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Instructions for use

Normetanephrine Urine RIA

Fast Track

REF **BA R-8500**

100 +2 +8 °C IVD CE 200 kBq

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the quantitative determination of Normetanephrine in urine.

First, Normetanephrine (Normetadrenaline) was quantitatively acylated.
The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of 125I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
This kit contains ^{125}Iodine (half life: 60 days), emitting ionizing X- (28 kev) and G- (35.5 kev) radiations.

The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

The reagents should be stored at 2 - 8 °C. Do not use components beyond the expiration date shown on the kit labels.

4.1 Contents of the kit

BA D-0023	REAC-TUBES	**Reaction Tubes**	2 x 50	ready for use
BA R-0012	ACYL-CONC	**Acylation Concentrate**	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
BA R-0025	PREC-REAG	**Precipitating Reagent**	1 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. *Mix thoroughly before use!*
BA R-0075	ACYL-DILUENT	**Acylation Diluent**	1x 4 mL	ready for use
BA R-0220	^{125}I NAD NMN	**125I – Noradrenaline - Normetanephrine**	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, yellow screw cap
BA R-8510	AS MN	**Normetanephrine Antiserum**	1 x 5.25 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA R-8601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-8602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-8603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-8604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-8605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-8606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-8611	ACYL-BUFF	**Acylation Buffer**	1 x 30 mL	ready for use
BA R-8619	HCL	**Hydrochloric Acid**	1 x 30 mL	ready for use, contains 0.25 M HCl, yellow coloured, green screw cap
BA R-8651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-8652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Polystyrene tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Suitable device for aspirating or decanting the tubes
- Gamma counter
- Vortex mixer
- Distilled water
- Temperature controlled water bath (37°C, 90°C) or similar heating device

5. Sample collection and storage

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.
Determine the total volume of urine excreted during 24 h for calculation of the results!
Storage: for longer period (up to 6 months) at -20°C.
Repeated freezing and thawing of the samples should be avoided.
Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents – with the exception of Precipitating Reagent - to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes accordingly. Duplicates are recommended.

⚠ *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge the tubes for 1 minute at 500xg to spin down adhering liquids.*

6.1 Preparation of reagents

Acylation Solution

⚠ *Before preparing the Acylation Solution make sure that the Acylation Diluent (BA R-0075) has reached room temperature (≥ 20°C) and forms a homogenous, crystal-free solution.*

Dilute the Acylation Concentrate (BA R-0012) 1 + 60 with Acylation-Diluent in a glass or polypropylene-vial.

Acylation Concentrate	10 µL	20 µL	25 µL	50 µL
Acylation-Diluent	600 µL	1.2 mL	1.5 mL	3 mL

⚠ *The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!*

6.2 Preparation and acylation

Hydrolysis

1.	Pipette **25 µL** of **standards**, **25 µL** of **controls,** and **25 µL** of **urine samples** into the respective **Reaction Tubes.**
2.	Add **250 µL Hydrochloric Acid** to all tubes.
3.	Mix thoroughly (vortex) and hydrolyze for **30 min.** at **90 °C**.
4.	Let the tubes cool down to room temperature.
⚠	***For the measurement of the free normetanephrine only, leave away steps 3 and 4.***

Acylation

1.	Pipette **250 µL** of **Acylation Buffer** into all tubes.
2.	Add **25 µL** of **Acylation Solution** to all tubes.
3.	Mix thoroughly (vortex) and acylate for **15 minutes** at **RT** (20-25°C).
4.	Add **1 mL dist. water** to all tubes.
⚠	The following volumes of the eluates are needed for the RIA: **Normetanephrine 25 µL**

6.3 Normetanephrine RIA

1.	Pipette **25 µL** of the **acylated Standard A** into the polysterene tubes for the **NSB**.
2.	Pipette **25 µL** of the **acylated standards, controls** and **samples** into the respective polysterene tubes.
3.	Pipette **50 µL** of the **^{125}I Normetanephrine** into **all tubes**.
4.	Pipette **50 µL** of **Normetanephrine Antiserum** into **all tubes** (***except totals and NSB***); mix thoroughly.
5.	Cover tubes. Incubate for **60 minutes** at **37°C.**
6.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 µL** into **all tubes** (***except totals***), and mix on a vortex.
7.	Incubate for **15 minutes** at **2 - 8 °C**.
8.	Centrifuge for **15 minutes** at **3 000 x g**, if possible in a refrigerated centrifuge.
9.	**Decant** or aspirate the **supernatant carefully** (***except totals***). Blot the tubes dry and leave them upside for 2 minutes.
10.	**Count** all tubes for **1 minute** in a gamma counter.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Normetanephrine (ng/mL=µg/L)	0	30	90	300	900	3 000
Normetanephrine (nmol/L)	0	164	491	1 638	4 914	16 380
Conversion:	Normetanephrine (ng/mL) x 5.46 = Normetanephrine (nmol/L)					

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

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

The amount of analyte excreted per day (µg/day) is calculated according to:

concentration of the sample (in µg/L) x volume of urine excreted per day (in L/day)

Example
The concentration of the sample read from the curve is 125 µg/L. The amount of urine collected during 24 hours is 1.3 L. Then the amount of analyte excreted during one day would be:

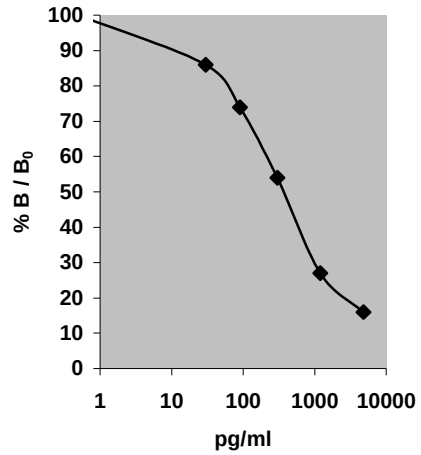
125 µg/L x 1.3 L/day = 162.5 µg/day

7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-report.

7.2 Typical calibration curve

NORMETANEPHRINE



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

Expected Reference Values		**Normetanephrine**
	Urine	< 600 µg/day

Analytical Sensitivity (Limit of Detection)		**Normetanephrine**
	Urine	11 ng/mL

	Substance	**Cross Reactivity (%)**
		Normetanephrine
Analytical Specificity (Cross Reactivity)	Derivatized Metanephrine	0.06
	Derivatized Normetanephrine	100
	Derivatized 3-methoxytyramine	0.08
	Adrenaline	< 0.001
	Noradrenaline	1.07
	Dopamine	0.001
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	< 0.001

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Normetanephrine	1	189 ± 12	6.6	Normetanephrine	1	230 ± 12	5.2
	2	1,095 ± 73	6.6		2	1,596 ± 100	6.3

Linearity			Range	Serial dilution up to	Mean (%)
	Normetanephrine	Urine	105 – 2,528 ng/mL	1:16	90

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Normetanephrine	Urine	100	86 – 111	

Method comparison versus HPLC*	Normetanephrine	Urine	HPLC = 1.1 RIA – 0.3	r = 0.99; n = 21

*The concentrations were assessed using both the RIA and the HPLC method (external QC samples from UK NEQAS). The correlation between RIA and HPLC is excellent. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content		CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!