510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232761

B Applicant

BioPorto Diagnostic Inc.

C Proprietary and Established Names

ProNephro AKI™ (NGAL)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PIG	Class II	21 CFR 862.1220 - Acute Kidney Injury Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Neutrophil gelatinase-associated lipocalin (NGAL)

C Type of Test:

Quantitative immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Immunoassay for the in vitro quantitative determination of neutrophil gelatinase-associated lipocalin (NGAL) in human urine.

Determination of NGAL is intended to be used in conjunction with clinical evaluation in pediatric patients (≥ 3 months to < 22 years) without underlying kidney disease admitted to the intensive care unit (ICU) for the management of cardiovascular or respiratory compromise or who have had a solid organ or bone marrow transplant.

ProNephro AKI™ (NGAL) is intended to be used in the first 24 hours of ICU admission. In patients with low SCr levels, indicative of no acute kidney injury (AKI) or AKI Stage 1, the test can be used as an aid to identify patients at risk to develop moderate to severe acute kidney injury (Stage 2/3 AKI) 48 to 72 hours after the time of assessment. In patients with an elevated SCr level, indicative of Stage 2/3 AKI, the test can be used as an aid to identify patients at risk to have persistent moderate to severe AKI (Stage 2/3) 48 to 72 hours after the assessment.

The particle-enhanced turbidimetric immunoassay is intended for use on the Roche cobas® c 501 clinical chemistry analyzer.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

This kit should only be used by qualified laboratory staff.

Pediatric urine NGAL results < cutoff of 125 ng/mL are indicative of a lower risk of developing or having persistent moderate to severe acute kidney injury (Stage 2 to 3 AKI) (within 48 to 72 hours), supported by a high negative predictive value as observed in the clinical study. However, the development or persistence of AKI is still possible for patients with NGAL results below this threshold.

Pediatric urine NGAL results ≥ cutoff of 125 ng/mL are indicative of a higher risk of developing or having persistent moderate to severe acute kidney injury (Stage 2 to 3 AKI) (within 48 to 72 hours). However, the development or persistence of AKI may not occur in patients with NGAL measurements above this threshold.

The ProNephro AKI™ (NGAL) test result cannot be used as a stand-alone result, but clinicians must interpret the significance of an NGAL result in conjunction with the patient's full clinical assessment.

Results are not to be used for diagnosis.

D Special Instrument Requirements:

Roche cobas c 501 clinical chemistry analyzer

IV Device/System Characteristics:

A Device Description:

The ProNephro AKI™ (NGAL) Reagent Kit contains the following reagents:

- R1: Reaction Buffer Reagent (17.9 mL) - a ready-to-use tris-buffer solution containing murine protein and preservative
- R2: Immunoparticle Suspension Reagent (7.65 mL) - a ready-to-use suspension of polystyrene microparticles coated with mouse monoclonal antibodies to NGAL that also contains preservative

The ProNephro AKI™ Reagent Kit provides enough reagents for 100 tests on the Roche cobas c 501 clinical chemistry analyzer.

The kit also includes a labeled Roche cassette and two funnels for transferring the reagent into the Roche cassette. The reagents must be transferred to a Roche cassette prior to loading onto the cobas c 501. This is done by pouring the R1 reagent into Position B of the cassette and R2 reagent into Position C. The operator will load the cassette onto the instrument and run the ProNephro AKI (NGAL) test per the instructions for use.

B Principle of Operation:

A pediatric urine sample is mixed with Reaction Buffer (R1). After a short incubation, the reaction is started by the addition of an immunoparticle suspension (microparticles coated with mouse monoclonal antibodies to NGAL) (R2). NGAL in the sample causes the immunoparticles to agglutinate. The degree of agglutination is quantified by the amount of light scattering, measured as absorption of light at a wavelength of 570 nm. The absorbance change is read approximately six minutes after the addition of R2. The NGAL concentration in the sample is determined by interpolation on a calibration curve prepared from calibrators of known concentrations. Calibrators and controls are prepared from recombinant NGAL that has been value assigned against an in-house master calibrator.

