EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR NEPHROCHECK® Test System

DECISION SUMMARY

A. DEN Number:

DEN130031

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the NEPHROCHECK® Test System.

C. Measurand:

The test reports an AKIRisk score derived from the measurement of Insulin-like growth factor-binding protein (IGFBP7) and tissue-inhibitor of metalloproteinases 2 (TIMP2).

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Astute Medical, Inc.

F. Proprietary and Established Names:

NEPHROCHECK® Test System

G. Regulatory Information:

1. New Regulation section:

   21 CFR 862.1220

2. Classification:

   Class II

3. Product code:

   PIG

4. Panel:

   75, Clinical Chemistry

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   Test results should be evaluated with other clinical and laboratory test information.

   Test results are not to be used as a standalone test.

   Test results should be used in patients 21 years of age and older.

   For prescription use only.

   This test system is for central laboratory use only. It is not for point-of-care use.

   Subject to special controls under regulation 21 CFR 862.1220

4. Special instrument requirements:

   ASTUTE140® Meter

I. Device Description:

The NEPHROCHECK® Test System is comprised of the NEPHROCHECK® Test Kit, the ASTUTE140 Meter, the NEPHROCHECK® Liquid Controls Kit, and the NEPHROCHECK® Calibration Verification (Cal Vers) Materials Kit.

The NEPHROCHECK® Test Kit includes the NEPHROCHECK® Test which is a single-use cartridge comprised of two immunoassays for the protein biomarkers insulin-like growth factor-binding protein (IGFBP7) and tissue-inhibitor of metalloproteinases 2 (TIMP2), on a membrane test strip enclosed in a plastic housing. Internal positive and negative procedural controls in each NEPHROCHECK® Test cartridge monitor the function of each test cartridge. If the automatic check of these procedural controls shows that the control value results are not within pre-defined limits, the Meter will display an error message and the Test result will not be reported. The concentrations of the TIMP-2 and IGFBP-7 proteins are used to derive the AKIRisk Score and these concentrations are not reported.

Also included in the kit is test buffer and the NEPHROCHECK® Test Conjugate Vial which contains murine monoclonal and goat polyclonal antibodies against TIMP-2 and IGFBP-7, fluorescent dye, stabilizers and excipients. A RFID Card that contains lot and calibration information is included with each kit. The RFID card must be loaded prior to using a new kit. Each kit can perform 25 tests.

The ASTUTE140® Meter is a bench-top analyzer that converts the fluorescent signal from each of the two immunoassays contained within the NEPHROCHECK® Test cartridge into the AKIRisk score. Only the AKIRisk score appears on the meter display. The ASTUTE140® Meter contains an internal printer that can print the AKIRisk score

The NEPHROCHECK® Low Liquid Control and NEPHROCHECK® High Liquid Control are bi-level, lyophilized control materials prepared from human urine containing human TIMP-2 and human IGFBP-7 proteins with protein stabilizers. TIMP-2 and IGFBP-7 proteins have been added to the urine to achieve specified target concentration levels. The expected concentrations and standard deviations are printed on the enclosed RFID cards. Each NEPHROCHECK® Liquid Control Kit Vial is intended for single use only.

The NEPHROCHECK® Calibration Verification Kit includes five levels of lyophilized material prepared from human urine, containing TIMP-2 and human IGFBP-7 to achieve specified target concentration levels that evenly span the reportable ranges of the AKIRisk Score. The expected concentrations and standard deviations of the individual biomarkers are embedded on a RFID card enclosed with the NEPHROCHECK® Calibration Verification Kit.

All human source material used to manufacture NEPHROCHECK® Liquid Controls and the NEPHROCHECK® Calibration Verification Kit was non-reactive for antigens to Hepatitis B (HBsAg), negative by tests for antibodies to HIV (HIV-1/HIV-s) and Hepatitis C (HCV), non-reactive for HIV-1 RNA and HCV RNA by licensed NAT, and non-reactive to Serological Test for Syphilis (STS) using testing methods approved by the FDA.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Not applicable

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

   Not applicable

3. Comparison with predicate:

   Not applicable

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

CLSI EP 5A-2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition

CLSI EP 6A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

CLSI EP25-A: Evaluation of Stability of In Vitro diagnostic Reagents; Approved Guideline

CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

L. Test Principle:

The NEPHROCHECK® Test is a sandwich fluorescent immunoassay. Test Buffer Solution and centrifuged urine supernatant are manually added by the operator to the Test Conjugate Vial containing the labeled fluorescent conjugate. A 100 µL aliquot of the urine/fluorescent conjugate mixture is dispensed into the sample port on the NEPHROCHECK® Test cartridge where it diffuses across a membrane containing the capture antibodies for TIMP-2 and IGFBP-7. After a brief waiting period, the NEPHROCHECK® Test Cartridge is inserted into the ASTUTE140® Meter for incubation, reading, result calculation and result display. The fluorescent signals from both biomarkers are incorporated into an algorithm to derive the AKIRisk Score. The NEPHROCHECK® Test result is displayed as a single value (AKIRisk score). The AKIRisk Score result is displayed on the Meter LCD screen in approximately 20 minutes from the addition of the specimen.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   Precision of the NEPHROCHECK® Test AKIRisk Score was evaluated using a 20 day precision study conducted at 3 clinical laboratories (1 internal, 2 external sites) according to CLSI Guideline EP5-A2 using 6 urine samples which include samples around the AKIRisk Score cutoff (Samples S3, S4, and S5) and samples to span the range of the AKIRisk Score (Samples S2, S6 and S7). All samples were tested 2 times a day in replicates of 2 using 3 reagent lots (1 per site) for 20 days (N=80 per sample for each lot/site). Precision results are provided below for all sites/lots combined, as well as for each individual site/lot.

All Sites/Lots

Test Specimen	Risk Score Mean	Within-Run		Between Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
S2	0.04	0.006	15	0.0	0.0	0.004	9.8	0.007	18.3
S3	0.14	0.011	8.1	0.004	3.2	0.009	6.8	0.015	11.0
S4	0.30	0.027	9.0	0.006	1.8	0.001	4.9	0.031	10.4
S5	0.56	0.048	8.5	0.0	0.0	0.039	6.9	0.061	10.9
S6	4.61	0.335	7.3	0.205	4.5	0.141	3.1	0.418	9.1
S7	8.55	0.844	9.9	0.217	2.5	0.493	5.8	1.001	11.7

Site 1/Lot 1

Test Specimen	Risk Score Mean	Within-Run		Between Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
S2	0.04	0.008	18.3	0.0	0.0	0.001	2.1	0.008	18.5
S3	0.13	0.012	9.5	0.007	5.1	0.006	4.7	0.015	11.8
S4	0.31	0.030	9.8	0.013	4.5	0.009	3.1	0.034	11.2
S5	0.53	0.059	11.2	0.0	0.0	0.025	4.7	0.064	12.1
S6	4.60	0.448	9.7	0.172	3.7	0.0	0.0	0.480	10.4
S7	8.552	0.894	10.5	0.0	0.0	0.262	3.1	0.932	10.9

Site 2/Lot 2

Test Specimen	Risk Score Mean	Within-Run		Between Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
S2	0.04	0.005	11.8	0.0	0.0	0.003	6.4	0.005	13.4
S3	0.15	0.011	7.4	0.004	2.4	0.005	3.7	0.013	8.6
S4	0.32	0.022	7.1	0.010	3.2	0.0	0.0	0.025	7.8
S5	0.55	0.046	8.3	0.020	3.6	0.030	5.4	0.058	10.5
S6	4.64	0.260	5.6	0.0	0.0	0.31	6.6	0.401	8.6
S7	8.10	0.544	6.7	0.147	1.8	0.502	6.2	0.754	9.3

Site 3/Lot 3

Test Specimen	Risk Score Mean	Within-Run		Between Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
S2	0.04	0.005	12.7	0.0	0.0	0.001	3.5	0.005	13.2
S3	0.13	0.010	7.3	0.002	1.0	0.005	3.9	0.011	8.4
S4	0.29	0.029	10.0	0.0	0.0	0.009	3.2	0.030	10.5
S5	0.60	0.035	5.9	0.0	0.0	0.022	3.6	0.042	6.9
S6	4.58	0.263	5.7	0.313	6.8	0.0	0.0	0.408	8.9
S7	9.04	1.017	11.2	0.351	3.9	0.0	0.0	1.076	11.9

An additional in-house 20 day precision study was conducted according to CLSI Guideline EP5-A2 using the NEPHROCHECK® Calibration Verifiers (5 levels) and the NEPHROCHECK® Liquid Controls (2 levels). All samples were tested 2 times a day in replicates of 2 using 1 reagent lots for 20 days (N=80). Precision results are

provided below.

Test Specimen	Risk Score Mean	Within-Run		Between Run		Between Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Cal Ver 1	0.09	0.10	9.7	0.0	0.0	0.0	5.1	0.10	11.0
Cal Ver 2	0.28	0.03	9.5	0.0	0.0	0.01	3.6	0.03	10.2
Cal Ver 3	0.96	0.07	7.7	0.03	3.4	0.02	1.8	0.08	8.6
Cal Ver 4	2.92	0.23	7.8	0.13	4.6	0.14	4.8	0.30	10.2
Cal Ver 5	6.67	0.49	7.3	0.18	2.7	0.12	1.8	0.53	8.0
Low Control	0.32	0.02	7.6	0.01	3.9	0.0	0.0	0.03	8.5
High Control	3.52	0.26	7.4	0.0	0.0	0.16	4.4	0.30	8.6

b. Linearity/assay reportable range:

The reportable range of the NEPHROCHECK® AKIRisk Score is 0.04 – 10.0. The markers that go into the score were assessed and found to be linear. However, the AKIRisk Score itself is not expected to be linear.

