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Instructions for Use

Metanephrine ELISA

Enzyme immunoassay for the quantitative determination of metanephrine in human urine.

REF **RE59181**

Σ **96**

2°C 8°C

EU: IVD

CE 2797

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REVISION HISTORY OF INSTRUCTIONS FOR USE

Changes from the previous version 2018-09 to actual version 2024-04	
Cover page	Layout change
Chapter 2	Additional chapter
Chapter 3	Update to scientific validity
Chapter 5	Additional information
Chapter 6	Update
Chapter 7	Update / Additional information
Chapter 8	Additional information
Chapter 13	Additional chapter
Chapter 15	Additional information
Chapter 16	Update and additional data
Chapter 17	Update and additional data
Chapter 18	Update and additional data
Chapter 19	Update literature
Symbol page	Layout change

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of metanephrine in human urine.

2. INTENDED PURPOSE

Enzyme immunoassay for the quantitative determination of metanephrine in human urine.
The test serves as an aid of diagnosis of catecholamine-secreting pheochromocytomas and paragangliomas as well as monitoring tool for therapy follow-up. Patient population includes adults suspected to be affected by neuroendocrine tumors. Pre-test in case of genetic predisposition (e.g. Von-Hippel-disease, and multiple endocrine neoplasia type 2) is advised.

The test kit is intended for professional use only.

The test kit is not for self-testing. The Metanephrine ELISA is NOT intended for near patient testing.

The test kit is intended for manual use and can be adapted to open ELISA processors.

3. SUMMARY AND EXPLANATION

The term metanephrines includes metanephrine, normetanephrine and 3-methoxytyramine, which are the metabolites of catecholamines (adrenalin, noradrenalin and dopamine). Metanephrine is naturally excreted into urine and certain tissues. At least 90 % of metanephrine and up to 40 % of normetanephrine are formed from metabolism of epinephrine and norepinephrine within the adrenals before release of these catecholamine into the circulation.[1]

In several diseases, e.g. pheochromocytomas, catecholamines and their metabolites metanephrine and normetanephrine are secreted in increasing amounts and associated with different clinical symptoms like severe hypertension palpitations and headache and are therefore applied for diagnostic purposes.[2] The diagnosis and the follow-up of tumor diseases of the nervous system are of special importance.[3]

This applies primarily to catecholamine-secreting tumors such as pheochromocytoma and paragangliomas.[2] Pheochromocytomas are rare tumors with 10 % being malignant.[4] They are neoplasms generally characterized by the autonomous production of catecholamines.[5] These catecholamine-producing tumors arise form chromaffin cells of the adrenal medulla or extra-adrenal paraganglionic tissue.[6] The inappropriate concentration of catecholamines may results in sustained or intermittent severe hypertension. Typical clinical symptoms include headaches, sweating and tachycardia.

The localization of the tumor, either in the adrenal medulla or extra-adrenal, can be found by measuring urinary metanephrines.[7]

Furthermore, a genetic screen should be applied due to heredity predispositions like von Hippel-Lindau disease or family members of patients with multiple endocrine neoplasia type 2 (MEN 2). [1]

After the adrenal masses are surgically removed, the diagnosis is postoperatively confirmed by histological examinations as well as by clinical follow-up.[5] Patients are re-examined after the surgery as well and metanephrine levels are checked.[8] Most pheochromocytomas are discovered in people aged 20 - 50 years but the tumor can develop at any age (Mayo Clinic, 2018).[9]

Metanephrines can be found in blood and urine and can be measured as free metanephrines or in a conjugated form after hydrolysis. Metanephrines in urine are commonly measured after an acid hydrolysis step that converts the large proportion of sulfate-conjugated metabolites from the free metabolite. For measuring total fractionated metanephrines, acid hydrolysis or enzymatic treatment is necessary before the assay. According to Lenders and colleagues urinary metanephrines may be more specific.[10]

4. TEST PRINCIPLE

The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of streptavidin alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards.

5. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only by GLP trained professionals.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Broken glass may cause injury. Handle glass vessels with caution.
5. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
6. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
8. Chemicals and prepared, used, unused or expired reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
9. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
10. All serious incidents that have occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
11. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
12. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites. Still the material should be handled with extreme caution.
13. Avoid contact with Stop solution. It may cause skin irritations and burns.
14. The summary of safety and performance is currently only available from the manufacturer on request. As soon as the European database on medical devices (Eudamed) (https://ec.europa.eu/tools/eudamed) is online, the summary of safety and performance will be uploaded by IBL's notified body and can be easily found using the following Basic UDI-DI 4049325IVR03010007CPF.

6. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2 - 8°C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents are stated in the corresponding chapters.

The unopened reagents are stable until the expiry date indicated. The kit is stable up to 6 months after the first opening (not exceeding the expiry date) when the Microtiter plate is packed in a tightly closed bag, the bottles are closed with their screw caps and the kit is stored at indicated storage temperature.

7. SPECIMEN COLLECTION AND STORAGE

Specimen
Urine

Specimen collection
It is possible to use spontaneous urine as well as 24-hour urine. The total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 - 15 mL of 6M HCl as preservative. Determine total volume for calculation of results.
Mix and centrifuge samples before use in the assay.
The in-vivo catecholamine and metanephrines release is influenced by several foods and drugs. Vitamin B, coffee and bananas, alpha-methyldopa, MAO and COMT inhibitors as well as medications related to hypertension or local anesthetics (tetracaine) should be discontinued for at least 72 hours prior to specimen collection.
Samples should not be taken from patients that took biotin-containing multivitamins or supplements within last 48 hours

Sample Collection Device
No special requirements.

Specimen storage
Samples can be stored at 2 - 8°C for 7 days
It is recommended to freeze samples and store at -20°C for long time storage (< 6 months).
Avoid repeated freeze-thaw cycles. Keep away from heat or direct sunlight.

8. MATERIALS SUPPLIED

Quantity	Symbol	Origin	Component
1 x 12 x 8	MTP	BIO	**Microtiter Plate** Break apart strips. MTP (12 strips of 8 wells each) coated with goat anti-rabbit antibody in solution containing bovine serum albumin. Vacuum dried.
1 x 8 mL	ANTISERUM	BIO	**Metanephrine Antiserum** Ready to use. Green colored. Rabbit anti-metanephrine antibody in phosphate buffer containing bovine serum albumin and < 0.1 % sodium azide (w/w).
1 x 8 mL	BIOTIN	BIO	**Metanephrine Biotin** Ready to use. Yellow colored. Contains Metanephrine Biotin, phosphate buffer bovine serum albumin, < 0.1 % sodium azide (w/w) and 0.1 M Hydrochloric acid (w/w)
1 x 400 µL	ENZCONJ CONC	BIO	**Enzyme Conjugate,** Concentrate (50x) Contains streptavidin alkaline phosphatase (from calf intestine), Tris buffer, bovine serum albumin and < 0.1 % sodium azide (w/w).
1 x 7 x 0.35 mL	CAL A-G		**Standard A-G** Ready to use. 0; 26; 64; 160; 400; 1000; 2500 µg/L 0; 0.13; 0.33; 0.81; 2.03; 5.08; 12.7 µmol/L Contains Metanephrine and 0.1 M Hydrochloric acid (w/w).
1 x 2 x 0.5 mL	CONTROL 1+2		**Control 1+2** Ready to use. Contains Metanephrine and 0.1 M Hydrochloric acid (w/w). Concentrations / acceptable ranges see QC certificate.
1 x 2.25 mL	ACYLREAG		**Acylation Reagent** Ready to use. Contains: dimethylformamide (DMF) mixture > 50 % (v/v).
2 x 50 x	HYDRTUB		**Hydrolyzation Tubes** Disposable polystyrene tubes (uncoated). Additional Hydrolysation tubes are available under REF KEZZ661.
1 x 20 mL	HCL		**HCl** Ready to use. 0.1 M Hydrochloric acid.
1 x 50 mL	ASSAYBUF CONC		**Assay Buffer,** Concentrate (10x) Contains phosphate buffer, bovine serum albumin and 1 % sodium azide.
1 x 11 mL	INDICATORBUF		**Indicator Buffer** Ready to use. Purple colored. Contains: Tris buffer, phenol red (color change at pH < 7.5) and ≤ 0.05 % Thimerosal (w/w).
2 x 100 mL	WASHBUF CONC		**Wash Buffer,** Concentrate (10x) Contains: Tris buffer, ≤ 0.6% Hydrochloric acid (w/w), 1 % Tween 20 (w/w), 0.2 % sodium azide (w/w).
1 x 13 mL	PNPP SUBS		**PNPP Substrate Solution** Ready to use. Contains: p-nitrophenyl phosphate (PNPP).
1 x 15 mL	PNPP STOP		**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
3 x	FOIL		**Adhesive Foil**

