December 7, 2023

BioPorto Diagnostic Inc.
Monika Bak
Director Regulatory Affairs
Tuborg Havnevej 15, st
Hellerup, 2900 Denmark

Re: K232761
Trade/Device Name: ProNephro AKI™ (NGAL)
Regulation Number: 21 CFR 862.1220
Regulation Name: Acute Kidney Injury Test System
Regulatory Class: Class II
Product Code: PIG
Dated: September 8, 2023
Received: September 8, 2023

Dear Monika Bak:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-torres -S

Marianela Perez-Torres, PhD
Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K232761

Device Name
ProNephro AKI™ (NGAL)

Indications for Use *(Describe)*
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 AKI (Stage 2/3) 48 to 72 hours after the 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary

ProNephro AKI™ (NGAL)

BioPorto Diagnostics Inc.
117 Fourth Avenue, Suite 202
Needham, MA 02494 USA

1. General Information

Submission Date: September 08, 2023

Submitter Information

Submitted By: BioPorto Diagnostics Inc.
117 Fourth Avenue Suite 202
Needham MA 02494
United States

Contact Person: Monika Bak
Director Regulatory Affairs
mbk@bioporto.com

510(k): K232761

2. Purpose for Submission

To obtain a substantial equivalence determination for the ProNephro AKI™ (NGAL) in a pediatric population (≥ 3 months to < 22 years) admitted to the pediatric intensive care unit (PICU).

3. Measurand

Neutrophil gelatinase-associated lipocalin (NGAL)

4. Type of Test

Quantitative, particle-enhanced turbidimetric immunoassay

5. Applicant

BioPorto Diagnostics Inc.

6. Proprietary and Established Names

ProNephro AKI™ (NGAL)

7. Regulatory Information

Assay

Trade Name:	ProNephro AKI™ (NGAL)
Classification:	Class II, 510(k) required
Regulation:	21 CFR 862.1220
Regulation Name:	Acute Kidney Injury Test System
Product Code:	PIG
Panel:	Clinical Chemistry

8. Indications for Use

8.1. Indications 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 AKI (Stage 2/3) 48 to 72 hours after the 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.

8.2. Special Conditions for Use Statement(s)

For in Vitro Diagnostics Use and Rx only

Warnings and Precautions for the Assay

- This kit should only be used by qualified laboratory staff.
- Do not shake the reagents.
- Use only clean containers if transferring reagents.
- Do not pour reagents back into the original containers once transferred.
- All specimens used in this test should be considered potentially infectious, and as such be treated with standard precautions.
- Powder-free gloves should be worn. Avoid direct skin contact. Should reagents come into contact with the skin, eyes or mouth, flush with plenty of water. Seek medical advice if any symptom is observed or when it is considered necessary.

- Reagents contain preservatives with 2-methyl-2H isothiazol-3-one (MIT) which may cause an allergic skin reaction. For more information, please refer to the safety data sheet.
- Do not switch caps on reagent containers as it may cause contamination or mix-up.
- Place calibrators in the correct order onto the instrument to ensure a correct calibration curve.
- Do not use reagents after the expiration date on the labels.
- Reagents with different lot numbers should not be mixed.
- All solutions and samples must be handled carefully and disposed of in accordance with national and local regulations.

8.3. Special Instrument Requirements

Roche cobas® c 501 clinical chemistry analyzer

9. Device Description

Table 1 describes the components required to run a ProNephro AKI™ (NGAL) test.

The ProNephro AKI™ (NGAL) Reagent Kit contains Reaction Buffer Reagent (R1) and Immunoparticle Suspension Reagent (R2). The R1 reagent is a ready-to-use tris-buffer solution containing murine protein and preservative. The R2 reagent is 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 finished kit will also include 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.

The ProNephro AKI™ (NGAL) Calibrator Kit consists of five (5) individual ready-to-use calibrator solutions (1 mL each) comprised of different concentrations (50-3000 ng/mL) of recombinant human NGAL in a HEPES buffer and a preservative.

The ProNephro AKI™ (NGAL) Control Kit contains ready-to-use High (500 ng/mL target concentration) and Low (200 ng/mL target concentration) Controls comprised of recombinant human NGAL in HEPES buffer and a preservative. There are three (3) 1 mL bottles of each level included with the kit.

