The NGAL Test™ CE IVD

The NGAL Test™ Reagent Kit
REF ST001CA

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INTENDED USE
The NGAL Test™ is a particle-enhanced turbidimetric immunoassay for the quantitative determination of neutrophil gelatinase-associated lipocalin (NGAL) in human urine, EDTA plasma and heparin plasma on automated clinical chemistry analyzers. NGAL measurements are useful in the diagnosis of acute kidney injury which may lead to acute renal failure.

SUMMARY AND EXPLANATION
Renal expression of NGAL increases dramatically in kidney injury from a variety of causes and NGAL is released into both urine and plasma. A large variety of renal disorders are associated with raised urinary and plasma levels of NGAL. Urinary NGAL serves as an early marker of acute kidney injury after cardiopulmonary bypass surgery[1,2] and both urinary and plasma levels of NGAL provide an early indication of acute renal injury in unselected patients in intensive care[3]. Raised urinary and plasma levels of NGAL have also been observed in patients with chronic kidney diseases[4], and persistently raised urinary levels after renal transplantation are associated with poor graft function[5].

TEST PRINCIPLE
The NGAL Test™ is a particle-enhanced turbidimetric immunoassay for the quantitative determination of NGAL in human urine and plasma. A sample of human urine or plasma is mixed with reaction buffer R1. After a short incubation, the reaction is started by the addition of an immunoparticle suspension (polystyrene microparticles coated with mouse monoclonal antibodies to NGAL). NGAL in the sample causes the immunoparticles to aggregate. The degree of aggregation is quantified by the amount of light scattering measured as absorption of light. The NGAL concentration in the sample is determined by interpolation on an established calibration curve.

REAGENTS
Composition
R1 Reaction Buffer, 35 mL, is a ready-to-use buffer solution containing murine protein and preservative.
R2 Immunoparticle Suspension, 7 mL, is a ready-to-use suspension of polystyrene microparticles coated with mouse monoclonal antibodies to NGAL, and contains preservative.

WARNINGS AND PRECAUTIONS
For In Vitro Diagnostic Use IVD
- Read all instructions before starting this test.
- This kit should only be used by qualified laboratory staff.
- Do not pipette by mouth.
- Do not shake the reagents.
- Use only clean containers if transferring reagents.
- Do not pour reagents back into their original containers once transferred.
- All specimens used in this test should be considered potentially infectious, and as such be treated with standard precautions. Powder-free gloves should be worn. Avoid direct skin contact. Should reagents come into contact with the skin, eyes or mouth, flush with plenty of water. Seek medical advice if any symptom is observed or when it is considered necessary.
- Do not switch caps on reagent containers as it may cause contamination or mix-up.
- Do not use reagents after the expiry date on the labels.
- Reagents with different lot numbers should not be mixed.
- All solutions supplied should be handled carefully and disposed of in accordance with national and local regulations.
- All equipment used in this test should be sterilized by one of the following methods:
  - Soak in 3.5% v/v glutaraldehyde for 30 minutes or longer.
  - Soak in 0.5% w/v sodium hypochlorite for 1 hour or longer.
  - Autoclave at 121°C for 20 minutes or longer.

STORAGE AND STABILITY
Shelf life at 2-8°C: See expiry date on the label.
Stability after opening: 4 weeks at 2-8°C.
On-board stability: 4 weeks at correct temperature (2-8°C) in appropriate containers.
On the Hitachi 917 analyzer, the manufacturer obtains satisfactory performance for up to 8 weeks on-board.

COLLECTION OF SPECIMENS
NGAL can be determined in human urine and plasma samples. Blood specimens should be collected aseptically into appropriate tubes by qualified staff using approved venepuncture techniques. Plasma should be prepared by standard techniques for laboratory testing. Urine should be centrifuged. The prepared specimens should be capped. If the assay cannot be performed within 24 hours or specimens are to be shipped, the specimens should be frozen at −70°C or below. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens.

SAMPLE STABILITY
Samples should be analyzed as soon as possible. However, both urine and plasma samples are stable for at least 1 day at room temperature (20-25°C) and for 3 days at 2-8°C. NGAL values are not significantly affected by 3 freeze/thaw cycles over 3 days.

