NEPHROCHECK® Test Kit Package Insert

Manufactured for
Astute Medical, Inc.
3550 General Atomics Ct.
Building 2
San Diego, CA 92121
USA

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.

Summary and Explanation of the Test

Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) is a soluble protein of about 26K Dalton molecular weight that is expressed in kidney and other tissues.[1] IGFBP-7 is thought to be involved or induced in several types of processes that have been associated with cellular injury.[2–8] Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) is a soluble protein of about 22K Dalton molecular weight that is expressed in kidney and other tissues.[9] TIMP-2 binds to and inhibits the activity of various metalloproteinases (MMPs).[10] TIMP-2 also activates MMP2. Through its action on the MMPs, TIMP-2 is thought to be involved or induced in several processes associated with leukocyte infiltration, cellular injury and disruption of cell contacts.[11–16] TIMP-2 and IGFBP-7 are also both involved with the phenomenon of G1 cell cycle arrest during the very early phases of cell injury.[17–20]

AKI engages a series of extremely complex cellular and molecular pathways involving endothelial, epithelial, inflammatory, and interstitial cells. These mechanisms include cell cycle, immunity, inflammation, and apoptosis pathways. Recently, it has been shown that, similar to other epithelia, renal tubular cells enter a short period of G1 cell-cycle arrest following injury from experimental sepsis[2] or ischemia.[21] It is believed that this prevents cells from dividing when the DNA may be damaged and arrests the process of cell division until the damage can be repaired lest resulting in the cell's demise or senescence.[18] TIMP-2 and IGFBP-7 are also known to be involved in the response to a wide variety of insults (inflammation, oxidative stress, ultraviolet radiation, drugs, and toxins).[19,20,22] This may help explain why they correspond to risk of AKI.

Studies to evaluate the combination of TIMP-2 and IGFBP-7 for risk assessment of human AKI are now entering the literature. In one publication[23], two multicenter observational studies in the intended use patients were performed. The first study enrolled 522 adults in three distinct cohorts (including patients with sepsis, shock, major surgery, and trauma) and examined over 300 potential AKI markers. The second study enrolled 744 adult subjects with critical illness and without evidence of AKI at enrollment; the final analysis cohort was a heterogeneous sample of 728 intended use patients. The primary endpoint was moderate to severe AKI within 12 hours of sample collection. The study revealed that urinary IGFBP-7 and TIMP-2, together, demonstrated an AUC of 0.80. Furthermore, [TIMP-2]·[IGFBP-7] significantly improved risk stratification when added to a nine-variable clinical model when analyzed using Cox proportional hazards model, generalized estimating equation, integrated discrimination improvement or net reclassification improvement.

AKI is one of the more prevalent and serious morbidities in hospitalized patients and is associated with a multitude of acute and chronic conditions.[24–29] The economic and public health burden of AKI is staggering with substantially increased mortality, morbidity, length of ICU stay and in-hospital costs, as well as longer term health consequences.[30–36] Tests to assess AKI provide important information to physicians and, in conjunction with other available clinical information, can aid physicians in optimizing patient management.[27,36–38]

Principles of the NEPHROCHECK® Test Procedure

The NEPHROCHECK® Test is a single-use cartridge comprised of assays for two protein biomarkers, TIMP-2, tissue-inhibitor of Metalloproteinase 2, and IGFBP-7, insulin-like growth factor-binding protein 7, on a membrane test strip enclosed in a plastic housing that employs a sandwich immunoassay technique. The test procedure involves the operator applying a fresh or thawed (i.e. previously frozen) clinical urine sample (mixed with labeled fluorescent conjugate) to the NEPHROCHECK® Test cartridge, and then inserting the Test cartridge into the ASTUTE140® Meter for incubation, reading, result calculation, and result display. The ASTUTE140® Meter is a bench-top/table-top analyzer that converts the fluorescent signal from each of the two immunoassays, TIMP-2 and IGFBP-7, contained within the NEPHROCHECK® Test cartridge into a single numerical result that is called the AKIRISK™ Score. For more information on the AKIRISK™ Score, please refer to “Results” and “Interpretation of Results”.

Materials Provided

The NEPHROCHECK® Test cartridge and NEPHROCHECK® Test Kit contain all the reagents needed for the generation of NEPHROCHECK® Test results in human adult urine specimens.

The NEPHROCHECK® Test cartridge and NEPHROCHECK® Test Conjugate Vial contain:

- Murine monoclonal and goat polyclonal antibodies against TIMP-2
- Murine monoclonal and goat polyclonal antibodies against IGFBP-7
- Fluorescent dye
- Stabilizers
- Excipients

The NEPHROCHECK® Test Kit (Part Number 500011) contains:

- NEPHROCHECK® Test 25
- NEPHROCHECK® Test Conjugate Vial CONJUGATE VIAL 25
- NEPHROCHECK® Test RFID Card RFID NEPHROCHECK 1
- NEPHROCHECK® Test Buffer (2 x 5 mL) BUFFER VIAL 1
- NEPHROCHECK® Test Kit Package Insert 1

Materials Required But Not Provided

- ASTUTE140® Meter (PN 500017)
- NEPHROCHECK® Liquid Control Kit (PN 500013)
- NEPHROCHECK® Calibration Verification Kit (PN 500009)
- NEPHROCHECK® Electronic Quality Control (PN 400016)
- Calibrated precision pipette, capable of dispensing 100 µL

Warnings and Precautions

- The NEPHROCHECK® Test should not be used as a “standalone test”. The NEPHROCHECK® Test result must be evaluated with other clinical and laboratory test information.
- The operator should use Standard Precautions when performing the NEPHROCHECK® Test or operating the ASTUTE140® Meter.
- For *in vitro* diagnostic use.
- The NEPHROCHECK® Test is intended for use in clinical laboratories and not for use in point-of-care settings.
- NEPHROCHECK® Test results should be interpreted within 12 hours of patient assessment for risk of AKI.
- Do not use the NEPHROCHECK® Test Kit beyond the expiration date printed on the outside of the box.
- Carefully follow the instructions and procedures described in this insert.
- Keep the NEPHROCHECK® Test cartridge and NEPHROCHECK® Conjugate Vial in the sealed pouch until ready for immediate use.
- Patient specimens, used NEPHROCHECK® Test cartridges and used pipette tips may be potentially infectious. Proper handling and disposal methods in compliance with federal and local regulations should be established.
- The NEPHROCHECK® Test is to be used only with the ASTUTE140® Meter, the NEPHROCHECK® Liquid Control Kit, and the NEPHROCHECK® Calibration Verification (Cal Vers) Kit.

- The NEPHROCHECK® Test Conjugate Vials contained in the NEPHROCHECK® Test Kit are to be used only with the NEPHROCHECK® Test cartridges contained in the same kit box. The NEPHROCHECK® Test Conjugate Vials are not to be used with cartridges that are contained in other boxes or provided with other products.
- The NEPHROCHECK® Test Kit requires the use of calibrated precision pipette(s). It is recommended that users review the proper procedures for the use of these devices in order to ensure accurate dispensing of volumes.
- In order to minimize contamination, pipette tips are to be discarded and a new one used for each new specimen.
- Patient identification schemes (i.e. IDs) that contain the following special characters "+", "&" or "@" should be entered into the ASTUTE140® Meter only with a barcode scanner—these characters should not be manually entered into the ASTUTE140® Meter using an external keyboard.
- Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he practices, to use or order the use of the device.

Storage and Handling Requirements

- Prior to using the NEPHROCHECK® Test Kit, inspect the kit components for damage. Do not use the NEPHROCHECK® Test Kit if any components are damaged.
- The NEPHROCHECK® Test Conjugate Vial material is lyophilized.
- The unopened NEPHROCHECK® Test Kit components are stable until the expiration date printed on the box when stored at 4–25°C (39.2–77°F).
- The opened NEPHROCHECK® Test cartridge is stable for 60 minutes at 22.9-24.8°C (73.2–76.6°F).
- The opened NEPHROCHECK® Test Buffer is stable to the expiration date printed on the bottle label or until 28 days after initial opening of the bottle (whichever occurs first) when the unused portion is properly stored at 4–25°C (39.2–77°F).
- Each NEPHROCHECK® Test cartridge and NEPHROCHECK® Test Conjugate Vial is intended for single use only.
- After completion of all tests included in the kit box, dispose of any remaining NEPHROCHECK® Test Buffer in accordance with local regulations.
- If kit materials are stored refrigerated, allow the kit components to reach operating temperature of 18–25°C (64–77°F) and operating humidity of 30–50% RH before opening the foil pouch.

