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VIDAS® NEPHROCHECK® (NEPH)

℞ only IVD

Intended Use

VIDAS® NEPHROCHECK® is an automated test for use on the VIDAS® 3 instrument for the immunoenzymatic quantitative determination of TIMP-2 (Tissue Inhibitor of Metalloproteinase-2) and IGFBP-7 (Insulin-like Growth Factor-Binding Protein 7) proteins in human urine using the ELFA technique (Enzyme Linked Fluorescent Assay) for calculation of the AKIRISK™ Score.

The VIDAS® NEPHROCHECK® assay 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 VIDAS® NEPHROCHECK® test is intended to be used in patients 21 years of age or older.

Summary and Explanation

Insulin-like Growth Factor-Binding Protein 7 (IGFBP-7) is a soluble protein of about 26 kilodaltons (kDa) 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 22 kDa 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.

It has been demonstrated that, similar to other epithelia, renal tubular cells enter a short period of G1 cell cycle arrest following injury from experimental sepsis or ischemia.[2,21] 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.

AKI is one of the more prevalent and serious morbidities in hospitalized patients and is associated with a multitude of acute and chronic conditions.[23-28] The economic and public health burden of AKI is staggering with substantially increased mortality, morbidity, length of intensive care unit stay and in-hospital costs, as well as longer term health consequences.[29-35]

Tests to assess AKI provide important information to physicians and, in conjunction with other available clinical information, can aid physicians in optimizing patient management.[26,35-37]

Principle

The assay principle combines a one-step sandwich enzyme immunoassay method with a final fluorescence detection (ELFA).

The single-use Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed single-use reagent strips.

All of the assay steps are performed automatically by the instrument.

The sample is transferred into the well containing anti-IGFBP-7 and anti-TIMP-2 antibodies labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture is cycled in and out of the SPR device several times. This

operation enables the two proteins to bind with the immunoglobulins that are fixed to the interior wall of the SPR device and to the conjugate to form a sandwich.

Unbound components are eliminated during washing steps.

Two detection steps are carried out successively. During each detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR device. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm.

For each protein, the intensity of the fluorescence is proportional to the concentration in the sample. At the end of the assay, the protein concentrations are calculated by the VIDAS® 3 instrument in relation to the two calibration curves, one corresponding to each protein, and encoded in the MLE (Master Lot Entry) data. The concentration from each protein, TIMP-2 and IGFBP-7, is converted into a single numerical result called the AKIRISK™ Score.

The VIDAS® NEPHROCHECK® Test result, known as the AKIRISK™ Score, is automatically calculated by the instrument as the product of the concentrations of the two biomarkers, in ng/mL, divided by 1000, according to the following formula:

AKIRISK™ Score = (TIMP-2 * IGFBP-7)/1000.

The AKIRISK™ Score is displayed on the instrument after the assay procedure is completed. The concentrations of the individual proteins are not displayed. The AKIRISK™ Score is displayed without units.

The results can then be printed out.

Content of the Kit (60 tests)

60 Strips[a] (NEPH)	STR	Ready-to-use.
60 SPR devices (NEPH) 2 x 30	SPR	Ready-to-use. Interior of SPR devices coated with: mouse monoclonal IgG anti-IGFBP-7 and anti-TIMP-2 + stabilizer of animal origin + preservative.
S1 Calibrator[b] (NEPH) 1 x 1.6 mL (liquid)	S1	Ready-to-use. Buffer containing proteins + stabilizer of animal origin + preservative. For each protein, MLE (Master Lot Entry) data indicate the acceptable range in "Relative Fluorescence Value" ["Calibrator (S1) RFV Range"].
C1 Control[b] (NEPH) 1 x 1.2 mL (liquid)	C1	Ready-to-use. Buffer containing proteins + stabilizer of animal origin + preservative. MLE data indicate the acceptable range for AKIRISK™ Score ["Control (C1) Score Value Range"].
Specifications for the factory master data required to calibrate the assay: MLE barcode printed on the box label.		
1 package insert downloadable from www.biomerieux.com		


[a] **DANGER** **WARNING** H290 / H315 / H317 / H318 / H319 / H335 / EUH208 / P261 / P280 / P302 + P352 / P305 + P351 + P338

[b] **WARNING** H317 / EUH208 / P261 / P280 / P302 + P352

Hazard statements

- H290: May be corrosive to metals.
- H315: Causes skin irritation.
- H317: May cause an allergic skin reaction.
- H318: Causes serious eye damage.
- H319: Causes serious eye irritation.
- H335: May cause respiratory irritation.
- EUH208: Contains 2-methyl-2H-isothiazolin-3-one. May produce an allergic reaction.

Precautionary statements

- P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.
- P302 + P352: IF ON SKIN: Wash with plenty of soap and water.
- P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, consult the Safety Data Sheet.

The SPR device
The interior of the SPR device is coated during production with anti-IGFBP-7 and anti-TIMP-2 monoclonal antibodies (mouse). Each SPR device is identified by the "NEPH" code. Only remove the required number of SPR devices from the pouch and carefully reseal the pouch after opening.