An NGAL concentration below the cut-off of 125 ng/mL in pediatric urine is indicative of lower risk of moderate to severe acute kidney injury (Stage 2/3 AKI) 48 to 72 hours after the time of assessment and is considered a negative result. Whereas a result greater than or equal to the cut-off of 125 ng/mL, indicates a risk of moderate to severe acute kidney injury (Stage 2/3 AKI) 48 to 72 hours after the time of assessment and is considered a positive result.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Nephrocheck Test System

B Predicate 510(k) Number(s):
DEN130031

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232761	DEN130031
Device Trade Name	ProNephro AKI™ (NGAL)	Nephrocheck® Test System
General Device Characteristic Similarities		
Intended Use/Indications for Use	Used in conjunction with clinical evaluation as an aid in the risk assessment for moderate or severe acute kidney injury (AKI)	Same
Test Type	Quantitative immunoassay	Same
Specimen Type	Urine	Same
General Device Characteristic Differences		
Test Technology	Particle Enhanced Turbidimetric Assay	Lateral Flow with Fluorescent Detection
Instrument	Roche Cobas c501 Clinical Chemistry Analyzer	ASTUTE140® Meter
Measurand	Neutrophil gelatinase-associated lipocalin (NGAL)	Insulin-like growth factor binding protein (IGFBP7) and tissue-inhibitor of metalloproteinases 2 (TIMP2)
Population	Pediatric (≥ 3 months to < 22 years)	Adult (≥ 21 years)
Units of Measurement	ng/mL	AKI Risk Score
Assay Measuring Range	50 – 3000 ng/mL	Score 0.04-10.0

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP06 2nd, Edition, Evaluation of the Linearity of Quantitative Measurement Procedures

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

CLSI EP07 3rd, Edition, Interference Testing in Clinical Chemistry.

CLSI EP28-A3C Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

ISO 14155 Third edition 2020-07, Clinical investigation of medical devices for human subjects - Good clinical practice

ISO 14971 Third Edition 2019-12, Medical devices - Application of risk management to medical devices

ISO 15223-1 Fourth edition 202-07, Medical devices- Symbols to be used with information to be supplied by the manufacturer – Part 1: General Requirements

ISO 17511 Second edition 2020-04, In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators trueness control materials and human samples

ISO 2859-1 Second edition 1999-11-15, Sampling procedures for inspection by attributes - Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection [Including: Technical Corrigendum 1 (2001) Amendment 1 (2011)]

IEC 62366-1 Edition 1.0. 2015-02, Medical devices - Part 1: Application of usability engineering to medical devices [Including CORRIGENDUM 1 (2016)]

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Precision studies were conducted following the recommendations in the CLSI EP05-03 guideline.

   Repeatability and Intermediate Precision

   The repeatability (within-run precision) and intermediate precision (within-laboratory precision) was evaluated internally on a single Roche cobas c 501 analyzer. The protocol consisted of testing nine native pediatric urine samples and sample pools with three lots of reagent for 20 days with two runs per day and three replicates per sample, for a total of 360 measurements/sample. The low and high control samples were tested each day at the beginning of the first run, between runs (except on Day 1), and at the end of the second run in two replicates.

The SD and %CV of the repeatability (within-run precision) and intermediate precision (within-laboratory precision) were calculated for each sample.

The table below summarizes the results of the repeatability and intermediate precision testing:

Sample	N	Mean NGAL (ng/mL)	Repeatability		Between-Run		Between-Day		Intermediate Precision (Within-Laboratory)	
			SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Sample 1	360	71	3.6	5.0	2.2	3.1	0.0	0.0	4.7	6.6
Sample 2	360	100	3.5	3.5	1.7	1.7	1.2	1.2	4.5	4.5
Sample 3	360	102	3.8	3.8	2.3	2.2	1.0	1.0	5.0	4.9
Sample 4	360	142	3.3	2.3	2.6	1.9	1.6	1.1	4.9	3.4
Sample 5	360	165	3.7	2.2	2.4	1.5	2.5	1.5	5.2	3.1
Sample 6	360	304	4.5	1.5	2.9	1.0	3.6	1.2	7.3	2.4
Sample 7	360	512	6.0	1.2	4.0	0.8	6.5	1.3	11.2	2.2
Sample 8	360	1049	12.4	1.2	8.5	0.8	12.8	1.2	22.7	2.2
Sample 9	360	2776	42.4	1.5	27.9	1.0	34.8	1.3	75.2	2.7
QC low	353	203	3.5	1.7	4.1	2.0	3.4	1.7	6.4	3.1
QC high	353	498	4.6	0.9	7.4	1.5	7.8	1.6	11.7	2.3