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 10; 50; 100; 1000 µL
2. Disposable glass tubes (12 x 75 mm)
3. Orbital shaker (200 - 900 rpm) (e.g. EAS 2/4, SLT)
4. Vortex mixer
5. Water bath, 90°C, 37°C
6. 8-Channel Micropipette with reagent reservoirs
7. Wash bottle, automated or semi-automated microtiter plate washing system
8. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600 - 650 nm)
9. Bidistilled or deionised water
10. Paper towels, pipette tips and timer
11. Waterproof marker for labelling the hydrolization tubes

10. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared and ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18 - 25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to perform duplicate measurements to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel micropipette for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

11. PRE-TEST SETUP INSTRUCTIONS

For manual and automatic version.

The contents of the kit for 96 determinations can be divided into 3 separate runs.

The volumes stated below are for one run with 4 strips (32 determinations).

If the customer wants to reduce the number of standards from 7 to 6 he can omit Standard G. The reportable range will then be reduced to 1000 µg/L.

If a larger number of strips is to be used, the volumes have to be changed accordingly.

11.1. Preparation of concentrated components

⚠	Do not mix up Metanephrine and Normetanephrine Enzyme Conjugate in case you use the Normetanephrine ELISA in parallel.

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	ASSAYBUF CONC	ad 150 mL	bidist. water	1:10		2 - 8°C	2 weeks
60 mL	WASHBUF CONC	ad 600 mL	bidist. water	1:10		2 - 8°C	4 weeks
120 µL	ENZCONJ CONC	with 6 mL	diluted Assay Buffer	1:51	Prepare freshly and use only once.	18 - 25°C	5 hours

11.2. Hydrolyzation of urine samples, Standards and Controls for total metanephrine (in hydrolyzation tubes)

⚠	The hydrolyzation step is necessary for the determination of total metanephrine. No hydrolyzation is required when assaying free metanephrine. Samples suspected to contain concentrations higher than 1500 µg/L have to be diluted with 0.1 M HCl before hydrolyzation step.

11.2.1. Sample preparation in the hydrolyzation tubes

1.	Pipette **10 µL** of each **Standard, Control and urine sample** into the respectively labelled hydrolyzation tubes.
2.	Pipette **40 µL** of **0.1 M HCl** into each tube.
3.	Close tubes. **Hydrolyze 1 hour** at **90°C** (check temperature with thermometer). Allow to cool down to room temperature afterwards. Vortex.
4.	Pipette **100 µL** of **Indicator Buffer** into each tube. Vortex.
5.	Pipette **20 µL** of **Acylation Reagent** into each tube. Vortex each tube immediately after pipetting. Take care that addition of acylation reagent into the content of the tubes is complete.
6.	Close tubes. **Incubate 15 minutes** at **18 - 25°C** (room temperature).
7.	Pipette **1 mL** of diluted **Assay Buffer** into each tube. Vortex.

