NEPHROCHECK® Test Kit Package Insert

For Export Only. Not for Sale in the United States.

Manufactured for
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Intended Use

The NEPHROCHECK® Test is an *in vitro* diagnostic device that quantitatively measures TIMP-2 (Tissue Inhibitor of Metalloproteinase 2) and IGFBP-7 (Insulin-like Growth Factor Binding Protein 7) proteins associated with kidney function in human urine by fluorescence immunoassay on the ASTUTE140® Meter. The test result is intended to be used in conjunction with clinical evaluation as an aid in the risk assessment of acute kidney injury in the critically ill. The NEPHROCHECK® Test is indicated for prescription use only.

Summary and Explanation

Acute kidney injury (AKI) is one of the more prevalent and serious morbidities in critically ill hospitalized patients and is associated with a multitude of acute and chronic conditions.[1–6] 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.[7–13] Tests to assess AKI provide important information to physicians and, in conjunction with other available clinical information, can aid physicians in optimizing subject management.[4,13–14]

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, 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 concentrations of TIMP-2 and IGFBP-7 contained within the NEPHROCHECK® Test cartridge into a single numerical result.

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 500003) containing:

- 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 Kit (PN 500000)
- NEPHROCHECK® Liquid Control Kit (PN 500005)
- NEPHROCHECK® Electronic Quality Control (EQC) Device (PN 400013)
- Calibrated precision pipette, capable of dispensing 100 µL

Warnings and Precautions

- For *in vitro* diagnostic use.
- The NEPHROCHECK® Test is intended for use by trained medical professionals.
- 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 and the NEPHROCHECK® Liquid Control 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 entered into the ASTUTE140® Meter using an external keyboard.

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 you encounter damage.
- 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 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 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, us 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 **Accepted**. 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 fresh or frozen 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 10 minutes at 1000 x g at 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.

   NOTE: Samples that will be tested > 20 hours after collection should be centrifuged and the supernatants flash frozen within 2 hours of sample collection. Supernatants should be stored at $\leq$ -70°C (-94°F) for future testing. Avoid repeated freezing and thawing of the supernatant.

Frozen / Refrigerated Samples

1. To test frozen or refrigerated samples, thaw or warm urine samples in a room temperature (18–25°C; 64.4–73.4°F) water bath until thawed and warmed to room temperature but no longer than 20 minutes.

2. Once the sample is thawed and/or warmed to room temperature, gently invert the sample tube 1–2 times to mix sample. Ensure sample is well-mixed before testing. Test the sample immediately after mixing.

   NOTE: Precipitates may be present in sample 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 patient sample into the water bath.

NEPHROCHECK® Test Procedure

NOTE:

- 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).

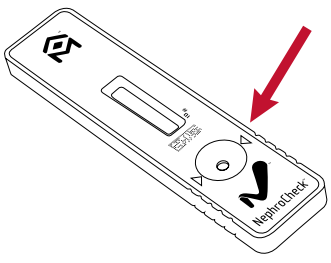
To perform the NEPHROCHECK® Test, follow these steps:

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. Remove the cap from the NEPHROCHECK® Test Conjugate Vial. Visually inspect the cap to ensure that the conjugate bead has not adhered to the cap. 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. 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.

      NOTE: The NEPHROCHECK® Test Buffer Solution is also used to run liquid controls. Do not discard the buffer solution until controls have been successfully passed.
   g. Using a new pipette tip, add 100 µL of centrifuged urine or liquid control sample to the NEPHROCHECK® Test Conjugate Vial. Mix thoroughly (mix at least three times using the pipette tip).
   h. Pipette 100 µL of mixed sample/conjugate solution onto the designated sample port on 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 side of the cartridge, place the cartridge inside 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.