Hook effect was evaluated at AKIRisk Score values up to 250. High AKIRisk Score samples were evaluated in replicates of 28. Hook effect was defined as results falling below the peak values of the highest calibrator. No high dose hook effect was observed for AKIRisk Score at these values.

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

Traceability:

The Astute140 Meter is factory calibrated. ASTUTE140® Meter calibration is based on two parameters: fluorescent intensity and linear positioning of the Meter's optics.

Internal standard solutions were developed by the sponsor using purified TIMP-2 and IGFBP-7. These standard solutions are used to make secondary calibrator materials for standardizing the production of the NEPHROCHECK® Test kits. The secondary calibrator materials are used to make working calibrators that are used to verify NEPHROCHECK® Control and Cal Vers values.

Value Assignment: Controls and Cal Vers:

The Cal Vers and Controls which are comprised of the two biomarkers are prepared gravimetrically. The Cal Vers and Controls are value assigned using the secondary calibrators. Individual biomarker values for each lot are embedded on a RFID card that is specific for each lot number.

The NEPHROCHECK® test kit open vial stability and the test cartridge open pouch stability protocols were reviewed and found acceptable. The results

support test kit stability of 16 weeks. Results support open pouch test cartridge stability of 30-50% RH and 64-77°F (18-25°C) for 30 minutes.

The real time stability study protocol and acceptance criteria were reviewed for the NEPHROCHECK® Controls and Cal Verifiers and found acceptable. Results support stability of 16 weeks at -4 to 39.2°F to (-20 to 4°C). Open vial stability study protocol and acceptance criteria for the NEPHROCHECK® Controls and NEPHROCHECK® Calibration Verifiers were reviewed and found acceptable. Results support open vial stability for the Controls and Cal Vers is 24 hours.

The study protocol and acceptance criteria for sample stability was reviewed and found acceptable. Results support stability of the urine supernatant up to 5 hours at 64.4-77°F (18-25°C).

d. Detection limit:

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were performed on three lots of NEPHROCHECK® test kits in accordance with CLSI EP17-A.

LoB Test Protocol
LoB was evaluated for AKIRisk Score using 3 urine samples that were treated to remove native levels of TIMP-2 and IGFBP-7. The samples were analyzed from 6 runs over 3 days for a total of 72 replicates per lot. The LoB was defined as the concentration at which there is a 95% probability that the sample does not yield an AKIRisk Score due to the absence of TIMP-2 and IGFBP-7.

LoD Test Protocol
LoD was evaluated on 6 low samples derived from apparently healthy donors with AKIRisk Score values ranging from the LoB to 4 times the LoB using 36 NEPHROCHECK® Tests from 3 lots for a total of 216 replicates. The LoD was calculated non-parametrically.

LoQ Test Protocol
LoQ was determined by comparing the LoD study results against limits developed to ensure quantitative AKIRisk Score test results. Results demonstrated that the total error is less than the LoD. Therefore, the LoQ = LoD.

The studies support the following limits of detection for the AKIRisk Score:

LoB = 0.0002
LoD = 0.002
LoQ = 0.002

The reportable range of the AKIRisk Score is 0.04 – 10.0. The LoQ is below the reportable range. Therefore, this LoQ supports the analytical validity of the NEPHROCHECK® test at the clinical cutoff of > 0.3 AKIRisk Score and for the claimed reportable range.

e. Analytical specificity:

Interference studies

Interference studies were conducted according to CLSI EP-7A2 using pooled human urine samples to determine whether the presence of potential interferents may interfere with the AKIRisk Score test results. Interferents were selected from medications, contrast agents, plasma expanders, other urine constituents, isoforms of TIMP and IGFBP, and other biomarkers which have been reported as elevated in the literature in AKI. The endogenous urine constituents were evaluated at 3 concentrations (low, mid-range and high). Exogenous substances were tested at low and high concentrations that were determined as the maximum amount eliminated in 24 hrs (low) and three times this concentration (high). Where the urinary excretion information was not available from literature, or other sources (specifically the drugs), the concentrations were determined as the maximum therapeutic dosage per liter of urine (low) and at three times this concentration (high). All other substances were evaluated at low and high concentrations. All samples were tested in replicates of 32, and the recovery of the AKIRisk Score in the presence of possible interferents calculated in comparison to the corresponding control sample. Interference was defined as any interferents that exhibited >±10% interference in the AKIRisk Score test result.

The tables below summarize the results.