Getting Started

Using the supplied RFID card, each NEPHROCHECK® Test lot must be registered into the ASTUTE140® Meter prior to first use.

Configure the ASTUTE140® Meter and run ASTUTE140® Electronic Quality Control (EQC) and NEPHROCHECK® Liquid Quality Control (LQC) procedures. (See "Installation" and "ASTUTE140® Meter Operation" in the ASTUTE140® Meter User Manual for detailed instructions.)

RFID Cards and Lot Registration

Each new ASTUTE140® Electronic Quality Control (EQC) Device, NEPHROCHECK® Liquid Control Kit and NEPHROCHECK® Test Kit is supplied with one or more RFID cards. These RFID cards contain lot specific product information such as product lot numbers, expiration dates, and calibration information. RFID cards must be used to transfer (or register) lot specific information for each new kit to the ASTUTE140® Meter prior to first use. To register a Kit or Device lot, locate the RFID card(s) included with the Kit or Device and perform the steps below. (See "ASTUTE140® Meter Operation" in the ASTUTE140® Meter User Manual for detailed instructions.)

NOTE: The NEPHROCHECK® Liquid Control Kit is supplied with two RFID cards, one card is for each level of control. The liquid control registration process must be carried out for each level of control.

How to Register RFID Cards with the ASTUTE140® Meter (Transfer Lot Specific Information)

1. From the **Main Menu**, use the navigation (arrow) keys to highlight and select the **Operator Menu** icon.
2. Press the right soft key to display the **Manage Lots** screen.
3. Use the soft key to select **Manage Test Lots** or use the arrow keys to highlight and select **Manage LQC Lots** or **Manage EQC Devices**.
4. A **Registered** screen will appear showing any lots previously registered (**Test Lots**, **LQC** or **EQC Devices**), press **Options** using the right soft key.
5. When the **Options** pop-up menu is displayed, use the arrow keys to highlight **Register Lot** (or **Device** for EQC) and press the right soft key to **Select**.
6. When prompted, hold the RFID card against the numeric keypad to register the information and press the right soft key to select **OK**.

7. If registered correctly, a screen indicating that the lot number (or Device) was successfully read from the RFID card will appear. Press the right soft key to select **Accept**. The lot or Device that was just registered should now appear in the list of registered lots or Devices.
8. If registered incorrectly, an error message will appear. Press the right soft key to select **OK** to close the error message. Repeat steps above. If registered incorrectly a second time, contact Astute Technical Support.
9. After use, return the RFID card to its sleeve and store it together with the lot number with which it arrived.
10. To register a second liquid control in a set or to register another Lot or Device, use the arrow keys to select **Register Lot** or **Device** from the **Options** pop-up menu and repeat the steps above.

Specimen Collection and Preparation

The NEPHROCHECK® Test is intended for use with adult human urine specimens only. Other specimen types have not been characterized.

Non-Frozen / Non-Refrigerated Samples

1. Collect a fresh urine sample of approximately 10 mL in a clean specimen collection cup without additives. For patients with indwelling bladder catheters, the collection bag should first be emptied and then a fresh sample of urine should be collected. Alternatively, the sample may be collected from an urometer, if present. Transport the urine sample to the laboratory that will run the NEPHROCHECK® Test.

   NOTE: Samples should be transferred to the laboratory and centrifuged within one hour of sample collection.
2. Thoroughly mix the urine in the specimen collection cup by inverting the container 8–10 times. Transfer the urine sample from the specimen collection cup to a clean centrifuge tube. Centrifuge the urine sample for approximately 10 minutes in refrigerated centrifuge set to an rcf of 1000 x g and temperature of 4°C (39.2°F). After centrifuging the sample, transfer the supernatant to a clean receptacle. Allow supernatant to reach room temperature and test the supernatant within 5 hours of sample collection. If testing cannot be completed within 5 hours of sample collection, supernatants may be refrigerated immediately after centrifugation and tested within 20 hours of sample collection.

Frozen / Refrigerated Samples

1. To test frozen or refrigerated samples, thaw or warm urine supernatants in a room temperature (18–25°C; 64.4–77°F) water bath until thawed and warmed to room temperature but no longer than 20 minutes.
2. Once the supernatant is thawed and/or warmed to room temperature, gently invert the sample tube 1–2 times to mix sample. Ensure supernatant is well-mixed before testing. Test the supernatant immediately after mixing.

   NOTE: Precipitates may be present in supernatant tube. Always invert the sample tube 1–2 times to ensure sample is well mixed before testing to ensure accurate measurement results.
3. Supernatants must be loaded into a NEPHROCHECK® Test cartridge within one hour of placing the supernatant into the water bath.
4. Avoid repeated freezing and thawing of the supernatant.

NEPHROCHECK® Test Procedure

- The Test procedure requires the use of a calibrated precision pipette for the following:
  - Addition of NEPHROCHECK® Test Buffer Solution and urine sample into the NEPHROCHECK® Test Conjugate Vial
  - Introduction of sample into the NEPHROCHECK® Test cartridge
- Prior to running the test, all NEPHROCHECK® Test Kit components must be at the operating temperature of 18–25°C (64–77°F).

To perform the NEPHROCHECK® Test, follow these steps:

1. Preparation:

   a. Highlight and select **Run Patient** on the ASTUTE140® Meter **Main Menu**.

   b. Manually enter the Patient ID or scan the Patient ID into the ASTUTE140® Meter using a barcode scanner (if connected). After confirming that the correct Patient ID and/or Sample ID have been entered, select **Run Patient**. The ASTUTE140® Meter drawer will automatically open. (NOTE: Patient identification schemes (i.e. IDs) that contain the following special characters “+”, “&” or “@” should be entered into the ASTUTE140® Meter only with a barcode scanner—these characters should not be entered into the ASTUTE140® Meter using an external keyboard.)

   c. Remove the new NEPHROCHECK® Test cartridge from the foil pouch and place on a flat surface.

d. Remove the NEPHROCHECK® Test Conjugate Vial from the pouch.

e. Each NEPHROCHECK® Test Conjugate Vial contains a single conjugate bead. Remove the cap from the NEPHROCHECK® Test Conjugate Vial. Visually inspect the cap and vial to ensure that the conjugate bead has not adhered to the cap and is present in the vial. If the bead has adhered to the cap, place the cap on the vial and tap three times. Repeat if necessary until the bead drops into the vial. Do not touch the bead or attempt to remove the bead from the cap by any other means.

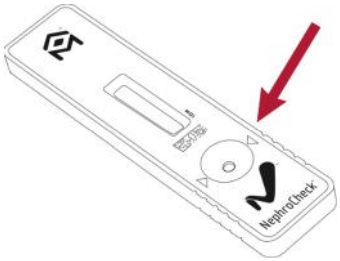
f. Pipette 100 µL of the NEPHROCHECK® Test Buffer Solution into the NEPHROCHECK® Test Conjugate Vial containing the conjugate bead. This will result in reconstitution of the conjugate bead into solution. Discard the pipette tip in accordance with local regulations.

NOTE: The conjugate liquid in the vial is to be used as soon as it is reconstituted.

NOTE: Each bottle of NEPHROCHECK® Test Buffer Solution contains enough buffer solution to run all of the tests supplied in the NEPHROCHECK® Test Kit. Do not discard the buffer solution until all tests supplied in the NEPHROCHECK® Test Kit have been used. Store the unused portion of the buffer at 4–25°C (39.2–77°F).

g. Using a new pipette tip, add 100 µL of centrifuged urine supernatant or liquid control sample to the NEPHROCHECK® Test Conjugate Vial that now contains the reconstituted conjugate bead solution. Mix thoroughly (mix at least three times using the pipette tip).

h. Pipette 100 µL of mixed sample/conjugate solution onto the sample port on the NEPHROCHECK® Test cartridge. Avoid introducing bubbles into the sample port when adding the sample / conjugate solution into the NEPHROCHECK® Test cartridge. Wait approximately one minute for the sample to be absorbed into the round well.