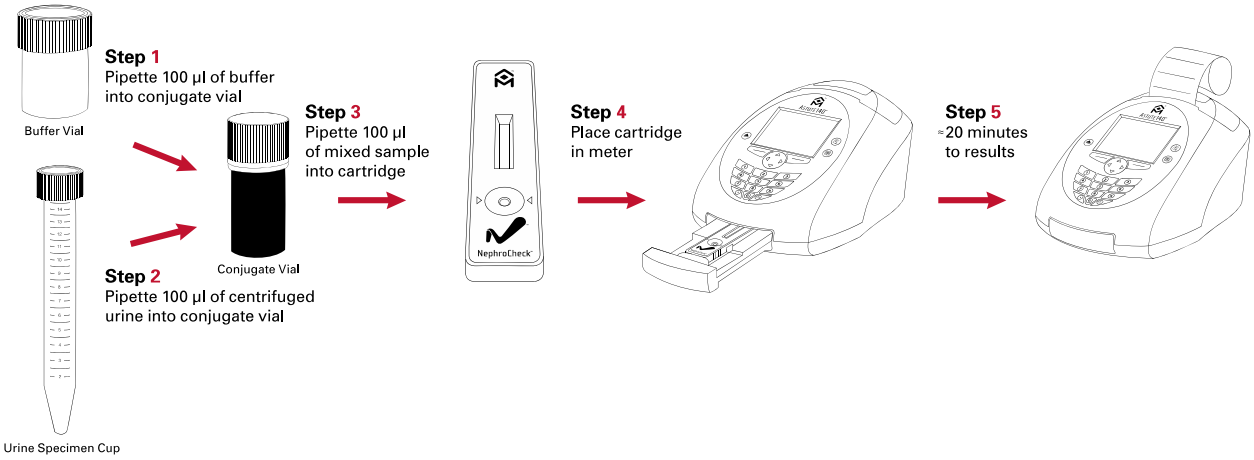
c. Eject the ASTUTE140® Meter drawer. Remove the NEPHROCHECK® Test cartridge and discard it and the conjugate vial in accordance with local regulations.

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").

NEPHROCHECK® Test Preparation Process



Results

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 risk result ("AKIRisk"). The NEPHROCHECK® Test result 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:

$$\text{NEPHROCHECK® Test Result ("AKIRisk")} = ([\text{TIMP-2}] * [\text{IGFBP-7}]) / 1000 \ (\text{units} = (\text{ng/ml})^2/1000)$$

The NEPHROCHECK® Test result 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.

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[15]. 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 internal controls (one positive and one negative control). These positive and negative controls are run automatically with every sample, in order to confirm the integrity of the NEPHROCHECK® Test cartridge and the performance of the ASTUTE140® Meter. If the automatic check of these internal 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 controls are in addition to the external NEPHROCHECK® Liquid Controls.

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

- In accordance with your laboratory standard quality control procedures

Limitations of the NEPHROCHECK® Test Procedure

Test results should be evaluated in the context of all clinical and laboratory data available. In those instances where the test results do not agree with the clinical evaluation, additional tests should be performed accordingly.

Performance Characteristics

Analytical Sensitivity

The limit-of-blank (LoB) was determined for each of the biomarker assays contained within the NEPHROCHECK® Test in accordance with the methods provided in CLSI guideline EP17-A[16]. A blank urine sample was evaluated on a total of 240 tests from three different lots of test kits (80 tests per lot). These data were collected over 40 separate runs that were conducted twice a day over 20 total days of testing. The limit-of-blank is the 95th percentile of the measured results. The limit-of-blank of each assay is presented below in **Table 1:**

Table 1

Biomarker	Limit-of-Blank
TIMP-2	0.6 ng/ml
IGFBP-7	0.7 ng/ml

In addition, the limit-of-detection (LoD) and limit-of-quantitation (LoQ) were also determined for each of the biomarker assays. Six human urine samples that contained low levels of both biomarkers were tested with 60 tests from three lots of test kits (20 tests per lot). These data were collected over 10 separate runs that were conducted twice a day over 5 total days of testing. The measured results were analyzed as described in CLSI guideline EP17-A[16]. Representative results of this analysis are presented below in **Table 2**:

Table 2

Biomarker	Limit-of-Detection	Limit-of-Quantitation
TIMP-2	1.1 ng/ml	1.1 ng/ml
IGFBP-7	3.6 ng/ml	3.6 ng/ml