Contrast Agents

Test Substance	High Test Conc. mg/L	Interference
Visipaque/Iodixanol	4,941	No
Omniscan/Gadodiamide	177	No
Omnipaque/Iohexol	14,085	No
Magnevist/Gadopentate	422	No
Optiray/Ioversol	4,944	No

Plasma Expanders

Test Substance	High Test Conc. mg/L	Interference
Dextran 40	22.2	No
Pentastarch	8.9	No
Hetastarch	5.9	No

Drugs and Other Substances

Test Substance	High Test Conc. mg/L	Significant Interference
Acetaminophen	200.72	No
Acetone	696.96	No
Acetylcysteine	1,664.54	No
Acetyl salicylic acid (Aspirin)	652.18	No
Acyclovir	52	No

Albumin	**1,250**	**Yes**
Albuterol	0.40	No
Amiodarone	6.08	No
Ammonia	1,000	No
Amoxicillin	75.27	No
Amphotericin	81.9	No
Ascorbic acid	29.94	No
Atorvastatin	80	No
Bicarbonates	2940	No
Bilirubin, conjugated	**72.07**	**Yes**
Bumetanide	30	No
Caffeine	59.81	No
Caspofungin	86.1	No
Cefepime	9.36	No
Ceftriaxone	810.30	No
Cephalexin	116.94	No
Ciprofloxacin	10.01	No
Clopidoqrel	225	No
Dexmedetomidine (Precidex)	0.21	No
Diltiazem (Cardizem)	43.2	No
Dopamine	0.9	No
Doripenem (Dobrimax)	1,050	No
Epinephrine	6	No
Ethacrynic acid	19.4	No
Ethanol	4,000	No
Fenoldopam	483.84	No
Fentanyl	100	No
Fluconazole	74.97	No
Fluvastatin	80	No
Furosemide	59.87	No
Gentamicin	10.04	No
Glucose	9,908.8	No
Hemoglobin	2000	No
Heparin	21.42	No
Hydralazine	600	No
Hydrochlorothiazide	6.02	No
Hydrocodone	0.20	No
Hydrocortisone	720	No
Ibuprofen	500.25	No
Insulin	0.003	No
Ketorolac	165.6	No
Lansoprazole	90	No
Linezolid	48	No
Lisinopril	0.30	No
Lorazepam	1.00	No
Low Molecular Weight Heparin	30	No
Mannitol	600	No

Metformin	39.99	No
Methylene blue	**3.89**	**Yes**
Metolazone	60	No
Metoprolol	5	No
Metronidazole	119.87	No
Midazolam	1.14	No
Morphine	0.5	No
Moxifloxacin	1200	No
Nitroglycerin	0.0237	No
Norepinephrine	204	No
Omeprazole	6.00	No
Ondanesteron	0.11	No
Pancuronium	8.4	No
Pantoprazole (Protonix)	85.2	No
Phenobarbital	100.10	No
Phenylephrine	30	No
Pravastatin	80	No
Prednisone (Prednisolone)	3.00	No
Propofol	16.01	No
Ranitidine	6.00	No
Riboflavin	12	No
Rocuronium	126	No
Theophylline	39.96	No
Tobramycin	24.06	No
Torsemide	12	No
Urobilinogen, synthetic	12	No
Valproic Acid	499.25	No
Vancomycin	100	No
Vasopressin	5	No
Vecuronium	21	No
Warfarin (Coumadin)	10.02	No

Common Urine Constituents

Test Substance	High Test Conc. mg/L	Significant Interference
Calcium	600	No
Chloride	5,600	No
Creatinine	1,800	No
Magnesium	240	No
Phosphate	2,800	No
Potassium	4,000	No
Sodium	3,600	No
Sulfate	4,800	No
Urea	23,000	No
Uric Acid	700	No

Potential Cross-Reactive Proteins

Protein	High Test Conc. mg/L	Significant Interference
Insulin like growth factor-Binding Protein 1 (IGFBP-1)	0.1	No
Insulin like growth factor-Binding Protein 2 (IGFBP-2)	0.25	No
Insulin like growth factor-Binding Protein 3 (IGFBP-3)	1.2	No
Insulin like growth factor-Binding Protein 4 (IGFBP-4)	1.2	No
Insulin like growth factor-Binding Protein 5 (IGFBP-5)	1.2	No
Insulin like growth factor-Binding Protein 6 (IGFBP-6)	1.2	No
Insulin like growth factor 1 (IGF-1)	1.5	No
Insulin like growth factor 2 {IGF-2)	1.5	No
Cysteine-rich motor neuron 1 protein (CRIM1)	1.2	No
Agrin	1.2	No
Serine protease HTRA1 (HTRA1)	1.2	No
Insulin-like growth factor binding protein-like 1 (IGFBP-1) (IGFBPL-1 (IGFBPL1)	1.2	No
Metalloproteinase inhibitor 1 (TIMP-1)	3	No
Metalloproteinase inhibitor 3 (TIMP-3)	2.5	No
Metalloproteinase inhibitor 4 (TIMP-4)	0.6	No
matrix metalloproteinase-2 (MMP-2)	0.03	No
matrix metalloproteinase-9 (MMP-9)	0.03	No