2. Run the NEPHROCHECK® Test:

a. Holding the NEPHROCHECK® Test cartridge by the grips on the sides of the cartridge, place the cartridge in the ASTUTE140® Meter drawer with the Astute Medical logo towards the inside of the meter drawer. Keep the NEPHROCHECK® Test cartridge horizontal and avoid tipping the test cartridge during placement into the ASTUTE140® Meter drawer.

b. Close the ASTUTE140® Meter drawer. In approximately 20 minutes, a single numerical test result will be displayed.

c. Eject the ASTUTE140® Meter drawer. Remove the NEPHROCHECK® Test cartridge and discard it and the conjugate vial in accordance with local regulations.

3. Review the NEPHROCHECK® Test Results:

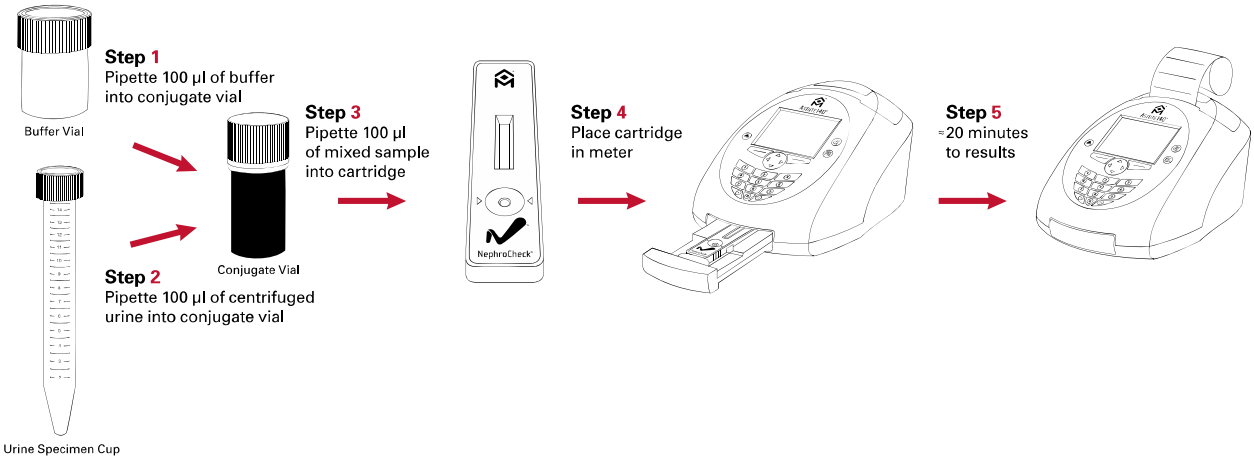
Upon completion of running the test, follow instructions in the ASTUTE140® Meter User Manual to print results (if desired) or upload results to the Laboratory Information System (LIS).

If the NEPHROCHECK® Test should fail, a Meter error message will indicate that the result is invalid and that a new cartridge should be run. If the procedure fails a second time, contact Astute Technical Support (See “Ordering and Contact Information”).

The ASTUTE140® Meter converts the fluorescent signal from each of the two immunoassays (TIMP-2 and IGFBP-7) contained within the NEPHROCHECK® Test cartridge into a single numerical result. The NEPHROCHECK® Test result (AKIRISK™ Score) is calculated by the ASTUTE140® Meter as the product of the measured concentrations of the two biomarkers, TIMP-2 and IGFBP-7 (measured as ng/mL), divided by 1000:

$$\text{NEPHROCHECK® Test Result (AKIRISK™ Score)} = \frac{[\text{TIMP-2}]*[\text{IGFBP-7}]}{1000} \quad (\text{units} = (\text{ng/ml})^2/1000)$$

NEPHROCHECK® Test Preparation Process



Results

The AKIRISK™ Score is displayed on the ASTUTE140® Meter screen after the NEPHROCHECK® Test procedure is completed. Results for the individual markers are not displayed. The Test result is displayed without units. Refer to the "Interpretation of Results" and "Reference Range" discussions for further information.

Interpretation of Results

A single cutoff of AKIRISK™ Score > 0.3 for the NEPHROCHECK® Test has been established based on the results from clinical studies to achieve high sensitivity while preserving acceptable specificity to identify the majority of subjects who will manifest moderate or severe AKI within 12 hours. Based upon results from clinical testing, intended use patients with AKIRISK™ Scores ≤ 0.3 are at lower risk of developing moderate to severe AKI within 12 hours of assessment than intended use patients with AKIRISK™ Scores > 0.3.

The AKIRISK™ Score reference range for apparently healthy subjects was 0.04 to 2.25 and for subjects with stable chronic morbidities was 0.05 to 2.20 (Refer to the "Reference Range" section for further information about these patient populations). In clinical studies (Study A and B) performed to validate the test in the intended use population, the following AKIRISK™ Scores were observed. In Study A, for the 408 intended use patients, the range (Central 95%) in AKIRISK™ Scores for subjects that did not have AKI was 0.04 to 2.62, while that for intended use patients with AKI was significantly elevated at 0.10 to 8.47. In Study B, for the 126 intended use patients, the range (Central 95%) in AKIRISK™ Scores for subjects that did not have AKI was 0.03 to 6.36, while the range for subjects with AKI was significantly elevated at 0.08 to 13.33. (Refer to the "Clinical Performance" section for further information regarding the clinical studies). Because the distributions of AKIRISK™ Scores for apparently healthy subjects, subjects with stable chronic morbidities (without acute illness), and intended use patients without AKI show overlap with the distribution of AKIRISK™ Scores for intended use patients with AKI, the NEPHROCHECK® Test results cannot be used as standalone results, and, based on the low positive predictive value (PPV), a result of an AKIRISK™ Score > 0.3 is not necessarily predictive of developing moderate to severe AKI within a specific time frame.

Standardization

Concentration results for each of the assays in the NEPHROCHECK® Test are traceable to reference standard solutions that contain defined mass (concentration) of TIMP-2 and IGFBP-7 proteins in accordance with EN ISO 17511.[39] The NEPHROCHECK® Test and NEPHROCHECK® Liquid Controls are traceable to the same reference standard solutions.

Quality Control Considerations

Each NEPHROCHECK® Test cartridge contains two detection zones used as procedural controls (one positive and one negative control) that indicate that the NEPHROCHECK® Test procedure was performed correctly. These positive and negative controls are run automatically with every sample. 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. These procedural controls are in addition to the external NEPHROCHECK® Liquid Controls and the ASTUTE140® Electronic Quality Control (EQC) Device.

Good Laboratory Practice suggests that external NEPHROCHECK® Liquid Controls be tested:

- Every 30 days
- With each new lot number of NEPHROCHECK® Test Kits
- With each new shipment of the NEPHROCHECK® Test Kits
- After ASTUTE140® Meter maintenance or servicing
- In accordance with your local, state, and/or federal regulations or accreditation requirements and standard quality control procedures

Good Laboratory Practice suggests that ASTUTE140® Electronic Quality Control (EQC) Device be tested:

- Daily
- After ASTUTE140® Meter maintenance or servicing
- In accordance with your local, state, and/or federal regulations or accreditation requirements and standard quality control procedures

Limitations of the NEPHROCHECK® Test Procedure

The NEPHROCHECK® Test result must be evaluated with other clinical and laboratory test information. The NEPHROCHECK® Test should not be used as a "standalone test".

Urinary albumin at concentrations above 125 mg/dL interfere with the NEPHROCHECK® Test results. Urinary albumin at concentrations above 3000 mg/dL cause an invalid test result. Urinary bilirubin at concentrations above 7.2 mg/dL interfere with the NEPHROCHECK® Test results. Use caution in interpreting NEPHROCHECK® Test results in patients with significant proteinuria or severe hyperbilirubinuria.

Methylene blue at concentrations above 0.49 mg/L interferes with NEPHROCHECK® Test results.

Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he practices, to use or order the use of the device.