Table 1: Test Components

Product Trade Name and Model Number	Kit Components
ProNephro AKI™ (NGAL) Reagent Kit ST101UB	Reaction Buffer (R1), 17.9 mL Immunoparticle Suspension (R2), 7.65 mL Roche cobas c 501 cassette and two funnels
ProNephro AKI™ (NGAL) Calibrator Kit ST102UA	Calibrator 1: 1 mL Calibrator 2: 1 mL Calibrator 3: 1 mL Calibrator 4: 1 mL Calibrator 5: 1 mL
ProNephro AKI™ (NGAL) Control Kit ST103UA	Control Low: 3 x1 mL Control High: 3 x1 mL

10. Substantial Equivalence Information

10.1. Predicate Device Name(s)

NEPHROCHECK® Test System

10.2. Predicate 510(k) Number(s)

DEN130031

10.3. Comparison With Predicate

Table 2: Predicate Device Comparison

Item	ProNephro AKI™ (NGAL)	NEPHROCHECK® Test System (Predicate Device)
K Number	K232761	DEN130031
Intended Use / Indications for Use	INDICATIONS 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 AKI (Stage 2/3) 48 to 72 hours after the 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.	INTENDED USE: The Astute Medical NEPHROCHECK® Test System is intended to be used in conjunction with clinical evaluation in patients who currently have or have had within the past 24 hours acute cardiovascular and or respiratory compromise and are ICU patients as an aid in the risk assessment for moderate or severe acute kidney injury (AKI) within 12 hours of patient assessment. The NEPHROCHECK® Test System is intended to be used in patients 21 years of age or older.
Classification	Class II	Same
Product Code	PIG	Same
Test Type	Quantitative immunoassay	Same
Test Technology	Particle Enhanced Turbidimetric assay	Lateral Flow with fluorescent detection

Item	ProNephro AKI™ (NGAL)	NEPHROCHECK® Test System (Predicate Device)
Instrument	Roche cobas c 501 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
Units of Measure	ng/mL	AKI Risk Score
Assay Measuring Range	50 ng/mL – 3000 ng/mL	Score 0.04 – 10.0
Specimen Type	Urine	Same
Controls	2 levels of controls: low and high	Same
Calibrators	N=5 levels across the measuring range of the assay	Same
Special Controls	Complies	Same
Repeatability (within-run) and Intermediate Precision (within-laboratory)	Repeatability (within-run): • 3.8 to 5% below the cutoff (125 ng/mL) • 0.9 to 2.3% from 125 to 500 ng/mL • 1.2 to 1.5% above 500 ng/mL Intermediate Precision (within-laboratory): • 4.9 to 6.6% below the cutoff (125 ng/mL) • 2.2 to 3.4% from 125 to 500 ng/mL • 2.2 to 2.7% above 500 ng/mL	Repeatability (within-run): • 9.5 to 9.7% below the cutoff score (0.3) • 7.4 to 7.8% above the cutoff score (0.3) Intermediate precision (within-laboratory): • 10.2 to 11% below the cutoff score (0.3) • 8.0 to 10.2% above the cutoff score (0.3)
Reproducibility (multi-site)	Total Imprecision: • 6.2 to 6.6% below the cutoff (125 ng/mL) • 3.6 to 4.8% from 125 to 500 ng/mL • 3.6 to 4.3% above 500 ng/mL	Total Imprecision: • 10.4 to 18.3% below and at the cutoff score (0.3) • 9.1 to 11.7% above the cutoff score (0.3)
Analytical Sensitivity	LoB = 9 ng/mL LoD = 14 ng/mL LoQ = 18 ng/mL	LoB = 0.0002 LoD = 0.002 LoQ = 0.002
Linearity	Linearity confirmed between 49 and 3105 ng/mL	The reportable range of the NEPHROCHECK AKI Risk Score is 0.4 to 10.0. The markers that go into the score were assessed and found to be linear. However, the AKI Risk Score itself is not expected to be linear.
High Dose Hook Effect	No Hook Effect at NGAL concentrations up to 40,000 ng/mL	No Hook Effect at AKI Risk Score values up to 250.