The table below summarizes the results of the lot-to-lot precision testing:

Sample	N	Mean NGAL (ng/mL)	Within-Lot*		Between-Lot	
			SD (ng//mL)	CV (%)	SD (ng/mL)	CV (%)
Sample 1	360	71	4.2	5.9	2.1	3.0
Sample 2	360	100	4.1	4.1	2.0	2.0
Sample 3	360	102	4.6	4.5	2.1	2.0
Sample 4	360	142	4.5	3.2	1.9	1.3
Sample 5	360	165	5.0	3.1	1.1	0.7
Sample 6	360	304	6.4	2.1	3.5	1.1
Sample 7	360	512	9.7	1.9	5.5	1.1
Sample 8	360	1049	19.7	1.9	11.3	1.1
Sample 9	360	2776	61.5	2.2	43.3	1.6
QC low	353	203	6.4	3.1	0.0	0.0
QC high	353	498	11.7	2.3	0.0	0.0

*The “Within-Lot” precision is a summation of the variances for the lower components: repeatability + between run + between day

Reproducibility

Reproducibility was evaluated on three Roche cobas c 501 analyzers at three sites (two external and one internal) with two operators per site. The protocol consisted of testing seven native pediatric urine samples and sample pools, and two controls with one reagent lot for

five days with two runs per day and three replicates per sample, for a total of 30 measurements/sample/site. QC samples were run at the beginning and end of each day for run qualification.

The table below summarizes the results of the reproducibility testing:

		Site 1		Site 2		Site 3		Overall Reproducibility	
Sample (N=30 per site)	**Mean NGAL (ng/mL)**	**SD (ng/mL)**	**CV (%)**	**SD (ng/mL)**	**CV (%)**	**SD (ng/mL)**	**CV (%)**	**SD (ng/mL)**	**CV (%)**
Sample 1	67	4.3	6.4	3.8	5.6	4.1	6.3	4.4	6.6
Sample 2	89	5.9	6.6	6.1	6.6	4.5	5.1	5.6	6.2
Sample 3	133	7.8	6.0	5.8	4.3	5.5	4.1	6.5	4.8
Sample 4	160	7.0	4.5	7.9	4.9	5.5	3.4	7.5	4.7
Sample 5	493	16.6	3.4	13.0	2.6	22.0	4.5	17.6	3.6
Sample 6	1213	46.3	3.8	36.9	3.0	43.0	3.6	43.4	3.6
Sample 7	2629	111.3	4.3	88.2	3.3	115.6	4.5	113.2	4.3
QC low	213	8.9	4.3	4.0	1.8	8.2	3.8	9.3	4.4
QC high	532	22.0	4.2	17.1	3.2	16.1	3.0	19.5	3.7

2. Linearity:

A linearity study was conducted following the recommendations in CLSI EP06-2nd edition guideline.

The protocol consisted of testing three different linearity sample sets, each consisting of a native pediatric urine sample pool with a high concentration above the measuring range, blended with a native pediatric urine sample pool with a low concentration below the measuring range, to create 14 levels of samples. Each linearity set was tested in one run, using one reagent lot on a single Roche cobas c 501 analyzer. Samples with an NGAL concentration below 150 ng/mL were tested in eight replicates. Samples with an NGAL concentration equal to or greater than 150 ng/mL were tested in four replicates.

The data was analyzed by weighted least square regression linear regression analysis. The relative deviation degree from linearity ranged between 0.1-13.3%.