Performance Characteristics

Analytical Sensitivity

Limit of Blank (LoB)

The NEPHROCHECK® Test has a Limit of Blank (LoB) of an AKIRISK™ Score of 0.0002. The LoB was determined using a protocol that followed the recommendations of CLSI EP17-A2.[40]

Limit of Detection (LoD)

The NEPHROCHECK® Test has a Limit of Detection (LoD) of an AKIRISK™ Score of 0.002. The LoD was determined using a protocol that followed the recommendations of CLSI EP17-A2.[40]

Limit of Quantitation (LoQ)

The NEPHROCHECK® Test has a Limit of Quantitation (LoQ) of an AKIRISK™ Score of 0.002. The LoQ was defined using a Total Error goal of 20 percent for each of the two biomarkers, TIMP-2 and IGFBP-7. The LoQ was determined using a protocol that followed the recommendations of CLSI EP17-A2.[40]

The LoB, LoD, and LoQ values for the AKIRISK™ Score are reported below in **Table 1**.

Table 1. Analytical Sensitivity

NEPHROCHECK® Test Result	Limit of Blank	Limit of Detection	Limit of Quantitation
AKIRISK™ Score	0.0002	0.002	0.002

Linearity

The reportable range of the NEPHROCHECK® Test AKIRISK™ Score is 0.04–10.00. The TIMP-2 and IGFBP7 markers used to derive the AKIRISK™ Score were assessed and found to be linear within the reportable range of the AKIRISK™ Score. However, the AKIRISK™ Score itself is not expected to be linear.

AKIRISK™ Scores that are outside the above reportable range are reported as either < 0.04 or > 10.00 by the ASTUTE140® Meter. If the AKIRISK™ Score is > 10.00, the specimen should not be diluted for retesting.

Precision

The reproducibility of the AKIRISK™ Score was determined in accordance with the methods provided in CLSI guideline EP5-A2.[42] As shown in **Table 2**, the AKIRISK™ Score exhibited a Total CV of 10.4% at the cutoff of > 0.3 and ranged between 9.1% and 18.0% across the reportable range.

The reproducibility of results was determined at three testing sites under intended use conditions using three NEPHROCHECK® Test Kit lots. Each site tested six urine samples (S2 – S7) spanning the measurable range of the NEPHROCHECK® Test. Samples S3, S4 and S5 (around the cutoff) exhibited the following AKIRISK™ Scores (mean result): 0.14, 0.3, and 0.56, respectively and observed precision (Total CV) of 11.0%, 10.4%, and 10.9%, respectively. Samples S2, S6 and S7 (designed to span the high and low ends of the AKIRisk Score' reportable range) exhibited the following AKIRISK™ Scores (mean results) of 0.04, 4.61 and 8.55, respectively and observed precision (Total CV) of 18.0%, 9.1% and 11.7%, respectively. Each site used a different test kit lot, set of ASTUTE140® Meters, and operators. Each urine sample was tested for at least 20 days, 2 test runs per day, 2 replicates per test run. The statistical analysis methods described in EP5-A2 were applied to the testing data to determine within-run, between run, between day, and total coefficient of variation values (CV's) across all three testing sites. These CV's are presented below in **Table 2**.

The NEPHROCHECK® Test is designed and manufactured so that each of the biomarker assays in the NEPHROCHECK® Test has imprecision of ≤15% within-run CV. The representative performance of the NEPHROCHECK® Test with regard to precision and reproducibility was characterized as described below.

Table 2. Precision

		S2	S3	S4	S5	S6	S7
AKIRISK™ Score	Mean Result	0.04	0.14	0.30	0.56	4.61	8.55
	Within Run SD	0.01	0.01	0.03	0.05	0.34	0.84
	Within Run CV	15.1%	8.1%	9.0%	8.5%	7.3%	9.9%
	Between Run SD	0.00	0.00	0.01	0.00	0.21	0.22
	Between Run CV	0.0%	3.2%	1.8%	0.0%	4.5%	2.5%
	Between Day SD	0.00	0.01	0.01	0.04	0.14	0.49
	Between Day CV	9.8%	6.8%	4.9%	6.9%	3.1%	5.8%
	Total SD	0.01	0.02	0.03	0.06	0.42	1.00
	Total CV	18.0%	11.0%	10.4%	10.9%	9.1%	11.7%

Interference Testing

Interfering Conditions

The effect of urine sample pH was evaluated for the NEPHROCHECK® Test. Two human urine pools were adjusted to target multiple pH values between approximately pH 4.2 and 9.9. One urine pool was prepared to have an AKIRISK™ Score of 0.26. The second urine pool was prepared to have an AKIRISK™ Score of 7.0. None of the urine pools or pH values tested exhibited significant interference. The recommended pH range for samples is 4.2 – 9.9.

The effect of urine specific gravity was evaluated for the NEPHROCHECK® Test. Two human urine pools were adjusted to target multiple specific gravity values between approximately 0.998 and 1.038. One urine pool was prepared to have an AKIRISK™ Score of 0.32. The second urine pool was prepared to have an AKIRISK™ Score of 6.9. None of the urine pools or specific gravity values tested exhibited significant interference. The recommended specific gravity range for samples is 0.998 – 1.038.

Interfering Substances

The substances listed below in **Table 3** and in the subsequent section entitled **"**Substances that Exhibited Interference**"** were evaluated for interference with the AKIRISK™ Score. Each substance was evaluated at multiple test concentrations in accordance with CLSI guideline EP7-A2.[43] Each substance was added to a human urine pool collected from apparently healthy donors was prepared to have an AKIRISK™ Score of approximately 0.28 – 0.36. This urine sample was then evaluated with 32 or more NEPHROCHECK® Tests. None of the substances listed in **Table 3** exhibited significant interference with AKIRISK™ Score at the maximum test concentrations listed below. However, the substances discussed in the section entitled **"**Substances that Exhibited Interference**"** did cause significant interference when added to urine as described below. The impact of these interferences on the AKIRISK™ Score is discussed below in the section entitled **"**Substances that Exhibited Interference**"**. A bias exceeding 10% is considered a significant interference.

Table 3. Substances with No Significant Interference

Substance	Test Conc. mg/L	Substance	Test Conc. mg/L
Dextran 40	22	Lansoprazole	90
Pentastarch	9	Linezolid	48
Hetastarch	6	Lisinopril	0.3
Visipaque (Iodixanol)	4941	Lorazepam	1
Omniscan (Gadodiamide)	177	Low Molecular Weight Heparin	30
Omnipaque (Iohexol)	14085	Mannitol	600
Magnevist (Gadopentate Dimeglumine)	422	Metformin	40
Optiray (Ioversol)	4944	Metolazone	60
Acetaminophen	201	Metoprolol	5
Acetone	697	Metronidazole	120
Acetylcysteine	1665	Midazolam	1
Aspirin	652	Morphine	1
Acyclovir	52	Moxifloxacin	1200
Albuterol	0.4	Nitroglycerin	0.02
Amiodarone	6	Norepinephrine	204
Ammonia	1000	Omeprazole	6
Amoxicillin	75	Ondanesteron	0.1
Amphotericin	82	Pancuronium	8
Ascorbic acid	30	Pantoprazole (Protonix)	85
Atorvastatin	80	Phenobarbital	100
Bicarbonates	2940	Phenylephrine	30
Bumetanide	30	Pravastatin	80
Caffeine	60	Prednisone (Prednisolone)	3
Caspofungin	86	Propofol	16
Cefepime	9	Ranitidine	6
Ceftriaxone	810	Riboflavin	12
Cephalexin	117	Rocuronium	126
Ciprofloxacin	10	Theophylline	40
Clopidogrel	225	Tobramycin	24
Dexmedetomidine (Precedex)	0.2	Torsemide	12
Diltiazem (Cardizem)	43	Urobilinogen	12
Dopamine	1	Valproic Acid (Valproate)	499
Doripenem	1050	Vancomycin	100
Epinephrine	6	Vasopressin	5
Ethacrynic acid	19	Vecuronium	21
Ethanol	4000	Warfarin (Coumadin)	10
Fenoldopam	484	Cystatin C	3
Fentanyl	100	Interleukin-18 (IL-18)	0.001
Fluconazole	75	Kidney Injury Molecule 1 (KIM 1)	0.02
Fluvastatin	80	Liver Type Fatty Acid Binding Protein (L-FABP)	1
Furosemide	60	N-acetyl-β-D-glucosaminidase (NAG)	0.00004
Gentamicin	10	Neutrophil Gelatinase Associated Lipocalin (NGAL)	3
Glucose	9909	Pi-Glutathione s-transferase (p-GST)	0.1
Hemoglobin	2000	Calcium	600
Heparin	21	Chloride	5600
Hydralazine	600	Creatinine	1800
Hydrochlorothiazide	6	Magnesium	240
Hydrocodone	0.2	Phosphate	2800
Hydrocortisone	720	Potassium	4000
Ibuprofen	500	Sodium	3600
Insulin	0.003	Sulfate	4800
Ketorolac	166	Urea	32000
		Uric Acid	700

Substances that Exhibited Interference

Urinary albumin at concentrations above 125 mg/dL interfere with the NEPHROCHECK® Test results. Urinary albumin at concentrations above 3000 mg/dL cause an invalid test result. Urinary bilirubin at concentrations above 7.2 mg/dL interfere with the NEPHROCHECK® Test results. Use caution in interpreting NEPHROCHECK® Test results in patients with significant proteinuria or severe hyperbilirubinuria.