Item	ProNephro AKI™ (NGAL)	NEPHROCHECK® Test System (Predicate Device)
Clinical Performance	**Complete Study Population (n=514):** Sensitivity (CI 95%): 72.3% (57.4-84.4%) Specificity (CI 95%): 86.3% (82.8-89.3%) PPV (CI 95%): 34.7% (28.5-41.4%) NPV (CI 95%): 96.9% (95.1-98-0%) **Group 1 – patients with Day 0 SCr indicative of no AKI or AKI Stage 1 (n=422):** Sensitivity (CI 95%): 61.1% (35.8-82.7%) Specificity (CI 95%): 88.6% (85.1-91.5%) PPV (CI 95%): 19.3% (10.1-31.9%) NPV (CI 95%): 98.1% (96.1-99.2%) **Group 2 – patients with Day 0 SCr indicative of AKI Stage 2/3 (n=92)** Sensitivity (CI 95%): 79.3% (60.3-92.0%) Specificity (CI 95%): 71.4% (58.7-82.1%) PPV (CI 95%): 56.1% (39.8-71.5%) NPV (CI 95%): 88.2% (76.1-95.6%)	Sensitivity (CI 95%): Study A, tested in 3 laboratories: 90-93% (83-99%) Study B, tested in 1 laboratory: 76% (60-91%) Specificity (CI 95%): Study A, tested in 3 laboratories: 45-49% (39-54%) Study B, tested in 1 laboratory: 51% (41-60%) PPV (CI 95%): Study A, tested in 3 laboratories: 26-27% (21-33%) Study B, tested in 1 laboratory: 31% (21-42%) NPV (CI 95%): Study A, tested in 3 laboratories: 96-97% (93-100%) Study B, tested in 1 laboratory: 88% (79-96%)

11. 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)]

12. Test Principle

ProNephro AKI (NGAL) is a particle-enhanced turbidimetric immunoassay for the quantitative determination of NGAL in pediatric urine. A urine sample is mixed with reaction buffer. After a short incubation, the reaction is started by the addition of an immunoparticle suspension (polystyrene microparticles coated with mouse monoclonal antibodies to NGAL). NGAL in the sample causes the immunoparticles to aggregate. The degree of aggregation is proportional to the loss of intensity of transmitted light, due to the scattering effect of particles suspended in solution. The NGAL concentration in the sample is determined by interpolation on an established calibration curve.

13. Performance Data – Analytical Studies

13.1. Precision

13.1.1. Repeatability and Intermediate Precision

Precision measurements were conducted to evaluate the repeatability (within-run precision) and intermediate precision (within-laboratory precision) according to CLSI guideline EP05-03. The protocol consisted of testing seven native pediatric urine samples and sample pools and two controls with three lots of reagent for 20 days with two runs per day and three replicates per sample (n=360/sample) on one cobas c 501 analyzer.

Table 3: Repeatability and Intermediated Precision Results

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

Table 4: Lot-to-Lot Imprecision

		Within-Lot*		Between-Lot	
	Mean (ng/mL)	Std Dev (ng/mL)	CV (%)	Std Dev (ng/mL)	CV (%)
Control Low	203	6.4	3.1	0.0	0.0
Control High	498	11.7	2.3	0.0	0.0
Ped. Urine 1	71	4.2	5.9	2.1	3.0
Ped. Urine 2	100	4.1	4.1	2.0	2.0
Ped. Urine 3	102	4.6	4.5	2.1	2.0
Ped. Urine 4	142	4.5	3.2	1.9	1.3
Ped. Urine 5	165	5.0	3.1	1.1	0.7
Ped. Urine 6	304	6.4	2.1	3.5	1.1
Ped. Urine 7	512	9.7	1.9	5.5	1.1
Ped. Urine 8	1049	19.7	1.9	11.3	1.1
Ped. Urine 9	2776	61.5	2.2	43.3	1.6

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

13.1.2. Reproducibility

Precision measurements were conducted to evaluate the reproducibility according to CLSI guideline EP05-03. 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 at three sites (one internal, two external) on one cobas c 501 analyzer per site and two operators per site. 30 data points were generated per sample and per site.

Table 5: Reproducibility Summary, per Site and Overall

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

13.2. Analytical Sensitivity

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with CLSI EP17-A2 requirements.

The Limit of Blank, Limit of Detection and Limit of Quantitation were below the lower limit of the measuring range.

The Limit of Blank is determined as the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95% (empirical value determined on the cobas c 501 analyzer: 9 ng/mL).

