrigendum 1).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   A precision study to assess repeatability and within-laboratory precision was conducted according to CLSI EP05-A3. Three native K_2 EDTA plasma samples and two controls were run in replicates of two per run, with two runs per day, over 20 days at one site. The results are summarized in the tables below:

Sample	Mean (µmol/L)	Repeatability		Within Laboratory	
		SD	CV(%)	SD	CV(%)
Plasma 1	53.9	3.7	6.9%	4.4	8.1%
Plasma 2	150	6.6	4.4%	6.8	4.6%
Plasma 3	443	11.8	2.7%	12.7	2.9%
Control 1	58.2	1.8	3.2%	2.2	3.8%
Control 2	215	3.4	1.6%	3.7	1.7%

A reproducibility study was conducted to assess instrument-to-instrument variability. Five human K_2 EDTA plasma samples with different analyte concentrations were run on three analyzers (i.e., AMI™). The samples were tested across three reagent lots (i.e., AMI™ Plasma Cartridge) at each site on each instrument with three replicates per run and two runs per day over five days. The between-lot, between-instrument, and reproducibility was calculated for each reagent lot. The results are summarized in the tables below:

Sample	Mean (µmol/L)	Between-Lot		Between-Instrument		Reproducibility	
		SD	CV(%)	SD	CV(%)	SD	CV(%)
Plasma 1	23.7	0.46	1.9%	1.24	5.2%	3.57	15.1%
Plasma 2	47.7	0	0.0%	1.08	2.3%	4.14	8.7%
Plasma 3	120	3.11	2.6%	0.61	0.5%	7.3	6.1%
Plasma 4	446	2.51	0.6%	0	0.0%	15.1	3.4%
Plasma 5	546	8.29	1.5%	1.13	0.2%	19.5	3.6%

2. Linearity:

Linearity was evaluated in accordance with the CLSI Guideline EP06-Ed2. 11 levels were prepared using a human K_2 EDTA plasma sample pool with ammonia concentration above the upper measuring range. Testing for each sample type was completed with two AMI™ Plasma Cartridge lots in one run each, 4 replicates per sample. The deviation from linearity did not exceed 2.8 µmol/L for values ≤ 10 µmol/L or -3.8% for values > 10 µmol/L. The results of the linearity study support the claimed measuring range of 13.2-567 µmol/L.

3. Analytical Specificity/Interference:

Interference was evaluated according to the CLSI EP07-A3 guideline. Testing was performed using human K_2 EDTA plasma samples with two ammonia levels (~39 and 229 µmol/L). The samples were assayed, and the ammonia concentrations of the spiked samples were compared to control samples without interferent. The tables below summarize the results indicating the highest interferent level at which bias between the samples with and without interferent is ≤ 10 µmol/L at ammonia concentrations < 100 µmol/L and ≤ 9.7% at ammonia concentrations ≥ 100 µmol/L.

Endogenous Interferences

Substance	Highest concentration at which no significant interference was observed
Albumin	5425 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	21 mg/dL
Glucose	600 mg/dL
Hemoglobin	100 mg/dL
Intralipid	1000 mg/dL
Total Protein	8520 mg/dL
Urea	135 mg/dL

Exogenous Interferences

Substance	Highest concentration at which no significant interference was observed
Acetaminophen	7.8 mg/dL
Ascorbic acid	5.25 mg/dL
Ethanol	600 mg/dL
Eltrombopag	3 mg/dL

The sponsor included the following limitation statements in the package insert:

Eltrombopag at therapeutic doses can be present in plasma at concentrations up to 4 mg/dL. Eltrombopag in plasma above 3 mg/dL can lead to positive bias of up to 25 µmol/L in ammonia measurement. Do not use this test on patients taking Eltrombopag. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Do not use hemolyzed samples.

4. Detection Limit and Assay Reportable Range:

Determination of the Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were conducted in accordance with the CLSI guideline EP17-A2.

Limit of Blank (LoB)
For determination of LoB, four analyte-free samples were measured in replicates of 5 using two AMI™ Plasma Cartridge lots over 3 days on one AMI™. In total 60 measured values of analyte free samples were obtained per lot. Data analysis was based on determination of the 95th percentile of the 60 measured values.

Limit of Detection (LoD)
For determination of LoD, four human K_2 EDTA plasma samples with low-analyte concentrations were measured in replicates of 5 using two AMI™ Plasma Cartridge lots over 3 days on one AMI™. In total 60 measured values of samples with low analyte

concentrations were obtained per lot. The LoD was calculated non-parametrically. The higher LoD of the 2 lots was chosen as the assay's LoD.

Limit of Quantitation (LoQ)
For the determination of LoQ, five human K_2 EDTA plasma samples with low-analyte concentrations were measured in replicates of 5 across two AMI™ Plasma Cartridge lots over 3 days for a total of 75 replicates per lot on one AMI™. The LoQ was defined as the concentration of analyte which has imprecision less than 20% CV.

The LoB, LoD and LoQ results are summarized below:

LoB	LoD	LoQ
6.2 µmol/L	10.8 µmol/L	13.2 µmol/L

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

The AMI™ assay is traceable to NIST-certified ammonium chloride.

Sample Stability: Sample stability studies validating K2-EDTA plasma storage conditions were reviewed and found acceptable to support 2 hours at 2-8 °C, 3 days at −20 °C ± 5 °C , and 20 days at −80 °C ± 5 °C.

6. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed comparing the ammonia assay on the AMI™ using AMI™ Plasma Cartridge to the predicate device, using a protocol based on CLSI EP09c-A3. A total of 99 human K_2-EDTA plasma samples (95 native and 4 contrived) with ammonia concentrations ranging from 23 to 488 µmol/L were evaluated with the candidate and comparator devices in singlicate. The linear regression analysis results between the candidate device (dependent variable, y) and the comparator device (x, comparator), are shown below:

N	Concentration Range (µmol/L)	Slope	Intercept	Correlation Coefficient (r)
99	23 to 488	1.003	0.04772	0.995

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Clinical Cut-Off

   Not applicable.

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

D Expected Values:

K2 EDTA plasma: <13.2 - 30 µmol/L (22.5 - 86.9 µg/dL)

The central 95% reference interval from apparently healthy adults was verified consistent with CLSI EP28-A3C. The sponsor includes the following information in the package insert:

It is the responsibility of each laboratory to confirm the validity of these intervals for the population it serves.

E Other Supportive Instrument Performance Characteristics Data:

1. Environmental Conditions: The sponsor provided adequate information to support the AMI™ and AMI™ Plasma Cartridge would work as intended in the environmental operating conditions claimed in the labeling.

2. Electrical safety and electromagnetic compatibility (EMC) testing were performed, and the system was found to be acceptable.

3. Software and cybersecurity documentation was reviewed and found to be acceptable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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