Methylene blue at concentrations above 0.49 mg/L interferes with NEPHROCHECK® Test results.

Potential Cross-Reactants

The AKIRISK™ Score was evaluated for cross-reactivity with the following proteins related to the biomarker assays in the NEPHROCHECK® Test (**Table 4**). These related proteins were evaluated at concentrations exceeding physiologically relevant levels. A test sample was prepared for each potentially cross-reacting protein by adding the protein of interest to a human urine pool was prepared to have an AKIRISK™ Score of approximately 0.28 – 0.36. For comparison, the same human urine pool that was used to prepare each test sample was used as a control sample. Each test and control sample was evaluated with 32 or more NEPHROCHECK® Tests. The biomarker concentration results for each test and control sample were compared to determine the percent cross-reactivity (percent cross-reactivity = (measured concentration in test sample – measured concentration in control sample) * 100 / cross reactant concentration) associated with each potentially cross-reacting protein. The results from this testing demonstrated that none of the potential cross-reactants presented below in **Table 4** exhibited significant cross-reactivity.

Table 4. Cross-Reactivity with Related Proteins

Potential Cross-Reactant	Cross-Reactant Concentration (ng/mL)	Potential Cross-Reactant	Cross-Reactant Concentration (ng/mL)
IGFBP-1	0.1	Agrin	1.2
IGFBP-2	0.25	HTRA1	1.2
IGFBP-3	1.2	IGFBPL1	1.2
IGFBP-4	1.2	TIMP-1	3
IGFBP-5	1.2	TIMP-3	2.5
IGFBP-6	1.2	TIMP-4	0.6
IGF-1	1.5	MMP-2	0.03
IGF-2	1.5	MMP-9	0.03
CRIM1	1.2		

Reference Ranges

Reference ranges were determined for two adult (at least 21 years of age) cohorts, apparently healthy subjects and subjects with stable chronic morbidities (without acute illness), and are shown in **Table 5**. Demographic and other information for the two cohorts is shown in **Table 6**. To determine the reference ranges, a urine specimen from each subject was measured with the NEPHROCHECK® Test at three independent laboratories and the results were analyzed independently for each laboratory. Results were comparable across the three laboratories. A wide range in the values of AKIRISK™ Scores was observed in the apparently healthy subjects and subjects with stable chronic morbidities (without acute illness). Because the distributions of AKIRISK™ Scores for apparently healthy subjects, subjects with stable chronic morbidities (without acute illness), and for intended use patients without AKI show overlap with the distribution of AKIRISK™ Scores for intended use patients with AKI, the NEPHROCHECK® Test results cannot be used as standalone results (Refer to the "Clinical Performance" section for further information about the intended use patients cohort). The reference range was defined by the central 95% as described in CLSI guideline C28-A3.[44] The overall reference range for apparently healthy subjects was 0.04 to 2.25 and for subjects with stable chronic morbidities was 0.05 to 2.20 when the results from all three laboratories were combined. The reference ranges were comparable for apparently healthy subjects and subjects with stable chronic morbidities, and for males and females. Each laboratory should establish a reference range that is representative of the patient population to be evaluated.

Table 5. Reference Ranges for Apparently Healthy Subjects and Subjects with Stable Chronic Morbidities by Testing Laboratory for Each of Three Testing Laboratories

		Laboratory 1		Laboratory 2		Laboratory 3	
Cohort	**Gender**	**Number of Subjects**	**AKIRISK™ Score Range***	**Number of Subjects**	**AKIRISK™ Score Range***	**Number of Subjects**	**AKIRISK™ Score Range***
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

*Based on Central 95%

Table 6. Demographic Characteristics of Apparently Healthy and Stable Chronic Morbidity Subjects

	Apparently Healthy Cohort Total N=378		Stable Chronic Morbidity Cohort Total N=372	
	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 (SD)	54	(17.3)	63	(14.7)
Median (IQR)	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
*IQR; interquartile range (Central 95%)

Clinical Performance

The clinical performance of the NEPHROCHECK® Test was evaluated in two clinical studies: Study A with a cohort of 408 intended use patients and Study B with a cohort of 126 intended use patients. The results from each of the two studies are presented in greater detail below, including a comparison of the results between the two studies.

Study A (n = 408 Intended Use Patients)

Clinical performance was evaluated in Study A with a cohort of 408 intended use patients. Adult subjects were prospectively enrolled at 23 geographically diverse hospitals in the United States. Patients with known moderate or severe acute kidney injury were excluded from enrollment. A urine specimen for measurement by the NEPHROCHECK® Test was collected at enrollment, frozen and stored at ≤-70°C until measurement. A urine specimen from each subject was measured with the NEPHROCHECK® Test at three independent laboratories.

Each subject in the intended use patient cohort was adjudicated by a Clinical Adjudication Committee (CAC). Of the 408 intended use patients, 337 (82.6%) were adjudicated as No AKI and 71 (17.4%) were adjudicated as AKI.

The results from the study demonstrate intended use patients with AKIRISK™ Scores ≤ 0.3 are at lower risk of developing moderate to severe AKI within 12 hours of assessment, and intended use patients with AKIRISK™ Scores > 0.3 are at greater risk of developing moderate to severe AKI within 12 hours of assessment.

Study B (n = 126 Intended Use Patients)

Clinical performance was also validated in a second study, Study B (n = 126 for intended use patients). Adult subjects were prospectively enrolled at 6 geographically diverse hospitals in the United States. A urine specimen was collected at enrollment, aliquoted, and the aliquots subjected to different processing conditions and then measured with the NEPHROCHECK® Test System to assess the effects of these various conditions on NEPHROCHECK® Test results.

In a retrospective analysis, each subject in the study was adjudicated by a Clinical Adjudication Committee (CAC) as No AKI or AKI. Of the 126 subjects, 97 (77.0%) were adjudicated as No AKI and 29 (23.0%) were adjudicated as AKI.

Table 7 shows the distribution of AKIRISK™ Scores > 0.3 versus ≤ 0.3 by CAC classification in a 2x2 table for Study A by laboratory and Study B.

Table 7. Comparison of AKIRISK™ Score (> 0.3 and ≤ 0.3) with AKI Status for (A) Laboratory 1 from Study A, (B) Laboratory 2 from Study A, (C) Laboratory 3 from Study A, and (D) Study B. Each laboratory in Study A is missing an AKIRISK™ Score from one subject (a different subject at each site). All 3 subjects without an AKIRISK™ Score were had an AKI Status of No AKI, resulting in 336 subjects No AKI subjects with AKIRISK™ Scores and 407 total subjects with AKIRISK™ Scores from each laboratory.