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentrated samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95%) (empirical value determined on the cobas c 501 analyzer: 14 ng/mL).

The Limit of Quantitation is the lowest analyte concentration that can be accurately quantified with a CV ≤ 20%. It has been determined using samples with low NGAL concentrations (empirical value determined on the cobas c 501 analyzer: 18 ng/mL).

13.3. Linearity

The linearity study was conducted to demonstrate that measurements across the claimed measuring range are linear. The study was performed in accordance with CLSI guideline EP06 2nd edition. Three linearity series were tested, each consisting of one pediatric urine samples pool with a high NGAL concentration (above the upper limit of the measuring range) diluted with one pediatric urine sample pool with a low NGAL concentration (below the lower limit of the measuring range) to create 14 samples per series, with concentrations covering and extending the measuring range. Each series was tested in one run with one reagent lot on one cobas c 501 analyzer.

The data support the claim that ProNephro AKI™ (NGAL) measurements are linear between 50 and 3000 ng/mL.

13.4. High-Dose Hook Effect

High-dose hook effect was evaluated on a single Roche cobas c 501 analyzer. The protocol consisted of testing two native pediatric samples with concentrations of approximately 40,000 ng/mL, using one reagent lot and four replicates per sample.

No sample showed a result that fell within the measuring range. No high-dose hook effect was observed up to 40,000 ng/mL NGAL.

13.5. Interference

The effect of the following endogenous substances, contrast agents, pH, and common and special pharmaceutical compounds on the assay's performance were tested. The protocol consisted of testing two pediatric samples pools with NGAL concentrations of approximately 150 ng/mL, and approximately 1,500 ng/mL, respectively, on one cobas c 501 analyzer with one reagent lot and six replicates for the lower sample, and four replicates for the higher sample. Interferences were tested up to the listed concentrations and showed non-significant interference.

Criterion: Recovery within ± 10% of initial value

Table 6: Endogenous Substances

Compound	Concentration (mg/L)
Albumin	60,000
Ammonium Chloride	2,000
Bilirubin, conjugated	800
Calcium Chloride	2,500
Citric Acid	2,500
Creatinine	10,000
Glucose	14,000
Hemoglobin	2,500
Immunoglobulin G	1,100
Magnesium	2,000
Oxalic Acid	300
Sodium Phosphate	4,000
Urea	38,000
Uric Acid	1,400
Urobilinogen	150

Table 7: Contrast Agents

Compound	Concentration (g/L)
Visipaque / Iodixanol	20
Omnipaque / Iohexol	60
Optiray / Ioversol	100

Sample pH: pH-values between 3.3 and 10.3 were tested and showed non-significant interference.

Table 8: Common Drugs

Compound	Concentration (mg/L)
Acetaminophen	3,000
Ascorbic Acid	4,000
N-Acetylcysteine	150
Ibuprofen	4,000
Levodopa	1,000
Methyldopa	2,000
Phenazopyridine	300
Salicyluric Acid	6,000

Table 9: Special Drugs and Immunosuppressive Agents

Compound	Concentration (mg/L)
Cefazolin	20,000
Cefepime	65,000
Ceftriaxone	90,000
Cyclosporine	200
Diazepam	500
Diphenhydramine	15,000
Everolimus	5
Famotidine	250
Fentanyl	100
Furosemide	3,500
Hydromorphone	300
Ketamine	2,500
Ketorolac	900
Levetiracetam	25,000
Lorazepam	300
Midazolam	3,000
Morphine	4,000
Mycophenolate Mofetil	1,000
Mycophenolic Acid Glucuronide (MPAG)	20,000
Ondansetron	500
Oxycodone	1,500
Piperacillin / Tazobactam	10,000 / 1,250
Prednisone	3
Prednisolone	3
Tacrolimus	2,5
Vancomycin	20,000

Cefepime was shown to interfere at concentrations above 4,062.5 mg/L. The maximum daily dose of Cefepime in pediatrics is 150 mg/kg and the calculated maximum concentration in the urine could be up to 6 g/L, depending on the age, weight, and urine output of the patient. The labeling will include a statement that patients being treated with Cefepime may have lower than expected NGAL results as interference was shown above a Cefepime concentration of 4 g/L.