Linearity

The linearity of the biomarker assays contained in the NEPHROCHECK® Test were evaluated in accordance with CLSI guideline EP6-A[17]. Three urine samples that contained various levels of TIMP-2 and IGFBP-7 were mixed with 3 separate urine samples that contained low levels of TIMP-2 and IGFBP-7. These samples were mixed to prepare 11 test samples with TIMP-2 concentrations from 0.8 ng/ml to 250 ng/ml and 10 test samples with IGFBP-7 concentrations from 26 ng/ml to 620 ng/ml. All samples were tested with at least 9 tests from a single lot of test kits. The concentration results for both TIMP-2 and IGFBP-7 were within 15% of their expected values for all test samples.

Measureable Ranges

TIMP-2: 1.2–225 ng/ml

IGFBP-7: 20–600 ng/ml

NEPHROCHECK® Test Result (“AKIRisk”): 0.02–135

NEPHROCHECK® Test results that are outside the above reportable range are reported as either < 0.02 or > 135.00 by the ASTUTE140® Meter.

Precision

The reproducibility of the biomarker assays contained in the NEPHROCHECK® Test was determined by testing multiple, human urine based control samples with three different lots of NEPHROCHECK® Tests. Testing was completed in accordance with the methods described in CLSI guideline EP5-A2[18]. Each control sample was evaluated on a total of at least 240 tests from three different lots of test kits (80 tests per lot). These data were collected over 40 separate runs that were conducted twice a day over at least 20 total days of testing. Study results were analyzed as described in CLSI guideline EP5-A2[18]. Representative results of this analysis are presented below in **Table 3**.

Table 3

Biomarker	Control Sample	Mean Concentration (ng/ml)	Within-Run Precision		Total Precision	
			SD	%CV	SD	%CV
TIMP-2	Control 1	2.7	0.3	10.7%	0.3	11.4%
	Control 2	139	11.1	8.0%	11.3	8.1%
IGFBP-7	Control 1	37.1	2.9	7.7%	2.9	7.9%
	Control 2	211	13.2	6.3%	14.0	6.6%

Interfering Substances

The following substances listed below in **Table 4** were evaluated for interference with the biomarker assays in the NEPHROCHECK® Test. These substances were evaluated in accordance with the methods described in CLSI guideline EP7-A2[19]. Each substance was added to a human urine pool that contained approximately 3 ng/ml TIMP-2 and 56 ng/ml IGFBP-7. 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 30 or more NEPHROCHECK® Tests. None of these substances interfered with TIMP-2 or IGFBP-7 assay results when added to urine at the concentrations listed below. A bias exceeding 15% (upper limit of 90% confidence interval) is considered a significant interference.

Table 4

Substance	Interferent Concentration
Acetone	12000 µmol/l
Ascorbic Acid	170 µmol/l
Sodium Bicarbonate	35000 µmol/l
Creatinine	442 µmol/l
Ethanol	86800 µmol/l
Glucose	55000 µmol/l
Hemoglobin	2000 µg/ml
Riboflavin	0.012 mg/ml
Urea	42900 µmol/l

The following substances listed below in **Table 5** were evaluated for interference with the biomarker assays contained in the NEPHROCHECK® Test. These substances were evaluated in accordance with the methods described in CLSI guideline EP7-A2[19] as described above. The following substances caused interference when added to urine at concentrations exceeding the interferent concentrations indicated below. A bias exceeding 15% (upper limit of 90% confidence interval) is considered a significant interference.

Table 5

Substance	Interferent Concentration
Albumin	1.25 mg/ml
Bilirubin, Conjugated	85.5 µmol/l

Interfering Conditions

The effect of urine sample pH was evaluated for each of the biomarker assays contained on the NEPHROCHECK® Test. Two human urine pools were adjusted to multiple pH values between pH 4 and 10. One urine pool contained approximately 3 ng/ml TIMP-2 and 60 ng/ml IGFBP-7. The other urine pool contained approximately 125 ng/ml TIMP-2 and 250 ng/ml IGFBP-7. For both urine pools, urine sample pH did not impact TIMP-2 or IGFBP-7 assay results. A bias exceeding 15% (upper limit of 90% confidence interval) is considered a significant interference.