PROCEDURE
Materials provided
R1 Reaction Buffer, 35 mL
R2 Immunoparticle Suspension, 7 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- The NGAL Test™ Calibrator Kit REF ST002CA
- The NGAL Test™ Control Kit REF ST003CA
- 0.9% w/v aqueous sodium chloride solution as zero calibrator
- Clinical chemistry analyzer
- Analyzer-specific application note (available for a number of analyzers)
- Analyzer-specific reagent containers for R1 and R2

ASSAY PROCEDURE
The assay should be carried out in accordance with the specific application note for the chemistry analyzer to be used. A general example of the assay procedure for an automated analyzer is given below:

1. Incubate 3 µL sample with 150 µL R1 at 37°C for 5 minutes
2. Add 50 µL R2
3. Read absorbance change at 570 nm for 5 minutes after the addition of R2
4. Calculate NGAL concentration from the absorbance change by interpolation on a calibration curve prepared with calibrators of known concentrations

APPLICATION PARAMETERS FOR THE HITACHI 917 ANALYZER

TEMPERATURE	37°C
ASSAY CODE	2point end-10
ASSAY POINT	18-34
SAMPLE VOLUME	3 (µL)
R1 VOLUME	150 (µL)
R2 VOLUME	50 (µL)
WAVELENGTH	800/570
CALIB. METHOD	SPLINE - 6-6
UNITS	ng/mL

NUMBER OF DETERMINATIONS
On a Hitachi 917, 1 mL of R2 Immunoparticle Suspension provides 20 cuvette readings of standards or samples. The dead volume of the analyzer and reagent container should be added when calculating the required amount of reagent.

LOADING OF REAGENTS
The NGAL Test™ Reagents are provided in containers that fit a number of commonly used analyzers. For use on other analyzers, transfer the contents of R1 and R2 into appropriate containers.

CALIBRATION
The NGAL Test™ Calibrator Kit REF ST002CA available from BioPorto Diagnostics should be used for calibration, with 0.9% w/v aqueous sodium chloride solution as zero calibrator.

Each laboratory should determine the appropriate frequency of calibration. Calibration should be repeated at least once a month and when a new reagent lot is used.

QUALITY CONTROL
For quality control, use The NGAL Test™ Control Kit REF ST003CA available from BioPorto Diagnostics. Quality control intervals and limits should be adapted to each laboratory's individual requirements. Each laboratory should establish corrective measures if values fall outside the limits.

MEASURING RANGE
The measuring range of The NGAL Test™ is 25 ng/mL to 3000 ng/mL on a Hitachi 917 analyzer. For information about measuring ranges on other analyzers, please refer to the analyzer-specific application note.

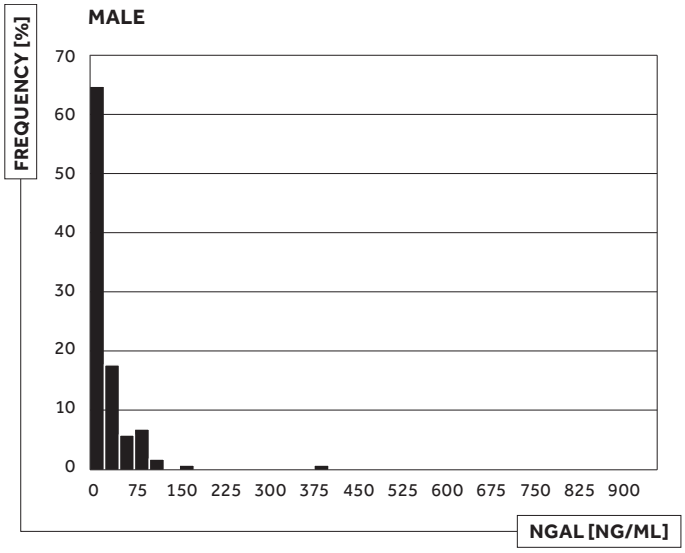
RESULTS
Different analyzers may use different curve fitting procedures for the calibrator results in accordance with the application parameters. The calibration curve must be validated with The NGAL Test™ Control Kit REF ST003CA as stated above and corrective measures taken if the results for Control High and Control Low fall outside the laboratory's established limits.

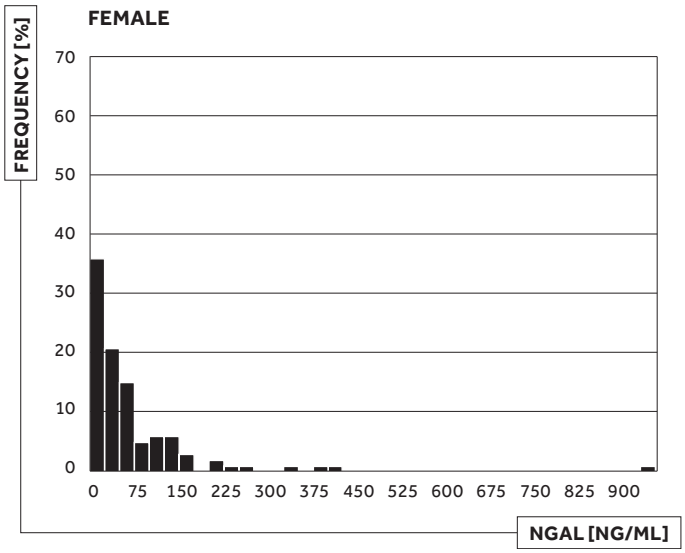
LIMITATIONS
The finding of a raised urinary or plasma level of NGAL cannot be independently diagnostic of any single pathology. A variety of pathologies other than renal disorders are associated with raised levels of urinary and/or plasma NGAL. Physicians must interpret the significance of any raised NGAL level in the light of the patient's clinical features.