The Reagent Strip
The strip consists of 10 wells covered with a labeled foil seal. The label comprises a barcode which mainly indicates the assay code, kit lot number, and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the VIDAS® NEPHROCHECK® (NEPH) Strip
The strip contains diethanolamine and sodium azide. Refer to the hazard statements “H” and precautionary statements “P” indicated above.[(a)]

Well	Reagents
1	Sample well: dispense 100 µL of calibrator, control, or sample.
2	Conjugate: buffer containing alkaline phosphatase-labeled anti-IGFBP-7 monoclonal IgG (mouse) + alkaline phosphatase-labeled anti-TIMP-2 monoclonal IgG (rabbit) + stabilizer of animal origin + preservative.
3	Wash buffer containing preservative.
4	Empty well.
5	Acid Wash buffer.
6-7-8	Wash buffer containing preservative.
9	Substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + preservative.
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + preservative.

Materials and Disposables Required but Not Provided

- Single-use pipette and/or micropipettes to dispense the appropriate volumes.
- Powderless disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instrument of the VIDAS® family: VIDAS® 3 with version 1.3.2 minimum, or higher.

Warnings and Precautions

- **For *in vitro* diagnostic use only.**
- **For professional use only, by qualified laboratory personnel in clinical laboratories.**
- **For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.**
- This kit does not contain products of human origin.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use the SPR devices if the pouch is pierced or if the dot sealing a SPR device has come unstuck.
- Do not use visibly deteriorated strips (damaged foil or plastic).

- Do not use reagents after the expiration date indicated on the box label.
- Do not mix reagents (or disposables) from different lots.
- VIDAS® NEPHROCHECK® assay reagents are only for use with the VIDAS® 3 instrument.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- Refer to the hazard statements “H” and precautionary statements “P” indicated above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (refer to the User Manual for user and preventive maintenance operations).

Storage Conditions

- Store the kit at +2°C/+8°C.
- Do not freeze reagents.
- **Store all unused reagents at +2°C/+8°C.**
- After opening the kit, check that the SPR pouches are correctly sealed and undamaged. If not, do not use the SPR devices.
- **After use, carefully reseal the pouch with the desiccant inside to maintain stability of the SPR devices, and return the complete kit to +2°C/+8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the box label, **except for SPR devices that are stable for 10 months at +2°C/+8°C after opening.**

Samples

Specimen type and collection

Urine (fresh or frozen). Adult human specimen only.

Types of tubes validated

- For sample collection: plastic tube or cup with no additive.
- After centrifugation: plastic tube with no additive.

It is recommended to validate collection tubes or cups before use as some contain substances which interfere with test results.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer’s recommendations for use.

Fresh sample preparation

The current WHO/DIL/LAB/99.1 document provides recommendations for sample preparation. [38]

For use of sample tubes, refer to the tube manufacturer’s recommendations for use.

1. Collect a fresh urine sample of approximatively 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.
2. Urine samples should be centrifuged within one hour of sample collection. If the urine sample is collected in a collection cup, mix the sample thoroughly by inverting the container 3 times, and then transfer the urine sample from the specimen collection cup into a clean centrifuge tube. Centrifuge the urine sample for 10 minutes at 1000 x g at +2°C/+25°C.
3. After centrifuging the sample, transfer the supernatant into a clean tube.
4. Test the supernatant within 5 hours of sample collection. If storage for more than 5 hours is needed, transfer the supernatant into a low binding protein tube.
5. If testing is completed within 24 hours, the sample must be stored at +2°C/+8°C.
6. If storage for more than 24 hours is needed, flash freeze the sample within 5 hours of sample collection, and store it at ≤ −60°C.

Frozen or refrigerated sample preparation
To test a frozen or refrigerated sample, thaw or warm the urine sample at room temperature (+18°C/+25°C), but no longer than 60 minutes. Ensure that the sample is properly thawed or warmed. After thawing, as precipitates may be present in the sample tube, gently invert the sample tube at least 3 times to homogenize the sample. Do not use a vortex-like mixer. Ensure that the sample is well-homogenized before testing to ensure accurate measurement results. **Test immediately after homogenizing.**

Sample stability
Fresh samples stored in closed primary tubes are stable at +18°C/+25°C for up to 5 hours. Fresh samples stored in low binding protein tubes are stable at +2°C/+8°C for up to 24 hours.

Frozen samples are stable at ≤ −60°C for up to 6 months, including up to 2 freeze/thaw cycles.

Do not store the frozen samples at temperatures above −60°C.

Sample dilution
Do not dilute the samples before using the VIDAS® NEPHROCHECK® assay.

Sample-related interference
It is recommended not to use turbid samples, and, if possible, to collect a new sample.

Refer to the section **PERFORMANCE – Study of drugs and other potentially interfering substances** for the compounds tested.

Instructions for Use

For complete instructions, see the Instrument User Manual.