Potential Biomarkers Elevated in AKI

Biomarker	High Test Conc. mg/L	Significant Interference
Cystatin C	2.6	No
lnterleukin-18 (IL-18)	0.001	No
Kidney Injury Molecule 1(KIM 1)	0.015	No
Liver Type Fatty Acid Binding Protein (L-FABP)	0.6	No
N-acetvH3-D-glucosaminidase (NAG)	4.4×10^{-5}	No
Neutrophil gelatinase associated lipocalcin (NGAL)	3	No
Pi-Glutathione s-transferase (p-GST)	0.05	No

No interference was observed from contrast agents, plasma expanders, urine constituents not stated above, isoforms of TIMP-2 and IGFBP-7, or other potential biomarkers reported in the literature as being elevated in AKI.

Urine albumin at concentrations above 1250 mg/L interferes with test results. Bilirubin at concentrations above 72.1 mg/L exhibits interference with test results.

Methylene blue at concentrations above 0.49 mg/L interferes with test results. These limitations are included in the labeling.

The effect of pH on AKIRisk Scores around and above the cutoff using samples with AKIRisk Score of 0.3 and 6.7 (at the higher end of ranges for TIMP-2 and IGFBP-7) was evaluated. Pooled human urine samples targeting these AKIRisk Scores were adjusted to target pH values of approximately 4, 6, 8, and 10. There was no effect from pH. The recommended pH range for samples is 4.2 – 9.9.

The effect of specific gravity on AKIRisk Scores around and above the cutoff using samples with AKIRisk Score of 0.3 and 6.9 (at the higher end of ranges for TIMP-2 and IGFBP-7) was evaluated. Pooled human urine samples targeting these AKIRisk Scores were adjusted to specific gravity of 1.001, 1.015 ±0.005, and > 1.0035. There was no effect from specific gravity on the AKIRisk Score. The recommended specific gravity range for samples is 0.998 – 1.038.

f. Assay cut-off:

The clinical study in M.3 below demonstrates that an AKIRisk Score > 0.3, when used in conjunction with clinical evaluation, aids in the risk assessment for moderate or severe acute kidney injury in patients who currently have or have had within the past 24 hours acute cardiovascular and or respiratory compromise and are ICU patients within twelve hours of patient assessment.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Not applicable. The NEPHROCHECK® Test is for human urine only.

3. Clinical studies:

The clinical performance of the NEPHROCHECK® Test at the cutoff of AKIRisk Score > 0.3 was evaluated in 2 clinical studies (Study A and Study B). Each of the 2 clinical studies was a multicenter prospective study in the intended use population at geographically diverse sites in the US. The intended use population was comprised of patients experiencing or having experienced within the past 24 hours acute cardiovascular or respiratory compromise and that were patients in the ICU, and these patients were followed for duration of the study (3 days). Patients with known moderate or severe acute kidney injury (e.g., RIFLE I, F, or KDIGO stage 2 or 3) were excluded from enrollment. Study A included 23 sites and enrolled 519 patients of which 408 completed the 3-day study and are denoted the evaluable cohort. Study B included 6 sites and enrolled 153 patients of whom 126

completed the 3-day study and are denoted the evaluable cohort. Unevaluable patients (Study A = 111 patients and Study B = 27 patients) were unevaluable because the patients did not complete the 3 day study due to removal of catheter or discharge from the ICU. Final diagnoses were adjudicated by an independent panel of expert nephrologists using the practice standards as "AKI" (moderate or severe AKI present) or "No AKI" (no moderate or severe AKI present) within 12 hours of enrollment. Urine specimens were collected at enrollment. For Study A, AKIRisk Score results were tested at 3 independent laboratories. For Study B, AKIRisk scores were tested at 1 laboratory. Adjudicators were blinded to the site diagnoses and AKIRisk Score results. Clinical performance data comparing AKIRisk score to final diagnosis (below) are provided for the evaluable cohorts. Exclusion of the unevaluable patients did not affect the conclusions of the study.