NORMAL REFERENCE RANGE
NGAL concentrations of self-declared apparently healthy subjects were measured in duplicate.

Sample type	Population	N	NGAL Median [ng/mL]	NGAL Range [ng/mL]; central 95%
Urine	Total	200	<25	<25 - 330
	Female	100	32	<25 - 397
	Male	100	<25	<25 - 136
EDTA plasma	Total	200	52	<25 - 102
	Female	100	53	<25 - 111
	Male	100	52	26 - 95
Heparin plasma	Total	200	53	<25 - 99
	Female	100	52	<25 - 113
	Male	100	54	26 - 97

In urine normal NGAL concentrations are significantly lower in males than in females (Wilcoxon-Mann-Whitney test, $p<0.0001$).





INTERPRETATION
Urinary and/or plasma concentrations of NGAL may be elevated in conditions that show no apparent relation to change in kidney function, including bacterial infections, other inflammatory disorders and certain carcinomas. BioPorto Diagnostics has determined that the NGAL concentration in an isolated sample of urine and/or EDTA plasma should exceed 250 ng/mL in order to indicate the presence of renal disorder, including acute kidney injury, without incurring the risk of an unacceptably high proportion of false positive diagnoses of renal disorder.

PERFORMANCE CHARACTERISTICS
The following data are representative of the performance of The NGAL Test™ on the Hitachi 917 analyzer. For information about performance characteristics on other analyzers please refer to the analyzer-specific application notes.

LIMIT OF DETECTION
The limit of detection was calculated according to the CLSI EP17 guidelines. It is 7.3 ng/mL for plasma and urine.

LIMIT OF QUANTIFICATION
The limit of quantification was below 25 ng/mL in plasma and urine defined as the NGAL concentration where a CV<20% was found.

PRECISION
Low and high controls (The NGAL Test™ Control Kit REF ST003CA) as well as a low, medium and high sample of urine, EDTA plasma and Heparin plasma were measured with double determinations in two separate runs per day for 20 days using three different reagent lots.

	Sample	Mean	CV			
			Total	Within Run	Between Run	Between Day
Controls	**Low**	202	3,3%	3,3%	0,0%	0,1%
	High	503	3,3%	3,2%	0,0%	0,7%
Urine	**Low**	112	2,8%	1,9%	1,5%	1,5%
	Medium	314	1,7%	0,8%	1,5%	0,0%
	High	2158	2,1%	0,8%	1,8%	0,7%
EDTA plasma	**Low**	97	3,1%	1,7%	2,6%	0,0%
	Medium	359	2,8%	0,7%	2,7%	0,0%
	High	2037	3,0%	0,9%	2,8%	0,0%
Heparin plasma	**Low**	116	1,8%	1,3%	0,7%	0,9%
	Medium	372	2,1%	0,5%	2,1%	0,0%
	High	1985	2,4%	0,6%	2,4%	0,0%

LINEARITY
The NGAL Test™ is linear from 25 ng/mL to 3000 ng/mL.

SECURITY RANGE
The NGAL Test™ showed no effect of antigen excess for NGAL concentrations below 40,000 ng/mL (the highest concentration tested).

INTERFERENCE
No interference was detected with hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5% v/v of a 10% v/v lipid emulsion (corresponding to 5 g/L).

LIABILITY
This Reagent Kit is only intended for the in vitro determination of NGAL in human urine or plasma. The Reagent Kit is only intended for use by qualified personnel carrying out research or diagnostic activities. If the recipient of this test passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at recipient's own risk secure in favor of BioPorto Diagnostics A/S all limitations of liability herein.

R1	Reaction Buffer		Consult instructions for use
R2	Immunoparticle Suspension		European Conformity
REF	Catalogue number		Use by
IVD	In vitro diagnostic medical device		Manufacturer
LOT	Batch code		Temperature limitation
IFU	Valid version of instructions		

REFERENCES

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4. Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, Buemi M (2008) Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis 52:595-605.
5. Hollmen ME, Kyllönen LE, Inkinen KA, Lalla ML, Salmela KT (2011) Urine neutrophil gelatinase-associated lipocalin is a marker of graft recovery after kidney transplantation. Kidney Int 79:89-98.

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