Reading VIDAS® PTC (Protocol Test Change) data and MLE data
When using the assay for the first time

With the external instrument barcode reader, **scan the barcodes (PTC and MLE) in the following order**:
1. Scan the PTC barcode(s), downloadable from www.biomerieux.com. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data on the box label.

When opening a new lot of reagents

With the external instrument barcode reader, scan the MLE data on the box label before performing the test.

Note: The master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** (refer to the User Manual).

Calibration
Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the MLE data have been entered, and then **every 56 days**. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf life of the kit.

The calibrator, identified by S1, must be tested in duplicate. For each protein, the calibrator value must be within the set RFV (Relative Fluorescence Value) range. If this is not the case, recalibrate using S1.

Kit controls
One control is included in each VIDAS® NEPHROCHECK® kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.

Results cannot be validated if the control value deviates from the expected values.

Note: The aim of the Kit Control is to validate calibration. Any other use of the Kit Control is under the customer's responsibility.

Procedure
1. **Remove the kit from storage at +2°C/+8°C and take out the required reagents. Carefully reseal the SPR pouch and return the kit to +2°C/+8°C.** The reagents can be used immediately.
2. Use one "NEPH" strip and one "NEPH" SPR device for each sample, control or calibrator to be tested. Make sure the SPR pouch has been carefully resealed after the required SPR devices have been removed.
3. The test is identified by the "NEPH" code on the instrument. The calibrator, identified by S1, must be tested in duplicate. The control, identified by C1, must be tested singly.

4. Mix the calibrator and control using a vortex-type mixer, or by inverting the vials at least 3 times.
5. **Do not mix samples using a vortex-type mixer. For optimal results, refer to all the paragraphs in the SAMPLES section.**
6. Before pipetting, ensure that the samples are free of bubbles.
7. For this test, the calibrator, control, and sample test portion is 100 µL.
8. Insert the "NEPH" SPR devices and "NEPH" strips into the instrument. Check to make sure the color labels with the assay code on the SPR devices and the Reagent Strips match.
9. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.
10. Close the vials and return them to the required temperature after pipetting.
11. The assay will be completed within **approximately 46 minutes.** After the assay is completed, remove the SPR devices and strips from the instrument.
12. Dispose of the used SPR devices and strips into an appropriate recipient.

Quality Control

Additional quality controls can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

Results and Interpretation

Once the assay is completed, results are analyzed automatically by the computer. For each protein, fluorescence is measured twice in the reagent strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR device is introduced into the substrate.

The second reading is taken after incubating the substrate with the enzyme that may be bound to the interior of the SPR device. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The concentrations of the two proteins are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistics model).

The AKIRISK™ Score is calculated by the instrument as the product of the measured concentrations of the two proteins, TIMP-2 and IGFBP-7 (measured as ng/mL), divided by 1000:

AKIRISK™ Score = ([TIMP-2] * [IGFBP-7])/1000 (units = $(ng/mL)^2/1000$).

The AKIRISK™ Score is displayed on the instrument after the assay procedure is completed. The concentrations of the individual proteins are not displayed. The AKIRISK™ Score is displayed without units.

Metrological traceability
Calibration of the VIDAS® NEPHROCHECK® assay is traceable to in-house reference calibrators for each of the two proteins.

Threshold and interpretation of results
A single cutoff of AKIRISK™ Score > 0.30 for the VIDAS® NEPHROCHECK® assay has been established based on the results from previous clinical studies

The AKIRISK™ Score displayed by the VIDAS® 3 instrument is based upon results from clinical testing among intended use patients.

The test results are interpreted as described in the following table:

AKIRISK™ Score	Interpretation
≤ 0.30	Intended use patients might not develop moderate to severe AKI within 12 hours of evaluation. Based on results from clinical testing*, intended use patients with AKIRISK™ Score ≤ 0.30 are at lower risk of developing moderate to severe AKI within 12 hours of assessment than intended use patients with AKIRISK™ Score > 0.30.

AKIRISK™ Score	Interpretation
> 0.30	Intended use patients could develop moderate to severe AKI within 12 hours of evaluation. Based on results from clinical testing*, intended use patients with AKIRISK™ Score > 0.30 are at increased risk of developing moderate to severe AKI within 12 hours of assessment than intended use patients with AKIRISK™ Score ≤ 0.30

* The data from Study A show a negative predictive value (NPV) of 95.5%, a false negative rate (FNR) of 10.1%, a positive predictive value (PPV) of 25.5%, and a false positive rate (FPR) of 54.8% (refer to CLINICAL PERFORMANCE section). The data from Study B show a negative predictive value (NPV) of 88.6%, a false negative rate (FNR) of 17.2%, a positive predictive value (PPV) of 29.3%, and a false positive rate (FPR) of 59.8%.