Pharmaceuticals

The following pharmaceuticals listed below in **Table 6** were evaluated for interference with the biomarker assays in the NEPHROCHECK® Test. These pharmaceuticals were evaluated in accordance with the methods described in CLSI guideline EP7-A2[19]. Each pharmaceutical was added to a human urine pool containing approximately 3 ng/ml TIMP-2 and 56 ng/ml IGFBP-7. 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 30 or more NEPHROCHECK® Tests. None of these substances interfered with TIMP-2 or IGFBP-7 assay results when added to urine at the concentrations listed below. A bias exceeding 15% (upper limit of 90% confidence interval) is considered a significant interference.

Table **6**

Test Substance	Interferent Concentration
Acetaminophen	1324 µmol/l
Amoxicillin	206 µmol/l
Aspirin	3620 µmol/l
Caffeine	308 µmol/l
Ciprofloxacin	30.2 µmol/l
Dopamine	5.87 µmol/l
Fentanyl	297 µmol/l
Furosemide	181 µmol/l
Heparin	3000 unit/l
Hydrocodone	0.67 µmol/l
Ibuprofen	2425 µmol/l
Insulin	0.071 unit/l
Lisinopril	0.74 µmol/l
Metoprolol	18.7 µmol/l
Midazolam	3.5 µmol/l
Morphine	1.75 µmol/l
Ondansetron	0.39 µmol/l
Propofol	89.8 µmol/l
Vancomycin	69 µmol/l

The following substances listed below in **Table 7** were evaluated for interference with the biomarker assays contained in the NEPHROCHECK® Test. These substances were evaluated in accordance with the methods described in CLSI guideline EP7-A2[19] as described above. The following substances caused interference when added to urine at concentrations exceeding the interferent concentrations indicated below. A bias exceeding 15% (upper limit of 90% confidence interval) is considered a significant interference.

Table 7

Test Substance	Interferent Concentration
Methylene Blue	1.3 µmol/l

Potential Cross-Reactants

The biomarker assays in the NEPHROCHECK® Test were evaluated for cross-reactivity with the following related proteins. A test sample was prepared for each potentially cross-reacting protein. This sample was prepared by adding the protein of interest to a human urine pool containing approximately 3 ng/ml TIMP-2 and 56 ng/ml IGFBP-7. 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 30 or more NEPHROCHECK® Tests. The biomarker concentration results for each test and control sample were compared to determine the percent cross-reactivity associated with each potentially cross-reacting protein. The results from this testing are presented below in **Table 8**.

Table 8

Cross-Reactivity with Related Proteins			
Potential Cross-Reactant	Cross-Reactant Concentration (ng/mL)	TIMP-2 % Cross-reactivity	IGFBP-7 % Cross-reactivity
IGF-1	1500	--	0
IGF-2	1500	--	0
IGFBP-1	100	--	-1.1%
IGFBP-2	250	--	-0.8%
TIMP-1	3000	0	--
TIMP-3	2500	0	--
TIMP-4	600	0	--