Study A/Laboratory 1

AKIRisk Score	**AKI Status**		**Total Number of NEPHROCHECK Test Results**
	AKI	**No AKI**	
AKIRISK Score > 0.3	65 (16.0%) TP	182 (44.7%) FP	247
AKIRISK Score ≤ 0.3	6 (1.5%) FN	154 (37.8%) TN	160
Total Number of NEPHROCHECK Test Results	71	336	407

Study A/Laboratory 2

AKIRisk Score	**AKI Status**		**Total Number of NEPHROCHECK Test Results**
	AKI	**No AKI**	
AKIRISK Score > 0.3	64 (15.7%) TP	172 (42.3%) FP	236
AKIRISK Score ≤ 0.3	7 (1.7%) FN	164 (40.3%) TN	171
Total Number of NEPHROCHECK Test Results	71	336	407

Study A/Laboratory 3

AKIRisk Score	AKI Status		Total Number of NEPHROCHECK Test Results
	AKI	No AKI	
AKIRISK Score > 0.3	66 (16.2%) TP	186 (45.7%) FP	252
AKIRISK Score ≤ 0.3	5 (1.2%) FN	150 (36.9%) TN	155
Total Number of NEPHROCHECK Test Results	71	336	407

Study B

AKIRisk Score	AKI Status		Total Number of NEPHROCHECK Test Results
	AKI	No AKI	
AKIRISK Score > 0.3	22 (17.5%) TP	48 (38.1%) FP	70
AKIRISK Score ≤ 0.3	7 (5.6%) FN	49 (38.9%) TN	56
Total Number of NEPHROCHECK Test Results	29	97	126

Test Clinical Performance Characteristics Study A

	Laboratory 1		Laboratory 2		Laboratory 3	
Statistic	**Value**	**95% CI**	**Value**	**95% CI**	**Value**	**95% CI**
Sensitivity (TPR)	0.92	0.85 , 0.98	0.90	0.83, 0.97	0.93	0.87, 0.99
Specificity (TNR)	0.46	0.40, 0.51	0.49	0.43, 0.54	0.45	0.39, 0.50
Negative Predictive Value (NPV)	0.96	0.93, 0.99	0.96	0.93, 0.99	0.97	0.94, 1.00
Positive Predictive Value (PPV)	0.26	0.21, 0.32	0.27	0.21, 0.33	0.26	0.21, 0.32
False Positive Rate	0.54	0.49, 0.60	0.51	0.46, 0.57	0.55	0.50, 0.61
False Negative Rate	0.08	0.02, 0.15	0.10	0.03, 0.17	0.07	0.01, 0.13

Test Clinical Performance Characteristics Study B

Statistic	Value	95% CI
Sensitivity (TPR)	0.76	0.60, 0.91
Specificity (TNR)	0.51	0.41, 0.60
Negative Predictive Value	0.88	0.79, 0.96
Positive Predictive Value	0.31	0.21, 0.42
False Positive Rate	0.49	0.40, 0.59
False Negative Rate	0.24	0.09, 0.40

a. Clinical Sensitivity:

See section M.3.

b. Clinical specificity:

See section M.3.

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

4. Clinical cut-off:

The clinical study above demonstrates that an AKIRisk Score > 0.3, when used in conjunction with clinical evaluation, aids in the risk assessment for moderate or severe acute kidney injury in patients who currently have or have had within the past 24 hours acute cardiovascular and or respiratory compromise and are ICU patients within twelve hours of patient assessment.

5. Expected values/Reference range:

A reference range study was performed to determine the AKIRisk Score in two adult (at least 21 years of age) cohorts—an apparently healthy subject population, and individuals with stable chronic morbidities (without acute illness). Demographic and other information for the two cohorts is shown below.

To determine the AKIRisk Score reference range, a urine specimen from each subject was collected and split into 3 aliquots and sent to 3 independent laboratories for measurement. Non-parametric analysis of the AKIRisk Score was performed for the results from each laboratory. There was no statistical difference among the three laboratories. A wide range of AKIRISK™ scores (0.04-2.42) was observed in the apparently healthy subjects and subjects with stable chronic morbidities (without acute illness). The reference range was defined by the central 95th percentile (2.5%–97.5%) as described in CLSI guideline C28-A3. Patient demographics of the two cohorts are presented below:

Demographic Characteristics of Apparently Healthy and Stable Chronic Morbidity Subjects

	Apparently Healthy Cohort		Stable Chronic Morbidity Cohort	
	N, Mean, or Median	**%, (SD), or [IQR]**	**N, Mean, or Median**	**%, (SD), or [IQR]**
Sex				
Female	191	50.5%	191	51.3%
Male	187	49.5%	181	48.7%
Race				
American Indian*	3	0.8%	6	1.6%
Asian	9	2.4%	10	2.7%
Black/African Amer.	43	11.4%	43	11.6%
Native Hawaiian♦	1	0.3%	3	0.8%
Caucasian	313	82.8%	300	80.6%
Other	9	2.4%	10	2.7%
Ethnicity				
Hispanic	43	11.4%	33	8.9%
Non-Hispanic	335	88.6%	339	91.1%
Age (years)				
Mean	54	(17.3)	63	(14.7)
Median	56	(40–68)	65	(53–75)
BMI (kg/m^2)				
Mean (SD)	27.5	(5.87)	30.8	(7.02)
Median [IQR]	26.8	[23.3–29.8]	29.8	[26.2–34.5]