Limitations of the Method

1. The VIDAS® NEPHROCHECK® assay should not be used as a "standalone test" as interference may be encountered with certain samples containing antibodies directed against reagent components.
2. The VIDAS® NEPHROCHECK® assay result must be evaluated with other clinical and laboratory test information.
3. Urine samples with AKIRISK™ Scores between 0.30 and 0.46 should also be tested with a urine dipstick test for the presence of hematuria. If the blood result is high positive (3+) or the urine sample has some indications of blood in the urine (e.g. pink/red color), the AKIRISK™ Score may be falsely elevated, and should not be used. Hemoglobin levels above 60 mg/L can lead to a 20% increase in the AKIRISK™ Score.

Reference Intervals

A reference interval study was performed based on the CLSI EP28-A3c recommendations.[39]

Reference intervals were determined independently for two adult cohorts, apparently healthy subjects (N=378) and subjects with stable chronic morbidities (without acute illness) (N=372).

The reference intervals were defined by the central 95% of the distribution of AKIRISK™ Scores, i.e. by the 2.5th and 97.5th percentiles. The results are presented in the table below.

Reference Intervals for Apparently Healthy Subjects and Subjects with Stable Chronic Morbidities

Cohort	Gender	Number of Subjects	AKIRISK™ Score Interval
Apparently Healthy Subjects	Female	191	[<0.04-2.81]
	Male	187	[<0.04-3.06]
	All	378	[<0.04-2.50]
Subjects with Stable Chronic Morbidities	Female	191	[<0.04-2.91]
	Male	181	[<0.04-2.63]
	All	372	[<0.04-2.66]

The overall reference interval for apparently heathy subjects was < 0.04 to 2.50. The overall reference interval for subjects with stable chronic morbidities was < 0.04 to 2.66. The reference intervals were comparable for apparently healthy subjects and subjects with stable chronic morbidities, and for males and females.

Demographic and other information for the two cohorts is shown in the table below.

Demographic Characteristics of Apparently Healthy and Stable Chronic Morbidity Subjects

Demographic Characteristics		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
Age (years)	Mean (SD)*	54.1	(17.3)	62.7	(14.7)
	Median (IQR)**	56.0	(40.0-68.0)	65.0	(53.0-75.0)

Demographic Characteristics		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
BMI (kg/m^2)	Mean (SD)*	27.5	(5.9)	30.8	(7.0)
	Median (IQR)**	26.8	(23.3-29.8)	29.8	(26.2-34.5)
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 American	43	(11.4)	43	(11.6)
	Native Hawaiian****	1	(0.3)	3	(0.8)
	White or 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)

*SD: Standard Deviation

**IQR: Interquartile Range (Central 50%)

***Includes Alaskan Native

****Includes Other Pacific Islander

Performance

Studies performed using the VIDAS® NEPHROCHECK® assay gave the following results.

Analytical Measuring Interval (AMI)

The Analytical Measuring Interval (AMI) is the range of values corresponding to the acceptable performance limits (precision and linearity).

The AMI for the AKIRISK™ Score is [0.04-10.00].

AKIRISK™ Scores that are outside the above reportable interval are reported as either < 0.04 or > 10.00 by the VIDAS® NEPHROCHECK® assay. If the AKIRISK™ Score is > 10.00, **the sample should not be diluted for retesting.**

Linearity

The TIMP-2 and IGFBP-7 proteins used to derive the AKIRISK™ Score were assessed and found to be linear on the measuring interval of the AKIRISK™ Score. However, the AKIRISK™ Score itself is not expected to be linear. Linearity was evaluated according to CLSI EP06-A recommendations.

Detection limits

	AKIRISK™ Score
Limit of Blank (LoB)	0.002
Limit of Detection (LoD)	0.003
Limit of Quantitation (LoQ)	0.003

LoB, LoD, and LoQ values were determined according to the CLSI EP17-A2 recommendations for each of the two proteins TIMP-2 and IGFBP-7. LoB, LoD, and LoQ values for the AKIRISK™ Score were derived from the values of the two proteins.

The Limit of Blank (LoB) is the concentration below which 95% of analyte-free samples are found.

The Limit of Detection (LoD) is the lowest concentration of analyte in a sample that can be distinguished from the analyte-free sample with a probability of 95% (observed result greater than the LoB with a 95% probability).

The Limit of Quantitation (LoQ) is the lowest concentration of analyte that can be detected and measured with an acceptable level of precision. For the VIDAS® NEPHROCHECK® assay, the acceptable level of precision corresponds to within-lot precision fixed at 20% CV for each of the two proteins TIMP-2 and IGFBP-7.

Precision

A precision study was performed according to CLSI EP05-A3 recommendations.[40]

A panel of human samples representing seven AKIRISK™ Score levels within the analytical measuring interval was analyzed on the VIDAS® 3 instrument. The following sources of variability were studied: repeatability, run, day, calibration, and lot/instrument/site.

Repeatability (within-run precision), within-lot/instrument/site precision, and total precision (between-lot/instrument/site) were estimated for each sample.

The values obtained with the VIDAS® 3 instrument are reported in the following table, as a guide.