The results of the linearity study support the claimed measuring range of 50 - 3000 ng/mL.

Automatic Dilution, High-Dose Hook Effect

Automatic dilution was evaluated by testing human recombinant NGAL spiked into three different pediatric urine sample pools at NGAL concentrations of approximately 40,000, 20,000, 10,000, and 5,000 ng/mL. The recombinant human (rh) NGAL was Amino-Acid-Analyzed (AAA) and thereby precisely quantified. Each sample was tested in four replicates, using the automatic re-run function of the analyzer when needed. The mean NGAL concentration after re-run and automatic dilution was compared to the target NGAL concentration, as determined by gravimetric dilution of a precisely quantified rhNGAL

preparation. Acceptance criteria was mean recovery ≤ ±10%. The results support the automatic 1:15 dilution.

High dose hook effect was evaluated by testing recombinant human NGAL spiked into three different pediatric urine sample pools at NGAL concentrations of approximately 80,000, 60,000, and 40,000, 20,000, 10,000, 5,000, 2.500 and 1.250 ng/mL NGAL. The results of the high-dose hook effect testing support the labeling statement that there is no high-dose hook effect up to 40,000 ng/mL NGAL.

3. Analytical Specificity/Interference:

Analytical specificity and interference testing was conducted following the recommendations in CLSI EP07-A3 and EP37-Ed1.

Interferents were selected from endogenous urine constituents, pH and Specific Gravity, cross reactants, contrast agents, common and special drugs.

All specificity and interference studies were performed using two sample pools with NGAL concentrations of approximately 150 ng/mL and 1500 ng/mL, respectively, prepared by blending a native pediatric urine sample with an elevated NGAL concentration with a commercially available pediatric urine pool with a low NGAL concentration. Acceptance criteria for the interference studies was mean recovery ≤ ±10%.

Endogenous Interference, pH and Specific Gravity
Endogenous interference, pH and specific gravity were evaluated on a single Roche cobas c 501 analyzer, using one reagent lot in one run. The lower concentration sample was tested in six replicates and the higher concentration sample was tested in four replicates. The endogenous urine constituents were evaluated at concentrations above the expected concentrations in the intended use population. Mean concentrations of the different urine constituents were evaluated using published data, and reduced urine volumes in pediatric patients with AKI were considered.

The tables below summarizes the results of the interference testing for endogenous substances, pH and SG:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Albumin	60,000 mg/L
Ammonium Chloride	2,000 mg/L
Bilirubin, conjugated	800 mg/L
Calcium Chloride	2,500 mg/L
Citric Acid	2,500 mg/L
Creatinine	10,000 mg/L
Glucose	14,000 mg/L
Hemoglobin	2,500 mg/L
Immunoglobulin G	1,100 mg/L
Magnesium	2,000 mg/L
Oxalic Acid	300 mg/L

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Sodium Phosphate	4,000 mg/L
Urea	38,000 mg/L
Uric Acid	1,400 mg/L
Urobilinogen	150 mg/L
pH	3.3-9.5
Specific Gravity (SG)	1.084

Cross-Reactivity

Cross-reactivity was evaluated on a single Roche cobas c 501 analyzer, using one reagent lot in one run. The lower concentration sample was tested in six replicates and the higher concentration sample was tested in four replicates. The maximum concentration of the potentially cross-reactive biomarkers tested was evaluated using published data, or package inserts/instructions for use for the respective biomarker assays, if available. If the potentially cross-reactive biomarker had been tested in AKI patients, at least the highest concentration mentioned in those studies was tested.

The table below summarizes the results of the interference testing for potentially cross-reactive biomarkers:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Cystatin C	8 mg/L
Interleukin 18	0.001 mg/L
Kidney Injury Molecule 1 (KIM-1)	0.015 mg/L
Liver Type Fatty Acid Binding Protein (L-FABP)	0.6 mg/L
N-acetyl H3-D-glucosaminidase (NAG)	15 U/L
Pi-Glutathione s-Transferase (p-GST)	0.5 mg/L
Insulin-like Growth Factor binding protein (IGFBP7)	0.03 mg/L
Tissue inhibitor of Metalloproteinase 2 (TIMP 2)	0.01 mg/L

Exogenous Interference – Contrast Agents

Potential interference of contrast agents was evaluated on a single Roche cobas c 501 analyzer, using one reagent lot in one run. The lower concentration sample was tested in six replicates, and the higher concentration sample in four replicates.