13.6. Analytical Specificity / Cross-Reactivity

Cross-reactivity studies were performed to determine if other biomarkers that have been reported to be elevated for patients with AKI interfere with ProNephro AKI (NGAL). The testing was performed in alignment with CLSI EP07-A3 requirements. The protocol consisted of testing two pediatric sample pools with NGAL concentrations of approximately 150 ng/mL, and approximately 1,500 ng/mL on one cobas c 501 analyzer with one reagent lot and six replicates for the lower sample, and four replicates for the higher sample. Cross-reactivity was tested up to the listed concentrations and no impact on results was observed.

Criterion: Recovery within ± 10% of initial value

Table 10: Potential Cross Reactants

Compound	Concentration (mg/L)
Alpha 1 Microglobulin (α1M)	1,000
Beta 2 Microglobulin (β2M)	15
Cystatin C	8
Interleukin 18	0.001
Kidney Injury Molecule 1 (KIM-1)	0.015
Liver Type Fatty Acid Binding Protein (L-FABP)	0.6
N-acetyl H3-D-glucosaminidase (NAG)	15 U/L
Pi-Glutathione s-Transferase (p-GST)	0.5
Insulin-like Growth Factor binding protein (IGFBP-7)	0.03
Tissue inhibitor of Metalloproteinase 2 (TIMP-2)	0.01

13.7. Specimen Stability

Analyte stability in pediatric urine samples was studied. Samples remain stable:

- 1 day at 15-30°C
- 3 days at 2-8°C
- 12 months at or below -70°C, up to 2 freeze/thaw cycles

13.8. Reagent Stability and Shelf-Life

Reagent stability and shelf-life studies were performed. The results of the reagent stability studies support the claims as reported in the IFU. The results for the shelf-life studies support a claim of 18 months.

14. Clinical Studies

14.1. Reference Range

In a multi-center, cross sectional, prospectively enrolled reference range study, the ProNephro AKI™ (NGAL) test was used on urine samples from 348 apparently healthy pediatric subjects between ≥ 90 days to < 22 years of age.

The majority of samples in this reference range study yielded results below the lower limit of the measuring range of 50 ng/mL. Even though the true concentration of these samples could be far below 50 ng/mL, the results were reported as "50 ng/mL" for the statistical evaluations.

The following results were obtained for the central 95% reference intervals:

Table 11: Summary of Results from the Reference Range Study

Age group ***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

Age group ***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*

14.2. Clinical Performance

A multi-center prospective clinical study called EARNEST was conducted at 6 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.

The clinical performance of ProNephro AKI™ (NGAL) was validated in a multi-center prospective clinical study called GUIDANCE 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 clinical performance evaluation was performed with 660 pediatric patients (≥ 90 days to < 22 years of

age) were enrolled at 15 sites across the US, with 514 evaluable subjects. The clinical data for each subject were captured and provided to at least two independent clinical experts for adjudication. The clinical performance of ProNephro AKI (NGAL) was evaluated against the adjudicated results.

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 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 hrs 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 hrs 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 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.

Table 11: Group 1 - GUIDANCE Study Results in Patients with Day 0 SCr indicative of no AKI or AKI stage 1 (n=422)

	AKI+	AKI-	**Total**
NGAL+	11	46	**57**
NGAL-	7	358	**365**
Total	**18**	**404**	**422**
Prevalence	18/422 (4.3%)		
Sensitivity (95 CI)	61.1% (35.8 - 82.7%)		
Specificity (95 CI)	88.6% (85.1 – 91.5%)		
Positive Predictive Value (95 CI)	19.3% (10.1 – 31.9%)		
Negative Predictive Value (95 CI)	98.1% (96.1 – 99.2%)		
Risk of AKI for NGAL negative Results	1.9%		
Risk of AKI for NGAL positive Results	19.3%		

Table 12: Group 2 - GUIDANCE Study Results in Patients with Day 0 SCr indicative of AKI stage 2/3 (n=92)

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

15. Proposed Labeling

The labeling satisfies the requirements of 21 CFR Parts 801 and 809 as well as the special controls for an Acute Kidney Injury Test System.

16. Conclusion

The complete results of the clinical and analytical studies submitted in this 510(k) premarket notification demonstrate that the ProNephro AKI™ (NGAL) test is substantially equivalent to the predicate device.