Sample	N	Mean	Repeatability (Within-run precision)		Within-lot/instrument/site precision		Total precision (between-lot/instrument/site)	
			Standard Deviation	CV (%)*	Standard Deviation	CV (%)*	Standard Deviation	CV (%)*
Sample 1	240	0.05	0.01	18.2	0.01	18.2	0.01	22.1
Sample 1**	239	0.05	0.01	10.7	0.01	10.7	0.01	17.4
Sample 2	240	0.05	0.01	10.5	0.01	12.5	0.01	16.1
Sample 3	240	0.19	0.01	5.7	0.01	6.7	0.01	7.3
Sample 4	240	0.71	0.04	5.5	0.05	6.8	0.07	10.2
Sample 5	240	1.73	0.07	3.9	0.08	4.9	0.14	7.9
Sample 6	240	4.28	0.19	4.5	0.22	5.0	0.24	5.6
Sample 7	240	7.48	0.37	4.9	0.40	5.3	0.49	6.5

* CV (%): Coefficient of Variation (%)

** An outlier with an AKIRISK™ Score of 0.15 was identified for Sample 1. The imprecision with and without the outlier is presented.

Analytical specificity

The analytical specificity of the VIDAS® NEPHROCHECK® assay was established by testing cross-reactive compounds according to CLSI document EP7-Ed3 recommendations. Cross-reactivity was evaluated by spiking urine samples with cross-reactive compounds.

The results demonstrated that none of the potential cross-reactants presented below exhibited significant cross-reactivity.

Potential Cross-Reactant	Cross-Reactant Concentration (mg/L)
Insulin like growth factor-Binding Protein 1 (IGFBP-1)	0.1
Insulin like growth factor-Binding Protein 2 (IGFBP-2)	0.25
Insulin like growth factor-Binding Protein 3 (IGFBP-3)	1.2
Insulin like growth factor-Binding Protein 4 (IGFBP-4)	1.2
Insulin like growth factor-Binding Protein 5 (IGFBP-5)	1.2
Insulin like growth factor-Binding Protein 6 (IGFBP-6)	1.2
Insulin like growth factor 1 (IGF-1)	1.5
Insulin like growth factor 2 (IGF-2)	1.5
Cysteine-rich motor neuron 1 protein (CRIM1)	1.2
Agrin	1.2
Serine protease HTRA1 (HTRA1)	1.2
Insulin-like growth factor binding protein-like 1 (IGFBPL1)	1.2
Metalloproteinase inhibitor 1 (TIMP-1)	3
Metalloproteinase inhibitor 3 (TIMP-3)	2.5

Potential Cross-Reactant	Cross-Reactant Concentration (mg/L)
Metalloproteinase inhibitor 4 (TIMP-4)	0.6
Matrix Metalloproteinase-2 (MMP-2)	0.03
Matrix Metalloproteinase-9 (MMP-9)	0.03

Study of drugs and other potentially interfering substances in urine samples

Potential interference by commonly used drugs and other substances was studied according to CLSI EP7-Ed3 recommendations.

None of the substances exhibited significant interference with AKIRISK™ Score at the maximum test concentrations listed below.

Drugs and Endogenous Substances	Maximum concentration (mg/L)
Acetaminophen (Paracetamol)	201
Acetone	697
Acetylcysteine	1665
Acetylsalicylic acid (Aspirin)	652
Acyclovir	66
Albumin	6900
Albuterol	0.4
Alkaline Phosphatase	600 U/L
Amiodarone	42
Ammonia	1000
Amoxicillin	75
Amphotericin	82
Ascorbic acid	52.5
Atorvastatin	80
Bicarbonates	2940
Bilirubin (conjugated)	400
Bumetanide	30
Caffeine	108
Caspofungin	86
Cefepime	9
Ceftriaxone	840
Cephalexin	126
Ciprofloxacin	12
Clopidogrel	225
Dexmedetomidine (Precedex)	0.2
Diltiazem (Cardizem)	43
Dipyrone (Metamizole/Noramidopyrine)	9600
Dopamine	1
Doripenem (Vetrenal)	1050
Epinephrine (Adrenaline)	6
Ethacrynic acid	19
Ethanol	6000
Fenoldopam	484
Fentanyl	100
Fluconazole	75

Drugs and Endogenous Substances	Maximum concentration (mg/L)
Fluorescein	1
Fluvastatin	80
Furosemide	60
Gentamicin	30
(D)-Glucose	9909
Human Anti Mouse Antibodies (HAMA)*	2
Hemoglobin	60
Heparin	21
Hydralazine	600
Hydrochlorothiazide	6
Hydrocodone	0.2
Hydrocortisone	720
Ibuprofen	500
Insulin	0.003
Ketorolac	166
Lansoprazole	90
Linezolid	48
Lisinopril	0.3
Lorazepam	1
Low Molecular Weight Heparin	30
Mannitol	18000
Metformin	40
Methylene blue	3.9
Metolazone	60
Metoprolol	5
Metronidazole	123
Midazolam	3.76
Morphine	7.8
Moxifloxacin	1200
Myoglobin	5
Nitroglycerin	0.02
Norepinephrine (Noradrenaline)	204
Omeprazole	8.4
Ondansetron	0.342
Pancuronium	8
Pantoprazole (Protonix)	85
Phenobarbital	690
Phenylephrine	30
Pravastatin	80
Prednisone (Prednisolone)	3
Propofol	48
Ranitidine	10.5
Riboflavin	12
Rocuronium	126