*****Includes Alaskan Native, ♦Includes Other Pacific Islander

Summarized results of the reference range study are below:

Reference Ranges for Apparently Healthy Subjects and Subjects with Stable Chronic Morbidities by Testing Laboratory for Each of Three Testing Laboratories

		Site 1		Site 2		Site 3	
Cohort	**Gender**	**Number of Subjects**	**Observed AKI Risk Scores***	**Number of Subjects**	**Observed AKI Risk Scores***	**Number of Subjects**	**Observed AKI Risk Scores***
Apparently Healthy Subjects	Female	191	0.04 - 2.42	191	0.04 - 2.17	191	0.04 - 2.58
	Male	185	0.04 - 2.33	187	0.04 - 2.10	187	0.05 - 2.35
	All	376	0.04 - 2.33	378	0.04 - 2.10	378	0.04 - 2.35
Subjects with Stable Chronic Morbidities	Female	191	0.04 - 2.20	191	0.04 - 1.93	191	0.04 - 2.28
	Male	179	0.06 - 2.23	181	0.06 - 2.13	181	0.06 - 2.36
	All	370	0.05 - 2.20	372	0.04 - 1.98	372	0.04 - 2.28

*Central 95%

N. Instrument Name:

Astute140 Meter

O. System Descriptions:

1. Modes of Operation:

   The Astute140 Meter is a small tabletop analyzer that can analyze one NEPHROCHECK® Test cartridge at a time. The operator must manually load the cartridge and remove it after the analysis has been completed.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

3. Specimen Identification:

   The Meter can be interfaced with a laboratory information system for data management via USB or Ethernet. It also has a PS/2 Port for connection to an optional barcode reader or keyboard.

4. Specimen Sampling and Handling:

   The Astute140 Meter can only accept one test cartridge at a time. Human urine is added to the kit Conjugate Vial and a 100 μL aliquot is dispensed onto the test cartridge for analysis.

5. Calibration:

   The Astute140 Meter is factory calibrated. Calibration is verified by the operator with an Electronic Quality Control cartridge and with liquid calibration verifiers.

   The NEPHROCHECK® Calibration Verification Kit contains 5 materials with specified target concentration levels that evenly span the reportable ranges of the biomarkers used in the algorithm to verify the AKIRisk Score . The expected concentrations and standard deviations are printed on the NEPHROCHECK® Expected Values Card enclosed with the NEPHROCHECK® Calibration Verification Kit.

6. Quality Control:

   Quality control is performed using an electronic QC cartridge and liquid low and high control materials. The electronic QC (EQC) is accomplished by comparing the output on the cartridge with a preset range of results. There are 2 internal QC lines on the EQC cartridge. The fluorescent intensities of the control lines must fall within a pre-set range for the test to be considered valid.

   Liquid QC consists of a high and low control. The QC lot, expiration, and biomarker ranges for each of TIMP-2 and IGFBP-7 are entered into the Astute140 Meter with a RFID card enclosed with each Control Kit. The RFID card must be loaded prior to running QC. The Astute140 Meter also has a "lock out" feature that prevents assays from being performed if either the EQC or liquid QC fails. QC results may be printed or downloaded into a data manager or LIS.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

The Astute 140 meter demonstrates compliance to EMC requirements including general requirements for laboratory use by meeting DIN EN 61326-1 as well as requirements for in vitro diagnostic medical equipment by meeting DIN EN 61326-2-6. In addition, the Astute 140 meter demonstrates compliance regarding information technology equipment and radio disturbances by meeting the requirements of DIN EN 55022, IEC/CISPR 22:2005.

The Astute140 Meter fulfilled EMI testing requirements for the RFID component under FCC part 15, subparts A, C, and I, ANSI C63:4 (2009), ANSI C95.1 (1992) and CISPR 16-4-2 (2003).

The Astute140 Meter underwent vibration testing based on EN 60068-2-64 and fall conditions based on EN 61010-1 to ensure that the meter met all reading, repeatability and functionality checks. The meter passed for all conditions.