(A) Laboratory 1, Study A	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

(B) Laboratory 2, Study A	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

(C) Laboratory 3, Study A	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

(D) Study B	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

The data in **Table 8** show that the NEPHROCHECK® Test performance, including high Test sensitivity at the cutoff of > 0.3, was validated in Study A. The data in **Table 8** show a sensitivity (which is the same as true positive rate or TPR), of 90-93%. This means that the AKIRISK™ Score was > 0.3 for 90-93% of the patients who manifested moderate to severe AKI within 12 hours of patient assessment for risk of AKI. The high sensitivity shows that the NEPHROCHECK® Test captured the majority of the AKI (positive) cases. **Table 8** also shows a false negative rate (FNR; equivalent to 1-sensitivity) of 7-10%, indicating that the AKIRISK™ Score was ≤ 0.3 for 7-10% of the patients who manifested moderate to severe AKI within 12 hours of patient assessment for risk of AKI. The data also show a specificity (equivalent to true negative rate or TNR) of 45-49%. This means that the AKIRISK™ Score was ≤ 0.3 for 45-49% of the patients who *did not* manifest moderate to severe AKI within 12 hours of patient assessment for risk of AKI. The data also show a false positive rate (FPR; equivalent to 1 – specificity) of 51-55%, indicating that the AKIRISK™ Score was > 0.3 for 51-55% of the patients (approximately 1 out of every 2 patients) who did *not* manifest moderate to severe AKI within 12 hours of patient assessment for risk of AKI. Therefore an AKIRISK™ Score of > 0.3 may not be predictive of developing moderate to severe AKI within 12 hours of patient assessment.

Table 8. NEPHROCHECK® Test Operating 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
FPR (1-Specificity)	0.54	0.49, 0.60	0.51	0.46, 0.57	0.55	0.50, 0.61
FNR (1- Sensitivity)	0.08	0.02, 0.15	0.10	0.03, 0.17	0.07	0.01, 0.13
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

The data in **Table 9** show the NEPHROCHECK® Test performance at the cutoff of AKIRISK™ Score > 0.3 in Study B.

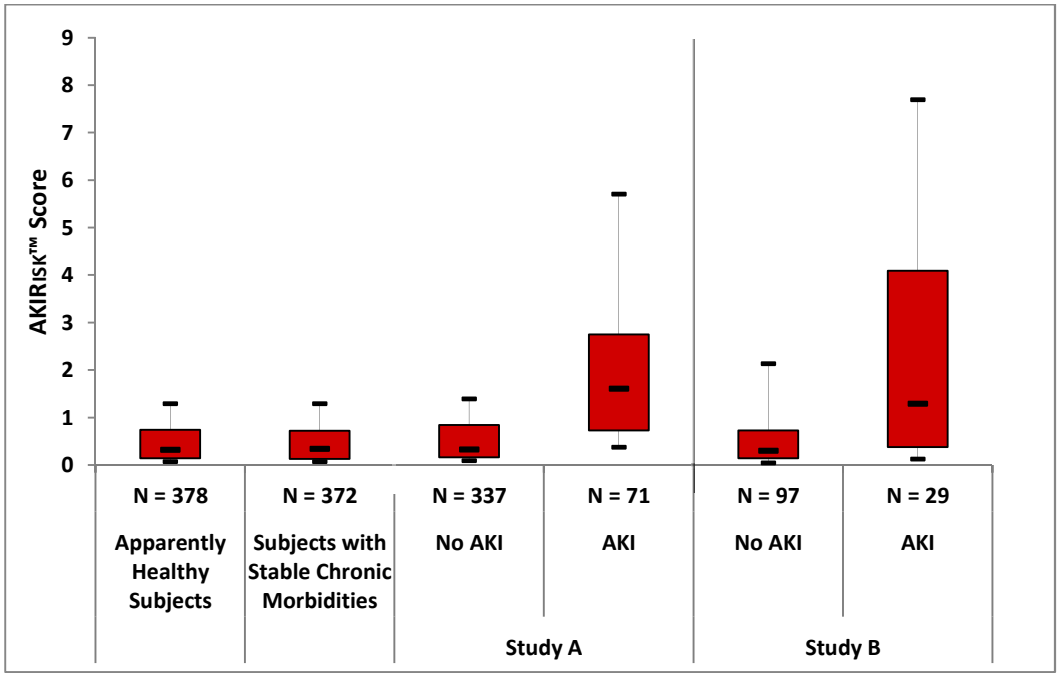
Table 9. NEPHROCHECK® Test Operating Characteristics (Study B)

Statistic	Value	95% CI
Sensitivity (TPR)	0.76	0.60, 0.91
Specificity (TNR)	0.51	0.41, 0.60
FPR (1-Specificity)	0.49	0.40, 0.59
FNR (1- Sensitivity)	0.24	0.09, 0.40
Negative Predictive Value (NPV)	0.88	0.79, 0.96
Positive Predictive Value (PPV)	0.31	0.21, 0.42

In Study A, the range of observed values of AKIRISK™ Scores was wider for Caucasians than for non-Caucasians for each cohort (apparently healthy subjects; subjects with stable chronic morbidities, but without acute illness; and for intended use patients). Logistic covariate analysis was performed to investigate whether race (Caucasian, non-Caucasian) or any of the following additional covariates: covariates, sex, age (≤65, >65) or BMI (≤28.3, >28.3), substantially affected the ability of the NEPHROCHECK® Test to discriminate AKI from No AKI. None of the four covariates had a statistically significant main effect, whereas the NEPHROCHECK® Test was highly significant in all four models ($p<0.0001$), therefore showing the NEPHROCHECK® Test performance is robust with respect to these covariates.

The distributions of AKIRisk™ Scores for the intended use patients from Study A and Study B that were No AKI and AKI are shown in **Figure 1**. Distributions of NEPHROCHECK® Test results for subjects in the apparently healthy and stable chronic morbidity cohorts (described in the Reference Range section) are also shown in **Figure 1** for comparison. Boxes and whiskers show interquartile ranges and 10th to 90th percentiles, respectively. The distributions are comparable for all groups without AKI, i.e. apparently healthy, stable chronic morbidities and No AKI intended use patients from Study A and Study B. These data show that AKIRISK™ Scores are not elevated (relative to results for apparently healthy subjects) for subjects with chronic comorbidities or for intended use patients without AKI. Conversely, AKIRISK™ Scores are substantially elevated for subjects for intended use patients.

Figure 1. AKIRISK™ Scores by Cohort



AKIRISK™ Scores from AKI subjects were significantly greater than those from No AKI subjects in both Studies A and B ($p < 0.0001$), showing the AKIRisk™ Score has significant ability to discriminate AKI from No AKI in both studies AKIRISK™ Scores in Study B were not significantly different from those in Study A ($p > 0.05$ for AKI and for No AKI), showing the AKIRisk™ Scores were not statistically different between the two studies. For No AKI subjects, the variability in AKIRISK™ Scores observed in Study B was slightly smaller than in Study A, as indicated by the slightly smaller interquartile range (25th-75th percentile) in Study B. This smaller variability is associated with a higher numerical point estimate for specificity in Study B compared with Study A (**Tables 8 and 9**). For AKI subjects, the variability in AKIRISK™ Scores observed in Study B is larger than in Study A, as indicated by the larger interquartile range (25th-75th percentile) in Study B. This larger variability is associated with a lower numerical point estimate for sensitivity in Study B compared with Study A (**Tables 8 and 9**).

Point estimates from both Studies (A and B) demonstrate that the NEPHROCHECK® Test identifies the majority of intended use patients at risk for AKI: 76% (sensitivity from Study B) to 90-93% (sensitivity from Study A).

Demographic and other baseline information for the intended use patients for Study A is shown in **Table 10** for all subjects and for AKI and No AKI subjects. The intended use patient cohort represents a diverse and heterogeneous population that is demographically comparable to ICU patient populations in the United States as reported in the literature.[45]

Table 10. Demographic Characteristics of Intended Use Patients for Study A by AKI Classification