Drugs and Endogenous Substances	Maximum concentration (mg/L)
Salicylic acid	599
Theophylline	60
Tobramycin	33
Torsemide (Torasemide)	12
Urobilnogen, synthetic	12
Valproic acid (Valproate)	499
Vancomycin	120
Vasopressin	5
Vecuronium	21
Warfarin (Coumadin)	75

* The expected levels of patients with HAMA experiencing proteinurea have not been established, so while no interference was observed at the level tested, use caution when interpreting results from patients with proteinuria and known HAMA.

Plasma Expanders	Maximum concentration (mg/L)
Dextran 40	22
Hetastarch (Hespan)	6
Pentastarch (Pentaspan)	9

Contrast Agents	Maximum concentration (mg/L)
Magnevist (Gadopentetate Dimeglumine)	422
Omnipaque (Iohexol)	14085
Omniscan (Gadodiamide)	177
Optiray (Ioversol)	4944
Visipaque (Iodixanol)	4941

Other Proteins Tested	Maximum concentration (mg/L)
Cystatin C	3
Interleukin-18 (IL-18)	0.001
Kidney Injury Molecule 1 (KIM 1)	0.02
Liver Type Fatty Acid Binding Protein (L-FABP)	1
N-acetyl-β-D-glucosaminidase (NAG)	0.00004
Neutrophil Gelatinase Associated Lipocalin (NGAL)	3
Pi-Glutathione s-transferase (p-GST)	0.1

Common Urine Constituents	Maximum concentration (mg/L)
Calcium	600
Chloride	5600
Creatinine	1800
Magnesium	240
Phosphate	1100
Potassium	4000

Common Urine Constituents	Maximum concentration (mg/L)
Sodium	3600
Sulfate	4800
Urea	32000
Uric acid	170

A bias exceeding 10% was considered as exhibiting significant interference.

No interference was observed for phosphate up to the urine concentrations of 1100 mg/L.

No interference was observed for albumin up to the urine concentration of 6900 mg/L. Testing specimens that contain albumin above this concentration may lead to results falsely increased by more than 10%.

Use caution when interpreting VIDAS® NEPHROCHECK® assay result in patients with significant proteinuria, or hyperphosphaturia.

Urine samples with AKIRISK™ Scores between 0.30 and 0.46 should also be tested with a urine dipstick test for the presence of hematuria. If the blood result is high positive (3+) or the urine sample has some indications of blood in the urine (e.g. pink/red color), the AKIRISK™ Score may be falsely elevated, and should not be used. Hemoglobin levels above 60 mg/L can lead to a 20% increase in the AKIRISK™ Score.

Effect of the urine sample pH
The effect of urine sample pH was evaluated for the VIDAS® NEPHROCHECK® Test. No significant interference was detected within the pH range [4-10].

Clinical Performance

The clinical performance of the VIDAS® NEPHROCHECK® assay was evaluated in two clinical studies. The results of the studies are presented below.

Study A (N= 399 Intended Use Patients)

The clinical performance of the VIDAS® NEPHROCHECK® assay was evaluated in Study A with a cohort of 399 intended use patients. Adult subjects were prospectively enrolled at 22 geographically diverse hospitals in the United States. Patients with known moderate or severe acute kidney injury were excluded from enrollment.

A urine specimen was collected at enrollment, frozen and stored at ≤ −60°C until measurement.

Four independent laboratories were involved in the specimen testing. A urine specimen from each subject was measured only once, at one of the four independent laboratories.

Each subject in the intended use patient cohort was adjudicated by a Clinical Adjudication Committee (CAC). Out of the 399 intended use patients, 330 (82.7%) were adjudicated as No AKI and 69 (17.3%) were adjudicated as AKI.

Study B (N= 126 Intended Use Patients)

Clinical performance was also evaluated in a second study, Study B (N= 126 intended use patients). Adult subjects were prospectively enrolled at 6 geographically diverse hospitals in the United States. A urine sample was collected at enrollment, aliquoted, and the aliquots subjected to different processing conditions. A urine sample from each subject was measured only once, at one independent laboratory.

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.

The VIDAS® NEPHROCHECK® test performance was validated at the cutoff of 0.30. The results from the two studies demonstrate intended use patients with AKIRISK™ Scores ≤ 0.30 are at lower risk of developing moderate to severe AKI within 12 hours of assessment, and intended use patients with AKIRISK™ Scores > 0.30 are at greater risk of developing moderate to severe AKI within 12 hours of assessment.