Clinical Performance

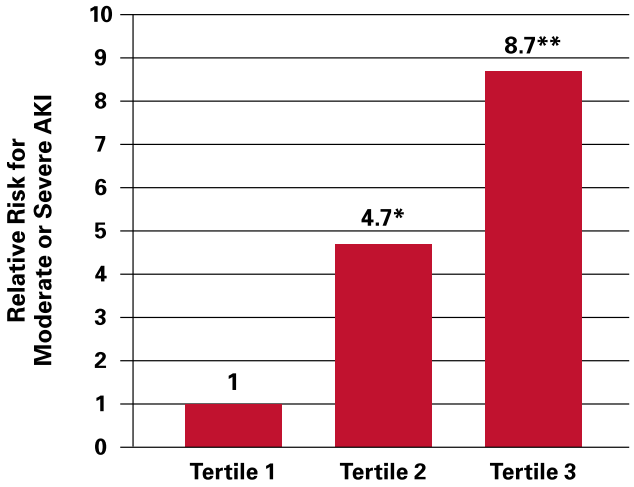
Critically Ill Study Cohort

Urine samples collected from critically ill adult subjects were used to validate the NEPHROCHECK® Test as an aid in the risk assessment of AKI in the critically ill. These samples were collected in three distinct cohorts at two sites in North America and one site in Europe. The sites enrolled subjects who were in the ICU or about to be admitted to the ICU and who had at least one risk factor for AKI, such as sepsis, hypotension, major trauma, hemorrhage, radiocontrast exposure or intravenous antibiotics. Data from the three cohorts were pooled to form the study cohort. Each subject in the study cohort had up to three urine biomarker samples collected within 18 hours after the time of enrollment. The study cohort comprised 586 subjects; 58% were male, 92% were white/Caucasian, and the mean (±SD) age was 62 (±15) years.

AKI status was determined using the full RIFLE criteria (based on serum creatinine and urine output values)[1]. An observation of RIFLE-I or RIFLE-F within the 12 hour interval starting from the time of each sample collection to 12 hours after the collection was classified as positive for moderate or severe AKI while absence of RIFLE-I or RIFLE-F within the 12 hour interval was classified as negative for moderate or severe AKI for the sample. Of the 586 subjects in the study cohort, 64 were classified as positive for moderate or severe AKI for at least one sample collection.

NEPHROCHECK® Test results for study cohort samples were divided into tertiles defined by the 33rd and 67th percentiles of values obtained for the entire study cohort. The 33rd and 67th percentiles corresponded to NEPHROCHECK® Test results of 0.14 and -0.43, respectively. The risk (corresponding to probability) of moderate or severe AKI was calculated for each tertile and was found to increase monotonically ($p < 0.0001$) with increasing tertile as follows: for tertile 1, risk = 2.1%; for tertile 2, risk = 9.8%; for tertile 3, risk = 18.2%. The relative risk of AKI was 4.7 and 8.7 for the second compared to the first tertile and the third compared to the first tertile, respectively (**Figure 1**).

Figure 1. Relative risk for moderate or severe AKI by tertiles of NEPHROCHECK® Test results. *p < 0.0001 for risk relative to the first tertile, **p < 0.0001 for risk relative to the first tertile and p < 0.03 for risk relative to the second tertile.



Apparently Healthy Cohort

NEPHROCHECK® Test results for urine samples collected from 383 apparently healthy adult subjects were used to establish the reference range for healthy subjects. Of this cohort, 45.6% were male and 68.1% were white/Caucasian. The mean (±SD) age was 57 (±16) years. Reference ranges were determined using the nonparametric method. The reference range corresponding to the 2.5th to 97.5th percentile was 0.03 to 1.93 for healthy subjects (**Table 9**). NEPHROCHECK® Test results at other commonly reported percentiles are provided in **Table 9**. For comparison, **Table 9** also provides results for samples collected from the subjects in the critically ill study cohort, grouped by maximum RIFLE stage within 12 hours of sample collection. These reference ranges are provided as guidelines only and are not intended to be critical values or medical decision limits. Each laboratory should establish its own reference intervals. Guidance for establishing reference intervals can be found in CLSI Guideline C28-A3c.[20]

Table 9. NEPHROCHECK® Test results at specified percentiles determined for samples collected from Healthy Subjects and Critically Ill Subjects. Samples from Critically Ill Subjects were grouped by maximum RIFLE stage within 12 hours of sample collection.[21]

	NEPHROCHECK® Test Results ("AKIRISK™")			
Percentile	Healthy Subjects	Critically Ill Subjects		
		No AKI	RIFLE R	RIFLE I or F
5	0.03	0.02	0.03	0.10
10	0.03	0.03	0.06	0.18
25	0.07	0.08	0.18	0.27
50	0.22	0.21	0.36	0.70
75	0.58	0.48	0.80	3.23
90	1.00	1.02	1.39	8.77
95	1.34	1.57	2.49	20.24
97.5	1.93	2.63	4.36	34.67

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