	All Total N=408		No AKI Total N=337		AKI Total N=71	
	N, Mean, or Median	%, SD, or IQR*	N, Mean, or Median	%, SD, or IQR*	N, Mean, or Median	%, SD, or IQR*
Sex						
Female	189	(46.3)	153	(45.4)	36	(50.7)
Male	219	(53.7)	184	(54.6)	35	(49.3)
Race						
Asian	2	(0.5)	1	(0.3)	1	(1.4)
Black/African Amer.	56	(13.7)	47	(13.9)	9	(12.7)
Caucasian	339	(83.1)	280	(83.1)	59	(83.1)
Unknown	6	(1.5)	6	(1.8)	0	(0.0)
Other	5	(1.2)	3	(0.9)	2	(2.8)
Ethnicity						
Hispanic	15	(3.7)	11	(3.3)	4	(5.6)
Non-Hispanic	356	(87.3)	295	(87.5)	61	(85.9)
Unknown	37	(9.1)	31	(9.2)	6	(8.5)
Age (years)						
Mean (SD)	63	(16.6)	63	(16.6)	62	(16.4)
Median (IQR)	65	(54–76)	65	(54–76)	64	(54–76)
BMI (kg/m^2)						
Mean (SD)	30.7	(9.22)	29.9	(8.66)	34.1	(10.87)
Median (IQR)	28.3	(24.8–34.4)	27.7	(24.3–33.5)	31.2	(25.8–37.9)
Reason for Hospital Admission‡						
Cardiovascular	146	(35.8)	117	(34.7)	29	(40.8)
Cerebrovascular	47	(11.5)	44	(13.1)	3	(4.2)
Sepsis	77	(18.9)	61	(18.1)	16	(22.5)
Respiratory/Pulmonary	171	(41.9)	142	(42.1)	29	(40.8)
Trauma	46	(11.3)	39	(11.6)	7	(9.9)
Surgery (Any)	120	(29.4)	107	(31.8)	13	(18.3)
Surgery (Emergency)	55	(13.5)	51	(15.1)	4	(5.6)
Surgery (Elective)	65	(15.9)	56	(16.6)	9	(12.7)
Gastrointestinal	49	(12.0)	40	(11.9)	9	(12.7)
Other	125	(30.6)	101	(30.0)	24	(33.8)
Reason for ICU Admission‡						
Cardiovascular	165	(40.4)	133	(39.5)	32	(45.1)
Cerebrovascular	52	(12.7)	48	(14.2)	4	(5.6)
Sepsis	97	(23.8)	75	(22.3)	22	(31.0)
Respiratory	206	(50.5)	170	(50.4)	36	(50.7)
Trauma	44	(10.8)	37	(11.0)	7	(9.9)
Surgery / Post-Op	128	(31.4)	112	(33.2)	16	(22.5)
Other	120	(29.4)	95	(28.2)	25	(35.2)
Type of ICU						
Medical	180	(44.9)	149	(45.0)	31	(44.3)
Surgical	70	(17.5)	59	(17.8)	11	(15.7)
Neurological	14	(3.5)	11	(3.3)	3	(4.3)
Trauma	27	(6.7)	20	(6.0)	7	(10.0)
Coronary Care Unit	10	(2.5)	7	(2.1)	3	(4.3)
Cardiac Surgery	38	(9.5)	33	(10.0)	5	(7.1)
Combined ICU	62	(15.5)	52	(15.7)	10	(14.3)

‡Subjects may have multiple reasons for admission; *IQR; interquartile range (Central 95%).

Literature References

1. Degeorges, A., Wang, F., Frierson, H. F., Jr., Seth, A., and Sikes, R. A. (2000) Distribution of IGFBP-rP1 in normal human tissues. *J. Histochem. Cytochem.* 48, 747-754.
2. Yang, Q. H., Liu, D. W., Long, Y., Liu, H. Z., Chai, W. Z., and Wang, X. T. (2009) Acute renal failure during sepsis: potential role of cell cycle regulation. *J. Infect.* 58, 459-464.
3. Burger, A. M., Leyland-Jones, B., Banerjee, K., Spyropoulos, D. D., and Seth, A. K. (2005) Essential roles of IGFBP-3 and IGFBP-rP1 in breast cancer. *Eur. J. Cancer* 41, 1515-1527.
4. Ahmed, S., Yamamoto, K., Sato, Y., Ogawa, T., Herrmann, A., Higashi, S., and Miyazaki, K. (2003) Proteolytic processing of IGFBP-related protein-1 (TAF/angiomodulin/mac25) modulates its biological activity. *Biochem. Biophys. Res. Commun.* 310, 612-618.
5. Sato, Y., Chen, Z., and Miyazaki, K. (2007) Strong suppression of tumor growth by insulin-like growth factor-binding protein-related protein 1/tumor-derived cell adhesion factor/mac25. *Cancer Sci.* 98, 1055-1063.
6. Usui, T., Murai, T., Tanaka, T., Yamaguchi, K., Nagakubo, D., Lee, C. M., Kiyomi, M., Tamura, S., Matsuzawa, Y., and Miyasaka, M. (2002) Characterization of mac25/angiomodulin expression by high endothelial venule cells in lymphoid tissues and its identification as an inducible marker for activated endothelial cells. *Int. Immunol.* 14, 1273-1282.
7. Sprenger, C. C., Vail, M. E., Evans, K., Simurdak, J., and Plymate, S. R. (2002) Over-expression of insulin-like growth factor binding protein-related protein-1(IGFBP-rP1/mac25) in the M12 prostate cancer cell line alters tumor growth by a delay in G1 and cyclin A associated apoptosis. *Oncogene* 21, 140-147.
8. Nousbeck, J., Sarig, O., Avidan, N., Indelman, M., Bergman, R., Ramon, M., Enk, C. D., and Sprecher, E. (2010) Insulin-like growth factor-binding protein 7 regulates keratinocyte proliferation, differentiation and apoptosis. *J. Invest Dermatol.* 130, 378-387.
9. Unigene, National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.633514.
10. Protein Knowledgebase, UniProtKB/Swiss-Prot; http://www.uniprot.org/uniprot/P16035.
11. Covington, M. D., Burghardt, R. C., and Parrish, A. R. (2006) Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14). *Am. J. Physiol Renal Physiol* 290, F43-F51.
12. Caron, A., Desrosiers, R. R., and Beliveau, R. (2005) Ischemia injury alters endothelial cell properties of kidney cortex: stimulation of MMP-9. *Exp. Cell Res.* 310, 105-116.
13. Mannello, F., Luchetti, F., Falcieri, E., and Papa, S. (2005) Multiple roles of matrix metalloproteinases during apoptosis. *Apoptosis.* 10, 19-24.
14. Le, N. T., Xue, M., Castelnoble, L. A., and Jackson, C. J. (2007) The dual personalities of matrix metalloproteinases in inflammation. *Front Biosci.* 12, 1475-1487.
15. Catania, J. M., Chen, G., and Parrish, A. R. (2007) Role of matrix metalloproteinases in renal pathophysiologies. *Am. J. Physiol Renal Physiol* 292, F905-F911.
16. Stetler-Stevenson, W. G. (2008) Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. *Sci. Signal.* 1, re6.
17. Devarajan, P. (2006) Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol.* 17, 1503–20.
18. Rodier, F., Campisi, J., Bhaumik, D. (2007) Two faces of p53: aging and tumor suppression. *Nucleic Acids Res.* 35, 7475–84.
19. Boonstra, J., Post, J.A. (2004) Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene.* 337, 1–13.
20. Seo, D.W., Li, H., Qu, C.K., Oh, J., Kim, Y.S., Diaz, T., Wei, B., Han, J.W., Stetler-Stevenson, W.G. (2006) Shp-1 mediates the antiproliferative activity of tissue inhibitor of metalloproteinase-2 in human microvascular endothelial cells. *J Biol Chem.* 281, 3711–21.
21. Witzgall, R., Brown, D., Schwarz, C., Bonventre, J.V. (1994) Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest.* 93, 2175–88.
22. Price, P.M., Safirstein, R.L., Megyesi, J. (2009) The cell cycle and acute kidney injury. *Kidney Int.* 76, 604–13.
23. Kashani, K., Al-Khafaji, A., Ardiles, T., Artigas, A., Bagshaw, S.M., et al. (2012) Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care*. 17, R25. http://ccforum.com/content/17/1/R25
24. Uchino, S., Kellum, J. A., Bellomo, R., Doig, G. S., Morimatsu, H., Morgera, S., Schetz, M., Tan, I., Bouman, C., Macedo, E., Gibney, N., Tolwani, A., and Ronco, C. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* **294**, 813-818 (2005).

25. Mehta, R. L., Pascual, M. T., Soroko, S., Savage, B. R., Himmelfarb, J., Ikizler, T. A., Paganini, E. P., and Chertow, G. M. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. *Kidney Int.* **66**, 1613-1621 (2004).

26. Waikar, S. S., Liu, K. D., and Chertow, G. M. Diagnosis, epidemiology and outcomes of acute kidney injury. *Clin. J. Am. Soc. Nephrol.* **3**, 844-861 (2008).