Study A	AKI status		Total number of VIDAS® NEPHROCHECK® test results
	AKI	No AKI	
AKIRISK™ Score > 0.30	62 True Positive	181 False Positive	243

Study A	AKI status		Total number of VIDAS® NEPHROCHECK® test results
	AKI	No AKI	
AKIRISK™ Score ≤ 0.30	7 False Negative	149 True Negative	156
Total number of VIDAS® NEPHROCHECK® test results	69	330	399

Study B	AKI status		Total number of VIDAS® NEPHROCHECK® test results
	AKI	No AKI	
AKIRISK™ Score > 0.30	24 True Positive	58 False Positive	82
AKIRISK™ Score ≤ 0.30	5 False Negative	39 True Negative	44
Total number of VIDAS® NEPHROCHECK® test results	29	97	126

The data in the Study A table below show a negative predictive value (NPV) of 95.5%. This means that 95.5% of the patients with an AKIRISK™ Score ≤ 0.30 have not manifested moderate to severe AKI within 12 hours of patient assessment for risk of AKI.

The data also show a positive predictive value (PPV) of 25.5%. This means that 25.5% of the patients with AKIRISK™ Score values > 0.30 have manifested moderate to severe AKI within 12 hours of patient assessment for risk of AKI.

Study A Statistics Cutoff: 0.30	Value %	[$_{95\%}$CI]* %
Sensitivity (TPR)	89.9	[80.5- 95.0]
Specificity (TNR)	45.2	[39.9-50.5]
FPR (1-Specificity)	54.8	[49.5-60.1]
FNR (1-Sensitivity)	10.1	[5.0-19.5]
Negative Predictive Value (NPV)	95.5	[91.0-98.2]
Positive Predictive Value (PPV)	25.5	[20.4-31.3]

* CI = Confidence Interval

The data in the table below show the VIDAS® NEPHROCHECK® test performance at the cutoff of AKIRISK™ Score of 0.30 in Study B.

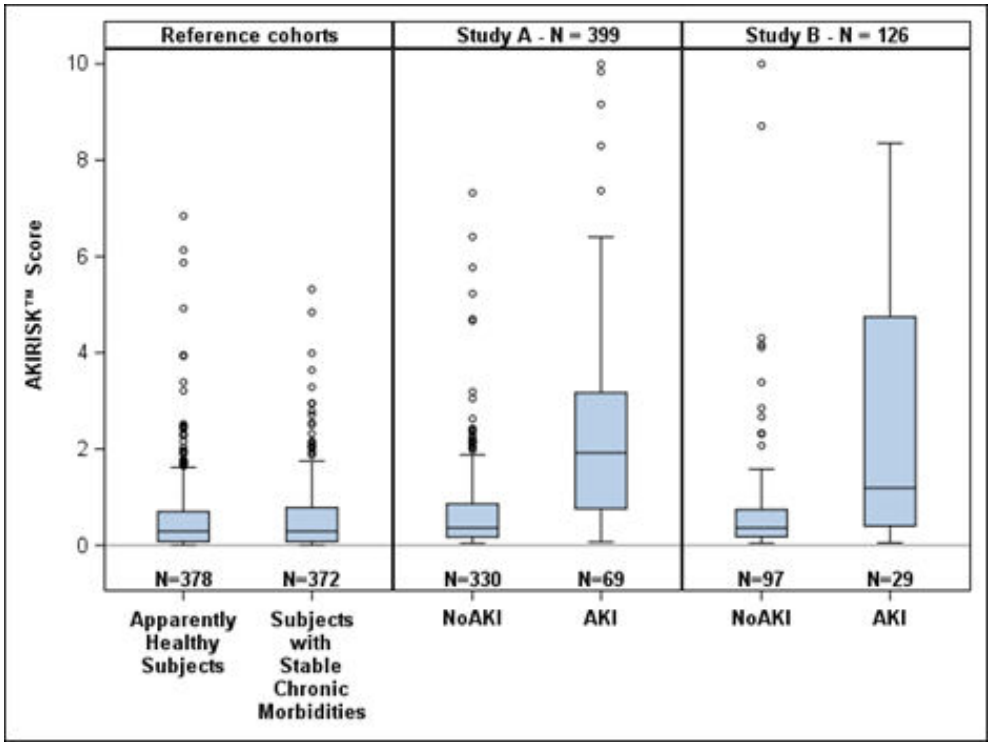
Study B Statistics Cutoff: 0.30	Value %	[$_{95\%}$CI]* %
Sensitivity (TPR)	82.8	[65.5-92.4]
Specificity (TNR)	40.2	[31.0-50.2]
FPR (1-Specificity)	59.8	[49.8-69.0]
FNR (1-Sensitivity)	17.2	[7.6-34.5]
Negative Predictive Value (NPV)	88.6	[76.0-95.0]
Positive Predictive Value (PPV)	29.3	[20.5-39.9]

* CI = Confidence Interval

The distributions of VIDAS® NEPHROCHECK® AKIRISK™ Scores 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 cohorts. Conversely, AKIRISK™ Scores are substantially elevated for intended use patients with AKI in the two cohorts compared

to results for apparently healthy subjects, subjects with chronic morbidities, or for intended use patients without AKI, showing that the AKIRISK™ Scores have ability to discriminate AKI from 'No AKI' patients.