12. TEST PROCEDURE

In microtiter plate for manual and automated version

1.	Pipette **50 µL** of each acylated **Standard**, acylated **Control** and acylated **patient sample** into the respective wells of the microtiter plate.
2.	Pipette **50 µL** of **Metanephrine Biotin** into each well.
3.	Pipette **50 µL** of **Metanephrine Antiserum** into each well.
4.	Cover plate with adhesive foil. Shake plate carefully. **Incubate 1 hour** at **18 - 25°C** on an orbital shaker **(500 rpm).**
5.	Remove adhesive foil. Discard incubation solution. Wash plate with an automate **6 x** with **250 µL** of diluted **Wash Buffer (3 x manually)**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **150 µL** of freshly prepared **Enzyme Conjugate** into each well.
7.	Cover plate with new adhesive foil. **Incubate 30 minutes** at **18 - 25°C** on an orbital shaker **(500 rpm)**.
8.	Remove adhesive foil. Discard incubation solution. Wash plate with an automate **6 x** with **250 µL** of diluted **Wash Buffer (3 x manually).** Remove excess solution by tapping the inverted plate on a paper towel.
9.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement to avoid formation of air bubbles.
10.	Pipette **100 µL** of **PNPP Substrate Solution** into each well.
11.	**Incubate 40 minutes** at **18 - 25°C** on an orbital shaker **(500 rpm)**.
12.	Stop the substrate reaction by adding **100 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
13.	**Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 600 - 650 nm) within **60 minutes** after pipetting of the Stop Solution.

13. AUTOMATION

Automated protocols can be provided for open ELISA systems: ThunderBolt®
For further information please contact: ProductSupport.IBL@tecan.com
For the use of products on automated instruments it is absolutely necessary to perform and maintain a validation according to legal requirements. A successful validation of the process is a prerequisite for diagnostic use. The responsibility for the implementation and documentation of validation in accordance with the country-specific requirements is assumed by the organization or institution.

14. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

Remarks for participants in the Quality Control Assessment Schemes

IBL is regularly taking part with the Metanephrine immunoassay in Quality Control Assessment Schemes. According to the organizers of the QC schemes they are using racematic (+/-) metanephrine for spiking of their specimen and not the biologically active form of metanephrine. Consequently, when measuring spiked samples in the high concentration range, the customer will find about 30 - 40 % lower results with the metanephrine immunoassay compared with HPLC.

If the organizers use native patient samples with elevated metanephrine concentrations, this problem does not occur. The reason for this is that the antibody used in the kit recognizes the biologically active form of metanephrine. Therefore, please check the preparation of specimen used in the QC scheme when interpreting your results.

15. CALCULATION OF RESULTS

The obtained ODs of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates could be omitted and the more plausible single value used).

The concentration of the samples can be read directly from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and re-assayed.

Calculate the 24 hour excretion for each urine sample: µg/24 h = µg/L x L/24 h

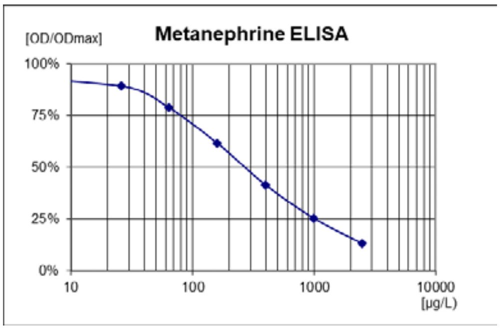
Conversion: 1 ng/mL = 1 µg/L

Metanephrine (µg/L) x 5.07 x 10^{-3} = µmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Metanephrine	OD_{Mean}	OD/OD_{max}
A	0.0 µg/L	1.867	100 %
B	26 µg/L	1.670	89 %
C	64 µg/L	1.473	79 %
D	160 µg/L	1.153	62 %
E	400 µg/L	0.776	42 %
F	1000 µg/L	0.473	25 %
G	2500 µg/L	0.245	13 %



Measuring Range: from 39.4 µg/L (LoQ as functional sensitivity) to 1559 µg/L (highest mean concentration measured in intra and inter assay precision study)

16. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests.