27. Kellum, J. A. Acute kidney injury. *Crit Care Med.* **36**, S141-S145 (2008).

28. Xue, J. L., Daniels, F., Star, R. A., Kimmel, P. L., Eggers, P. W., Molitoris, B. A., Himmelfarb, J., and Collins, A. J. Incidence and mortality of acute renal failure in Medicare beneficiaries, 1992 to 2001. *J. Am. Soc. Nephrol.* **17**, 1135-1142 (2006).

29. McCullough, P. A., Adam, A., Becker, C. R., Davidson, C., Lameire, N., Stacul, F., and Tumlin, J. Epidemiology and prognostic implications of contrast-induced nephropathy. *Am. J. Cardiol.* **98**, 5K-13K (2006).

30. Joannidis, M., Metnitz, B., Bauer, P., Schusterschitz, N., Moreno, R., Druml, W., and Metnitz, P. G. Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. *Intensive Care Med.* **35**, 1692-1702 (2009).

31. Dasta, J. F., Kane-Gill, S. L., Durtschi, A. J., Pathak, D. S., and Kellum, J. A. Costs and outcomes of acute kidney injury (AKI) following cardiac surgery. *Nephrol. Dial. Transplant.* **23**, 1970-1974 (2008).

32. Bagshaw, S. M., George, C., Dinu, I., and Bellomo, R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephrol. Dial. Transplant.* **23**, 1203-1210 (2008).

33. Hoste, E. A., Clermont, G., Kersten, A., Venkataraman, R., Angus, D. C., De, B. D., and Kellum, J. A. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. *Crit Care* **10**, R73 (2006).

34. Chertow, G. M., Burdick, E., Honour, M., Bonventre, J. V., and Bates, D. W. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J. Am. Soc. Nephrol.* **16**, 3365-3370 (2005).

35. Amdur, R. L., Chawla, L. S., Amodeo, S., Kimmel, P. L., and Palant, C. E. Outcomes following diagnosis of acute renal failure in U.S. veterans: focus on acute tubular necrosis. *Kidney Int.* **76**, 1089-1097 (2009).

36. Ishani, A., Xue, J. L., Himmelfarb, J., Eggers, P. W., Kimmel, P. L., Molitoris, B. A., and Collins, A. J. Acute kidney injury increases risk of ESRD among elderly. *J. Am. Soc. Nephrol.* **20**, 223-228 (2009).

37. Mehta, R. L., Kellum, J. A., Shah, S. V., Molitoris, B. A., Ronco, C., Warnock, D. G., and Levin, A. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* **11**, R31 (2007).

38. International Society of Nephrology. (2012) KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int.* ***Suppl. 2****, 1-138.* http://www.kdigo.org/clinical_practice_guidelines/pdf/KDIGO%20AKI%20Guideline.pdf

39. ISO 17511:2003. In vitro diagnostic medical devices-Measurement of quantities in biological samples-Metrological traceability of values assigned to calibrator and control materials. ISO, Geneva, Switzerland.

40. CLSI Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP17-A (ISBN 1-56238-551-8), 2004.

41. CLSI Protocols for Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline. NCCLS Document EP21-A (ISBN 1-56238-502-X) 2003.

42. CLSI Protocols for Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline Second Edition. CLSI Document EP5-A2 (ISBN 1-56238-542-9) 2004.

43. CLSI Protocols for Interference Testing in Clinical Chemistry; Approved Guideline Second Edition. CLSI Document EP7-A2 (ISBN 1-56238-584-4) 2005.

44. CLSI Protocols for Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline Third Edition. CLSI Document C28-A3 (ISBN 1-56238-682-4) 2008.

45. Lilly, C.M., Zuckerman, I.H., Badawi, O. and Riker, R.R. Benchmark Data From More Than 240,000 Adults That Reflect the Current Practice of Critical Care in the United States. *Chest* **140**;1232-1242 (2011).

Ordering and Contact Information

NEPHROCHECK® Test Kit (PN 500011)

For questions regarding the use or performance of the NEPHROCHECK® Test Kit or any Astute Medical, Inc. product, please contact Astute Technical Support.

Astute Medical, Inc.
3550 General Atomics Ct.
Building 2
San Diego, CA 92121
USA
Phone: +1 (855) 317-2788 (Monday thru Friday, 8am – 5pm PST)
Fax: +1 (858) 332-0690
Email: technicalsupport@astutemedical.com
Website: www.astutemedical.com

Symbol Glossary

	Manufacturer
	Consult instructions for use
	*In vitro* diagnostic medical device
	Catalog number
	Batch code
	Use by YYYY-MM-DD
	Do not reuse
	Temperature limitation
	Biological risks
	Sufficient for
	Contents of package
	Conjugate vial
	Buffer vial
	Test Buffer Solution
	RFID Lot
	NEPHROCHECK® RFID Card
	Test Kit

End User License Agreement

PURCHASE AND/OR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THE TERMS AND CONDITIONS OF THIS END USER LICENSE AGREEMENT

Astute Medical, Inc. (together with its affiliates, “Astute”) hereby grants to the purchaser/user (“you”, “your”) of this product the limited license to use this product solely for the purpose as specified on the approved label therefor. You hereby agree that you shall use this product solely for such purpose and for no other purpose.

If you do not agree with each of the terms and conditions set forth in this End User License Agreement, please contact Astute within ten (10) days after

receipt of this product to return the unused and unopened product for a full refund.

LIMITED WARRANTY. FOR THE APPLICABLE WARRANTY PERIOD, ASTUTE WARRANTS THAT THIS PRODUCT SHALL BE (A) OF GOOD QUALITY AND FREE OF MATERIAL DEFECTS, (B) FUNCTION IN ACCORDANCE WITH THE MATERIAL SPECIFICATIONS REFERENCED IN THE PRODUCT MANUAL, AND (C) APPROVED BY THE PROPER GOVERNMENTAL AGENCIES REQUIRED FOR THE SALE OF PRODUCTS FOR THEIR INTENDED USE AS DESCRIBED IN THE APPLICABLE PRODUCT MANUAL OR INSERT THROUGHOUT THE PRINTED EXPIRATION DATE, OR IN THE CASE OF THE ASTUTE140® METER FOR A PERIOD OF TWELVE (12) MONTHS FROM THE DATE OF SHIPMENT (THE "LIMITED WARRANTY"). IF THIS PRODUCT FAILS TO MEET THE REQUIREMENTS OF THIS LIMITED WARRANTY, THEN AS YOUR SOLE REMEDY, ASTUTE SHALL EITHER REPAIR OR REPLACE, AT ASTUTE DISCRETION, THIS PRODUCT.

EXCEPT FOR THE LIMITED WARRANTY STATED IN THIS SECTION, TO THE EXTENT PERMITTED UNDER APPLICABLE LAW, ASTUTE DISCLAIMS ANY AND ALL WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO, ANY WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND NON-INFRINGEMENT REGARDING THIS PRODUCT.

ASTUTE'S MAXIMUM LIABILITY FOR ANY CUSTOMER CLAIM SHALL NOT EXCEED THE NET PRODUCT PRICE PAID THEREFOR.

NO PARTY SHALL BE LIABLE TO ANY OTHER PARTY FOR SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, OR LOSS OF BUSINESS, PROFITS, DATA OR REVENUE, EVEN IF A PARTY RECEIVES NOTICE IN ADVANCE THAT THESE KINDS OF DAMAGES MIGHT RESULT.

The Limited Warranty above shall not apply if this product has been subjected to physical abuse, misuse, abnormal use, use inconsistent with the product manual or insert, fraud, tampering, unusual physical stress, negligence or accidents.

Any warranty claim pursuant to the Limited Warranty shall be made in writing within the applicable Limited Warranty period.

You agree to use this product in strict accordance with all applicable local, state and federal laws, regulations and guidelines, and industry practices.

You further agree that you shall not resell or otherwise transfer this product to any other person or entity, without the prior express written approval of Astute Medical, Inc. Information about commercial resale or distribution of the products of Astute Medical, Inc. may be obtained by e-mailing us at info@astutemedical.com or by writing to us at Astute Medical Inc., General Atomics Court, MS 02/641, San Diego, CA, 92121, USA.

No amendment or addition to this End User License Agreement shall be binding upon the parties unless reduced to writing and signed by the respective authorized officers of the parties.