Q. Proposed Labeling:

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

R. Identified Risks and Required Mitigations:

Identified Potential Risks	**Required Mitigation Measures**
o **Incorrect interpretation of test results**	Special controls (1), (2), and (3)
o **Incorrect test results**	Special control (3)

S. Benefit/Risk Analysis:

Summary	
Summary of the Benefits (s)	The NEPHROCHECK® Test System aids in the risk assessment for moderate or severe AKI *in critically ill patients* within twelve hours of patient assessment for risk of AKI. The main benefit from the use of the NEPHROCHECK® Test is to provide an opportunity to improve patient management for acute kidney injury by aiding physicians in identifying patients at risk for AKI. Identifying patients at risk for AKI may aid in the prevention of progression to CKD or possibly death and allow treatment to potentially reverse the injury to the kidney. Since AKI diagnosis is currently commonly delayed, identifying patients at higher risk before development of AKI may allow for better management and treatment and, therefore, serve an unmet medical need.
Summary of the Risk(s)	The potentially harmful events associated with the use of the NEPHROCHECK® Test are from inappropriate clinical decisions based on undetected instances of incorrect interpretation of test results and incorrect test results. A false negative test result may result in a patient being misclassified into the non-high risk group, potentially resulting in a temporary delay in implementation of the kidney-sparing strategies for high risk patients. Although the impact is potentially mitigated through labeling, (i.e., in the case of a low NEPHROCHECK® Test, (i.e. AKIRisk result $\leq$ 0.3) the user is instructed not to alter standard of care management), should the user not follow the instructions and, instead act on the basis of a low test result, the duration of impact of a false negative test result would only last until the results from additional testing (e.g., serum creatinine elevation, decrease in urine output) become available. A false positive test result could contribute to a patient being misclassified into a high risk group and could result in subjecting the patient to further, unnecessary diagnostic testing (e.g., more frequent

	serum creatinine measurements or more frequent urine output monitoring) or temporary worsening of the primary disease state due to, for example, temporary selection of less nephrotoxic treatments or diagnostics that may be less efficacious. The duration of the potential impact of a false positive test result would be for a limited period of time since the subsequent diagnostic workup for patients at high risk for AKI (e.g., monitoring of serum creatinine and urine output) soon clarify the patient's renal status. These risks can be mitigated via the special controls, including an intensive training program which includes the performance, limitations, and interpretation of the test.
Summary of Other Factors	Acute kidney injury (AKI) is one of the more prevalent and serious morbidities in critically ill hospitalized patients and is associated substantially increased mortality, morbidity, length of ICU stay and in-hospital cost as well as longer term health consequences, including increased risk for chronic kidney disease (CKD). Use of the NEPHROCHECK® Test System aids in avoidance of suboptimal patient management or delay in optimal management of patients at risk for AKI. In contrast to such conditions as myocardial infarction, AKI may not present with signs and symptoms sufficient to guide risk assessment. However, the literature states that "there is no reliable way for a clinician to use this information to establish a clear risk profile", and no laboratory test method exists for risk assessment of AKI. Currently, there are no other *in vitro* diagnostic devices cleared or approved for the NEPHROCHECK® Test's indicated use. Results of the clinical studies demonstrate that the NEPHROCHECK® Test is able to provide a benefit that outweighs risk for the intended use patient population. The magnitude of the benefit as well as the probability that a patient for whom the device is intended will experience a benefit was assessed through the clinical performance of the test in two studies. In summary, the test benefits outweigh the risks for use of the test as an adjunct to other clinical criteria to identify patients in the intended use population who are at risk for moderate or severe AKI. The relatively low specificity strongly suggests that some patients who are not at risk for developing AKI may exhibit a positive test result. This reiterates the necessity to consider other standard, accepted clinical criteria in the assessment of AKI risk in patients in the intended use population.
Conclusions Do the probable benefits outweigh the probable risks?	The ability of the NEPHROCHECK® Test to aid patients in identifying patients at risk for moderate or severe AKI offers significant potential benefit. These patients can benefit from the kidney-sparing management strategies. Compared to these benefits,

	the NEPHROCHECK® Test System poses risks that are adequately mitigated by general and special controls. Thus, the probable benefits outweigh the probable risks.

T. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 862.1220. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. This device is classified under the following:

Product Code: PIG

Device Type: Acute kidney injury test system

Class: II (special controls)

Regulation: 21 CFR 862.1220

(a) *Identification*. An acute kidney injury test system is intended to measure one or more analytes in human samples as an aid in the assessment of a patient's risk for developing acute kidney injury. Test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods.

(b) *Classification.* Class II (special controls). Acute kidney injury test systems must comply with the following special controls:

1) Premarket notification submissions must detail an appropriate end user device training program that will be offered while marketing the device as part of your efforts to mitigate the risk of failure to correctly interpret test results.

2) As part of the risk management activities performed as part of your 21 CFR 820.30 design controls, you must document the appropriate end user device training program provided in your premarket notification submission to satisfy special control (1) that will be offered while marketing the device as part of your efforts to mitigate the risk of incorrect interpretation of test results.

3) Robust clinical data demonstrating the positive predictive value, negative predictive value, sensitivity and specificity of the test in the intended use population must be submitted as part of the premarket notification submission.