The table below summarizes the results of the interference testing for contrast agents:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Visipaque / Iodixanol	20 g/L
Omnipaque / Iohexol	60 g/L
Optiray / Ioversol	100 g/L

Exogenous Interference – Common and Special Drugs

A risk assessment was performed to determine special drugs which may be most likely to interfere with ProNephro AKI ™ (NGAL) in pediatric patients. This risk analysis included research of published data and obtaining data from PICU sites to determine drugs most prescribed for pediatric patients with impaired kidney function and their maximum dosages given. The list was narrowed down by excluding drugs that are known to not be excreted in the urine. If urine concentrations of drugs had been established, a concentration of at least 3x the reported concentration was tested. If urine concentrations were unknown, the maximum daily therapeutic dose was calculated based on mean body weight per age group and at least 3x that therapeutic concentration was tested.

Exogenous interference by common and special drugs was evaluated on a single Roche cobas c 501 analyzer, in one run, using in total five different reagent lots and four different lots of calibrators and controls. The lower concentration sample was tested in six replicates, the higher concentration sample in four replicates.

The tables below summarize the results of the interference testing for common and special drugs:

Common Drug Interference Summary:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Acetaminophen	3,000 mg/L
Ascorbic Acid	4,000 mg/L
N-Acetylcysteine	150 mg/L
Ibuprofen	4,000 mg/L
Levodopa	1,000 mg/L
Methyldopa	2,000 mg/L
Phenazopyridine	300 mg/L
Salicyluric Acid	6,000 mg/L

Special Drugs Interference Summary:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Cefazolin	20,000 mg/L
Cefepime	4063 mg/L
Ceftriaxone	45,000 mg/L
Diazepam	500 mg/L
Diphenhydramine	11,250 mg/L
Famotidine	250 mg/L
Fentanyl	100 mg/L
Furosemide	3,500 mg/L
Hydromorphone	300 mg/L
Ketamine	2,500 mg/L
Levetiracetam	25,000 mg/L
Lorazepam	300 mg/L

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Midazolam	3,000 mg/L
Morphine	4,000 mg/L
Ondansetron	500 mg/L
Oxycodone	1,500 mg/L
Vancomycin	20,000 mg/L

The results of the special drugs interference testing performed with ProNephro AKI ™ (NGAL) met the sponsor's criteria, except for Cefepime, Ceftriaxone, and Diphenhydramine. Additional testing was done for these drugs at lower concentrations until interference was no longer observed and the results met the sponsor's acceptance criteria of a mean recovery ≤ ± 10%.

The sponsor included the following statement in the ProNephro AKI™ (NGAL) Instructions for Use:

Cefepime was shown to interfere at concentrations above 4,063 mg/L. Patients being treated with Cefepime may have lower than expected NGAL results.

Exogenous Interference – Immunosuppressive Agents and Special Drugs
In addition, the sponsor conducted a study to evaluate exogenous interference by immunosuppressive agents and special drugs. Exogenous interference by immunosuppressive agents and special drugs was evaluated on a single Roche cobas c 501 analyzer. The study protocol consisted of testing two different pediatric urine sample pools with NGAL concentrations of approximately 150 and 1500 ng/mL in one run with one reagent lot. The lower concentration sample was tested in six replicates and the higher concentration sample was tested in four replicates. The sample pools were prepared by blending one native pediatric urine sample with an elevated NGAL concentration and one pediatric urine pool with a low NGAL concentration. High concentration stock solutions of the potentially interfering immunosuppressive agents and special drugs were prepared in a suitable solvent (water, ethanol, DMSO), or the potentially interfering immunosuppressive agents and special drugs were spiked directly into the pediatric urine sample pools.