It is recommended that each laboratory establishes its own range of normal values.

16.1. Reference interval in healthy population

The results 146 24-hour urinary samples from apparently healthy individuals were tested with Metanephrine ELISA, the metanephrine concentration (µg/24h) was found in agreement with the levels published for urinary metanephrine in the literature.

	N	Lowest Value [µg/24h]	Mean Value [µg/24h]	Highest Value [µg/24h]	90 % percentile	MN > upper limit (N)	Expected according to literature
female (18 - 63 years)	51	81	198	521	389	15 (29 %)	237 µg/24h, upper limit reference range Gerlo and Sevens *et al.* (1994) [11]
male (18 - 76 years)	95	65	234	570	403	16 (17 %)	355 µg/24h upper limit reference range Gerlo and Sevens *et al.* (1994) [11]
total (18 - 76 years)	146	65	222	570	402	40 (27 %)	43 - 260 µg/24h; Thomas L (2020) [12]

It is strongly recommended to follow the patient preparation and sample collection as indicated due to the high influence of food and medicaments in the metanephrines release.

16.2. Metanephrine levels in affected population

In affected individuals with pheochromocytoma usually higher metanephrines values are observed. Levels of urinary fractioned metanephrine are 1490 nmol/24h (294 µg/24h) with a highest value of 2200 nmol/24h (434 µg/24h) according to Grouzmann *et al.* (2010) [13] or higher than 1531 nmol/24h (302 µg/24h) according to Perry *et al.* (2007). [14]

A clear medical decision point for the analyte metanephrine in urine regarding the indicated diseases cannot be identified. The increased values of urinary metanephrine are consistent as indicator, but the concentration of metanephrine measured in affected and healthy population may overlap and the result itself is not constantly reported above a threshold in identified literature.

17. LIMITATIONS OF THE PROCEDURE

Samples from patients that took biotin-containing multivitamins or supplements may contain biotin amounts which will cause interference with the assay. Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross-reactivities, see PERFORMANCE.

	Component	Concentration
The following substances do not have a significant effect (+/- 20 % of expected value) on the test results up to the stated concentrations	Human serum albumin	50 mg/mL
	Creatinine	5 mg/mL
	Whole blood	1 % (v/v)

18. PERFORMANCE

Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity
(±)-Synephrine	0.8 %
L-(−)-Normetanephrine (+)-bitartrate salt monohydrate	0.2 %
Dopamine hydrochloride	0.1 %
(±)-Normetanephrine (+)-bitartrate salt	0.1 %
3- Methoxytyramine hydrochloride	0.1 %
Homovanillic acid	0.1 %
Vanillmandelic acid	0.1 %
Caffeic acid	0.1 %
Epinephrine	0.1 %
DL-Normetanephrine hydrochloride	0.2 %

Limit of Blank (LoB)

The LoB study was performed with five blank samples (Standard A), measured in 4 replicates per sample over three days with two kit lots. Limit of Blank: 19.2 µg/L

Limit of Detection (LoD)

The LoD study was performed using five different low concentrated samples, measured in 4 replicates per sample over three days with two kit lots. Limit of Detection: 31.8 µg/L

Limit of Quantitation (LoQ) as Functional Sensitivity

The functional sensitivity study was performed using 8 low concentrated samples, measured in 4 replicates over three days with two kit lots. LoQ (functional sensitivity): 39.4 µg/L

Trueness: Calibration/ Metrological Traceability

The assay Metanephrine ELISA, intended for the quantitative determination of metanephrine in human urine, is metrological traceable to SI unit µg/L (= ng/mL). Metanephrine concentrations assigned to the kit standards, controls and human urine samples are traceable by calibration of standards to reference measurement procedure LC-MS according to EN ISO 17511:2021.

The metrological traceability is validated by comparison to HPLC reference measurement.

The resulting Passing-Bablok fit IBL ELISA (µg/L) = 21.79 + 1.026 HPLC (µg/L) and the correlation r = 0.927 demonstrates good comparability between both methods. The internal reference material is commutable between IBL ELISA and HPLC.