AKIRISK™ Scores from AKI subjects were significantly greater than those from 'No AKI' subjects in both Studies A and B ($p < 0.05$). AKIRISK™ Scores in Study B were not significantly different from those in Study A ($p \geq 0.05$), showing the AKIRISK™ Scores were not statistically different between the two studies.



Demographic and other baseline information for the US intended use patients of clinical study A is shown in the table below for all subjects and for 'AKI' and 'No AKI' subjects.

Demographic Characteristics		All Total N = 399	No AKI Total N = 330	AKI Total N = 69
		N (%), Mean (SD), Median (IQR)	N (%), Mean (SD), Median (IQR)	N (%), Mean (SD), Median (IQR)
Age (years)	Mean (SD)*	62.8 (16.6)	63.0 (16.6)	62.3 (16.6)
	Median (IQR)**	65.0 (53.0-76.0)	65.0 (53.0-76.0)	64.0 (54.0-76.0)
BMI (kg/m^2)	Mean (SD)*	30.6 (9.1)	29.8 (8.5)	34.3 (10.9)
	Median (IQR)**	28.2 (24.7-34.3)	27.6 (24.2-33.2)	31.2 (25.9-38.9)
Sex	Female	185 (46.4)	150 (45.5)	35 (50.7)
	Male	214 (53.6)	180 (54.5)	34 (49.3)
Race	Asian	2 (0.5)	1 (0.3)	1 (1.4)
	Black or African American	55 (13.8)	46 (13.9)	9 (13.0)
	Native Hawaiian or other Pacific Islander	1 (0.3)	0 (0.0)	1 (1.4)
	Other	4 (1.0)	3 (0.9)	1 (1.4)
	Unknown	6 (1.5)	6 (1.8)	0 (0.0)
	White or Caucasian	331 (83.0)	274 (83.0)	57 (82.6)
Ethnicity	Hispanic or Latino	15 (3.8)	11 (3.3)	4 (5.8)
	Non-Hispanic or Non-Latino	347 (87.0)	288 (87.3)	59 (85.5)
	Unknown	37 (9.3)	31 (9.4)	6 (8.7)

Demographic Characteristics		All Total N = 399	No AKI Total N = 330	AKI Total N = 69
		N (%), Mean (SD), Median (IQR)	N (%), Mean (SD), Median (IQR)	N (%), Mean (SD), Median (IQR)
Reason for Hospital Admission***	Cardiovascular	143 (35.8)	115 (34.8)	28 (40.6)
	Cerebrovascular	44 (11.0)	41 (12.4)	3 (4.3)
	Sepsis	76 (19.0)	60 (18.2)	16 (23.2)
	Respiratory/Pulmonary	168 (42.1)	140 (42.4)	28 (40.6)
	Trauma	45 (11.3)	38 (11.5)	7 (10.1)
	Surgery (Any)	120 (30.1)	107 (32.4)	13 (18.8)
	Surgery (Emergency)	55 (13.8)	51 (15.5)	4 (5.8)
	Surgery (Elective)	65 (16.3)	56 (17.0)	9 (13.0)
	Gastrointestinal	48 (12.0)	39 (11.8)	9 (13.0)
	Other	123 (30.8)	100 (30.3)	23 (33.3)
Reason for ICU Admission***	Cardiovascular	162 (40.6)	131 (39.7)	31 (44.9)
	Cerebrovascular	49 (12.3)	45 (13.6)	4 (5.8)
	Sepsis	95 (23.8)	73 (22.1)	22 (31.9)
	Respiratory	201 (50.4)	167 (50.6)	34 (49.3)
	Trauma	43 (10.8)	36 (10.9)	7 (10.1)
	Surgery/Post-Op	127 (31.8)	111 (33.6)	16 (23.2)
	Other	119 (29.8)	94 (28.5)	25 (36.2)
Type of ICU	Cardiac surgery	37 (9.3)	32 (9.7)	5 (7.2)
	Combined ICU	60 (15.0)	50 (15.2)	10 (14.5)
	Coronary Care Unit	10 (2.5)	7 (2.1)	3 (4.3)
	Medical	177 (44.4)	148 (44.8)	29 (42.0)
	Neurological	12 (3.0)	9 (2.7)	3 (4.3)
	Other	7 (1.8)	6 (1.8)	1 (1.4)
	Surgical	69 (17.3)	58 (17.6)	11 (15.9)
	Trauma	27 (6.8)	20 (6.1)	7 (10.1)

*SD: Standard Deviation

**IQR: Interquartile Range (Central 50%)

***Subjects may have multiple reasons for admission.

Waste Disposal

Dispose of used or unused reagents, as well as any other contaminated disposable materials, following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced, according to their nature and degree of hazardousness, and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

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Index of Symbols

Symbol	Meaning
	Catalogue number
	*In Vitro* Diagnostic Medical Device
	For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

Limited Warranty

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Revision History

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2022-07	057209-01	N/A	Not applicable (First publication)

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