The tables below summarize the results of the interference testing for immunosuppressive agents and special drugs:

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Prednisone	3 mg/L
Prednisolone	3 mg/L
Tacrolimus	2.5 mg/L
Cyclosporine	200 mg/L
Sirolimus	2 mg/L
Everolimus	5 mg/L
Mycophenolate Mofetil	1000 mg/L
Mycophenolic Acid Glucuronide (MPAG)	20,000 mg/L
Tazobactam	20,000 mg/L

Potentially Interfering Substance	Maximum concentration tested that demonstrated no significant interference
Piperacillin	10,000 mg/L
Piperacillin/Tazobactam 1/0.125	10,000 mg/L/1250 mg/L
Ketorolac	900 mg/L

4. Assay Reportable Range:

   The analytical measuring interval is 50 to 3000 ng/mL.

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

   Traceability
   The device is traceable to an internal reference standard (master calibrator), i.e. recombinant human NGAL. The sponsor stated that no internationally recognized reference material for NGAL is available. BioPorto has developed a recombinant internal reference material.

   Sample Stability
   Stability of stored specimens (room temperature, refrigerated storage and frozen storage) and the effect of freezing/thawing on the measurement of NGAL on the Roche cobas c 501 analyzer were evaluated. The acceptance criteria for all stability studies were recovery within ±10%.

   Urine specimens were shown to be stable for one day at 15-30°C, 3 days at 2-8°C and 12 months at or below -70°C, up to 2 freeze/thaw cycles. Specimens were shown to withstand 2 freeze-thaw cycles when stored at or below -70°C.

6. Detection Limit:

   Limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) studies were performed following the recommendations in CLSI EP17-A2 guideline.

   To estimate LoB, six analyte-free samples were tested using two lots of reagent in five runs distributed over five days with three replicates per sample on a single Roche cobas c 501 analyzer. In total, 90 determinations for analyte-free samples were obtained for each lot. The LoB was defined as the concentration at which there is a 95% probability that the sample does not yield a result. The results of the LoB study performed with ProNephro AKI™ (NGAL) support an LoB claim in the labeling of 9 ng/mL.

   To estimate LoD, six samples with low analyte concentrations were tested with two lots of reagent in five runs distributed over five days with three replicates per sample. LoD was evaluated on a single Roche cobas c 501 analyzer. In total, 60 determinations for samples with low analyte concentrations were obtained for each lot. The LoD was defined as the lowest amount of analyte in a sample that can be detected with a 95% probability.
   The results of the LoD study performed with ProNephro AKI ™ (NGAL) support an LoD claim in the labeling of 14 ng/mL.

To estimate LoQ, four samples with analyte concentrations close to the expected LoQ were tested with two lots of reagent in five runs distributed over five days with three replicates per sample. LoQ was evaluated on a single Roche cobas c 501 analyzer. The LoQ was defined as the lowest concentration of analyte in a sample which can be quantified with an intermediate precision of no more than 20% CV. The results of the LoQ study performed with ProNephro AKI ™ (NGAL) support an LoQ claim in the labeling of 18 ng/mL.

7. Assay Cut-Off:

   Please refer to Section VII.D below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable. Please refer to section VII.C.

2. Matrix Comparison:

   Not applicable, assay is indicated for use with urine samples only.

C Clinical Studies:

1. Clinical Sensitivity:

   See below.

2. Clinical Specificity:

   See below.

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

   The clinical performance of ProNephro AKI™ (NGAL) was validated in a multi-center prospective clinical study based on the established cutoff (125 ng/mL) identifying patients at risk of moderate to severe acute kidney injury (Stage 2 to 3 AKI) 48 to 72 hours after the time of assessment. The study enrolled 660 pediatric patients (≥ 90 days to < 22 years of age) at 15 sites across the US, with 514 evaluable subjects. The study population was comprised of patients having at least one of the following leading to admission to the PICU, or occurring within 12 hours of admission to the PICU: vasoactive medication administration, mechanical ventilation, history of solid organ transplantation (renal transplantation included only if more than 3 months prior), history of bone marrow transplantation or hypotension, (having received ≥ 40 ml/kg of resuscitative fluid in pre-ICU (within 6 hours prior to ICU admission) or in first 12 hours of ICU). Urine samples were collected from each enrolled subject in the first 24 hours of ICU admission, were centrifuged and stored frozen until shipping to the clinical testing site. Testing was performed on one Roche cobas c 501 analyzer. Samples were tested randomized in single determinations. Blood samples were drawn at four timepoints (Day 0, Day 1, Day 2, and Day 3) from enrolled subjects for serum/plasma creatinine testing. Most screen failures were due to either missing urine or