The combined uncertainty of measurement is calculated as 15.2 % according to the 'Guide to the expression of uncertainty in measurement' (JCGM 100:2008; GUM 1995 with minor corrections).

Method comparison versus commercial ELISA

The method comparison study was conducted during one day of testing by one operator. The run was performed with high and low kit controls and with different human urine samples. Each sample was tested in duplicate using one reagent lot.

y	x	n	r	y = ax + b
Metanephrine ELISA (IBL)	Other commercial Metanephrine Urine ELISA	75	0.964	y = 0.89x + 50.40

Method comparison versus reference method

The method comparison study was conducted during five days of testing by one operator. The run was performed with high and low kit controls and with different human urine samples. Each sample was tested in duplicate using one reagent lot.

y	x	n	r	y = ax + b
Metanephrine ELISA (IBL)	HPLC	151	0.926	y = 1.041x + 19.94

Linearity

Four urine samples having different metanephrine levels were serially diluted in zero standard (up to 1:8) and the concentrations of the diluted samples were assayed in the ELISA. Each dilution was measured in duplicates in one assay run. The mean recovery of the diluted samples was 105 % (range 85 - 118 %) the concentration range 57 µg/L up to 1533 µg/L.

Recovery

Increasing amounts of metanephrine (3 - 2457 nmol/L) were added to three different urine samples and all samples (spiked and unspiked) were assayed in one run. The mean recovery including all urine samples was 96 % (range 86 - 120 %) with a concentration range 40 µg/L up to 1186 µg/L.

Precision

The intra assay study was conducted by testing three different urine samples in one run. Each sample was tested 20 times, in one run, by one operator, in a day, with one lot.

Intra-Assay			
Sample	**Mean conc.**	**SD**	**CV**
1	115.9 µg/L	10.2 µg/L	8.8 %
2	544.2 µg/L	45.9 µg/L	8.4 %
3	1477 µg/L	74.5 µg/L	5.0 %

The mean intra assay variation CV is 7.4 % (range 5.0 % – 8.8 %) with a concentration range from 115.9 µg/L up to 1477 µg/L.

The inter assay precision was conducted by measuring three different urine samples in one run. Each sample was tested 20 times. In total, four different operators took part in the measurements over 2 days using one lot.

Inter-Assay			
Sample	**Mean conc.**	**SD**	**CV**
1	111 µg/L	13 µg/L	11.3 %
2	228 µg/L	21 µg/L	9.3 %
3	1559 µg/L	114 µg/L	7.3 %

The mean inter assay variation is 9.3 % (range 7.3 % - 11 %) with a concentration range from 111 µg/L up to 1559 µg/L.

The inter lot precision data was established by evaluation of FRC (final release control) runs and measuring range determination measurements of 6 QC urine samples using data from 29 runs (samples in double determination), 17 different operators and 12 different lots.

Inter-Lot			
Sample	**Mean conc.**	**SD**	**CV**
1	43.5 µg/L	8.4 µg/L	19.3 %
2	122.9 µg/L	15.7 µg/L	12.8 %
3	127.6 µg/L	13.9 µg/L	10.9 %
4	221.9 µg/L	22.7 µg/L	10.2 %
5	531.4 µg/L	45.4 µg/L	8.5 %
6	1499.8 µg/L	181.5 µg/L	12.1 %

The mean inter lot variation CV is 12.3 % (range 8.5 % - 19.3 %) with a concentration range from 43.5 µg/L up to 1499.8 µg/L.

19. PRODUCT LITERATURE REFERENCES

[1] Eisenhofer, G., Lenders, J. W., Linehan, W. M., Walther, M. M., Goldstein, D. S., & Keiser, H. R. (1999). Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel–Lindau disease and multiple endocrine neoplasia type 2. New England Journal of Medicine, 340(24), 1872-1879.

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Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
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Generic table, not all symbols are present in the product

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