blood collection; exclusion of the unevaluable patients did not affect the conclusions of the study.

The indication for use does not exclude patients with diagnosed AKI per KDIGO guidelines or patients with elevated sCr values prior to or within the first 12 hours of ICU admission. These patients were included in the GUIDANCE study. For these patients the ProNephro AKI™ (NGAL) test is used to assess the risk of persistent AKI 48 to 72 hours after the time of the NGAL assessment.

The subgroups of patients within the intended use population can be summarized into patients that present:

1. Without a sCr value indicative of Stage 2-3 AKI at ICU admission,
   a. AND developing Stage 2-3 AKI 48 to 72 hours after the time of assessment,
   b. OR not developing Stage 2-3 AKI 48 to 72 hours after the time of assessment.
2. With an elevated sCr value indicative of Stage 2-3 AKI at ICU admission,
   a. AND have persisting Stage 2-3 AKI 48 to 72 hours after the time of assessment,
   b. OR do not have persisting Stage 2-3 AKI 48 to 72 hours after the time of assessment.

The clinical data for each subject were captured and provided to at least two independent clinical experts for adjudication. Adjudicators were blinded to the site diagnoses and NGAL results. Subjects were adjudicated for the determination of moderate to severe AKI by an expert panel of clinicians following KDIGO guidelines and using clinical judgment. Urine output information was not available for all subjects in the clinical studies, and adjudication was done based on SCr values only. Of the 514 evaluable subjects in combined groups 1 and 2, 47 AKI (Stage 2 or 3) positive subjects were determined by adjudication (9.1% prevalence).

The clinical performance of ProNephro AKI™ (NGAL) was evaluated against adjudicated results.

For the overall evaluable subjects, sensitivity and specificity and their corresponding 95% confidence interval (CI) were calculated to be 72.3% (57.4-84.4%) and 86.3% (82.8-89.3%) respectively. The negative predictive value (NPV) was 96.9% (95.1-98.0% 9) and the positive predictive value (PPV) was 34.7% (28.5-41.5%).

The following clinical performance characteristics for sensitivity, specificity, negative predictive value, and positive predictive value were calculated for the two subgroups of patients within the intended use population when assessed separately.

Group 1 Study Results – Day 0 sCr indicates no AKI or AKI stage 1 (n=422)

	AKI+	**AKI-**	**Total**
NGAL+	11	46	57
NGAL-	7	358	365
Total	18	404	422
Prevalence	4.3% (18/422)		

	AKI+	AKI-	Total
Risk of AKI for NGAL Negative Results	1.9%		
Risk of AKI for NGAL Positive Results	19.3%		
Sensitivity (95% CI)	61.1% (35.8% to 82.7%)		
Specificity (95% CI)	88.6% (85.1% to 91.5%)		
PPV (95% CI)	19.3% (10.0% to 31.9%)		
NPV (95% CI)	98.1% (96.1% to 99.2%)		

Group 2 Study Results – Day 0 sCr indicates AKI stage 2/3 (n=91)

	AKI+	AKI-	Total
NGAL+	23	18	41
NGAL-	6	45	51
Total	29	63	92
Prevalence	31.5% (29/92)		
Risk of AKI for NGAL Negative Results	11.8%		
Risk of AKI for NGAL Positive Results	56.1%		
Sensitivity (95% CI)	79.3% (60.3% to 92.0%)		
Specificity (95% CI)	71.4% (58.7% to 82.1%)		
PPV (95% CI)	56.1% (39.8% to 71.5%)		
NPV (95% CI)	88.2% (76.1% to 95.6%)		

The sponsor has satisfied the requirements per 21 CFR 862.1220(b)(3).

To mitigate the risk of incorrect interpretation of test results, the sponsor provided details and documentation of the end user device training program that will be offered while marketing the device. The content of the end user training and assessment was reviewed and found to be acceptable to satisfy the requirements per 21 CFR 862.1220(b)(1) and (b)(2).

D Clinical Cut-Off:

A multi-center prospective clinical study was conducted at six clinical sites to establish the NGAL cutoff to identify patients at risk of moderate to severe acute kidney injury (Stage 2 to 3 AKI) 48 to 72 hours after the time of assessment. 257 pediatric patients (≥ 90 days to < 22 years of age) were enrolled in the study with 203 evaluable subjects. The cut-off was established at 125 ng/mL with the sample collection timepoint within the first 24 hours of ICU admission.

E Expected Values/Reference Range:

A reference range study was performed to determine the NGAL concentrations in apparently healthy pediatric subjects (ages from 3 months to 22 years). Samples were collected from four geographically diverse sites in the US to ensure a variety of healthy patient samples. Demographic and other information for the enrolled subjects is shown in the tables below.

Number and Percentage of Female and Male Specimens by Age:

Gender	Number of evaluable subjects	≥3 month to <2 years of age	≥2 years to <12 years of age	≥12 years to <22 years of age
Female	159 (45.7%)	31 (42.5%)	64 (46.0%)	64 (47.1%)
Male	189 (54.3%)	42 (57.5%)	75 (54.0%)	72 (52.9%)
Total	348	73	139	136

Demographics:

Factor	Mean ± SD	Median	Range
Age	9.3 ± 6.0	9.2	0.26 to 21.7
Race	**N**	**%**	
American Indian/Alaskan Native	0	0.0	
Asian	16	4.6	
Black/African American	12	3.4	
Native Hawaiian or Pacific Islander	0	0.0	
White	303	87.1	
Unknown/Prefer not to answer	6	1.7	
Other	11	3.2	
Ethnicity*			
Hispanic/Latino	36	10.3	
Not Hispanic/Latino	306	87.9	
Not Reported	6	1.7	

To determine the NGAL reference range, a urine specimen from each subject was collected and stored frozen until testing at an internal lab on one Roche cobas c 501 analyzer.

Samples were excluded due to urinary tract infection (UTI) if at least one of the following criteria was fulfilled:

1. Moderate to high level of leukocyte
2. Nitrite positive

The pediatric population included 348 evaluable subjects in three age groups from ≥3 months to <2 years of age, ≥2 years to <12 years of age, and ≥12 years to <22 years of age. There was an even distribution of males and females represented in the study, although the total number of subjects in the youngest age group was slightly lower overall.

Results of the reference range study are summarized below:

MALE subjects	**N**	**Median**	**Central 95% (2.5^{th}-97.5^{th})**	**N / % NGAL results ≥ 125 ng/mL**
≥ 90 days to < 22 years (all)	189	≤50	≤50 - ≤50	0 / 0
≥ 90 days to < 2 years	42	≤50	≤50 – 59	0 / 0
≥ 2 years to < 12 years	75	≤50	≤50 - ≤50	0 / 0
≥ 12 years to < 22 years	72	≤50	≤50 - ≤50	0 / 0

FEMALE subjects	**N**	**Median**	**Central 95% (2.5^{th}-97.5^{th})**	**N / % NGAL results ≥ 125 ng/mL**
≥ 90 days to < 22 years (all)	159	≤50	≤50 - 96	2 / 1.26*
≥ 90 days to < 2 years	31	≤50	≤50 - 107	0/0*
≥ 2 years to < 12 years	64	≤50	≤50 - 67	1 / 1.56*
≥ 12 years to < 22 years	64	≤50	≤50 - 101	1 / 1.56*

**Elevated NGAL values might be attributed to a borderline UTI result which did not justify the exclusion of the subject from the reference range study, as per predefined exclusion criteria in the study protocol.*

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.