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Instructions for use

Nephrines (Urine) RIA

^{125}I – Radioimmunoassay for the determination of
metanephrine and normetanephrine in urine.

For research use only, not for use in diagnostic procedures.

REF

IB88526

Σ
2 x 100



400 kBq

1. Introduction**1.1 Intended use and principle of the test**

¹²⁵I – Radioimmunoassay for the determination of metanephrine and normetanephrine in urine. For research use only, not for use in diagnostic procedures.

First, metanephrine (metadrenaline) and normetanephrine (normetadrenaline) are 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. Determination of unknown samples is achieved by comparing their activity with a standard curve prepared with known standards.

⚠ *The anti-Metanephrine antibodies used in this test kit only recognise the biologically relevant L-forms of Metanephrine. Commercially available synthetic Metanephrine is always a mixture of the D- and L-forms. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic Metanephrine is used to enrich native samples. As only about 50% of the synthetic Metanephrine – the L-portion – will be detected by use of this kit, spiked samples will be underestimated. Therefore native samples containing solely the L-form should be used.*

1.2 Background

Metanephrine and normetanephrine are the metabolites of the catecholamines epinephrine and norepinephrine, respectively. They are metabolized to vanillylmandelic acid or excreted with the urine.

Subjects with pheochromocytoma or other tumors derived from neuroendocrine cells show elevated urinary levels of total metanephrines.

As catecholamine secretion from neuroendocrine cells might show high variations, urine samples collected over a period of 24 hours are used to average these fluctuations.

2. Procedural cautions, guidelines, warnings and limitations**2.1 Precautions, guidelines and warnings**

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) This assay was validated for a certain type of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
- (3) The principles of Good Laboratory Practice (GLP) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) All kit reagents - with the exception of Precipitating Reagent – and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (6) For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
- (7) The radioactive material (¹²⁵Iodine, half life 60 days, emitting ionizing X-radiation with 28 keV and G-radiation with 35.5 keV) may be received, acquired, possessed and used only by physicians, laboratories or hospitals. In compliance with regulations, a copy of the customer's current radioisotope license must be on file with the supplier. Orders cannot be shipped until the license is received by the supplier (Radiation Protection Act of June 30, 1989).
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (10) Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
- (11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (12) A standard curve must be established for each run.
- (13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- (14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (15) For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.

- (16) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (17) The results obtained with this test kit are for research use only, not for use in diagnostic procedures.
- (18) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, the buffer capacity of the Acylation Buffer is insufficient. As a consequence metanephrine and normetanephrine will not be acylated quantitatively.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of (nor-)metanephrine level in the sample.

2.2.3 High-Dose-Hook effect


No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C.

4. Materials

4.1 Content of the kit

BA D-0023	REAC-TUBES	Reaction Tubes - Ready to use
Content:	Reaction Tubes in a resealable pouch	
Volume:	2 x 50 tubes	
BA R-0012	ACYL-CONC	Acylation Concentrate - Concentrated
Content:	Concentrated acylation reagent	
Volume:	1 x 0.5 ml/vial	
Hazards identification:		
	H 314 Causes severe skin burns and eye damage.	
BA R-0075	ACYL-DILUENT	Acylation Diluent - Ready to use
Content:	Dimethylsulfoxide	
Volume:	1 x 4 ml/vial, dark grey cap	
BA R-0025	PREC-REAG	Precipitating Reagent - Ready to use
Content:	Goat anti-rabbit serum in PEG phosphate buffer	
Volume:	2 x 55 ml/vial, white cap	
BA R-8619	HCL	Hydrochloric Acid - Ready to use
Content:	0.25 M hydrochloric acid, yellow coloured	
Volume:	1 x 30 ml/vial, dark green cap	

BA R-0120 ¹²⁵I ADR MN **¹²⁵I – Metanephrine** - Ready to use
 Content: ¹²⁵I labeled Metanephrine, red coloured
 Volume: 1 x 5.5 ml/vial, blue cap

Hazards identification:



Radioactive, activity < 200 kBq

BA R-0220 ¹²⁵I NAD NMN **¹²⁵I – Normetanephrine** - Ready to use
 Content: ¹²⁵I labeled Normetanephrine, red coloured

Volume: 1 x 5.5 ml/vial, yellow cap

Hazards identification:



Radioactive, activity < 200 kBq

BA R-8410 AS MN **Metanephrine Antiserum** - Ready to use

Content: Rabbit anti-metanephrine antibody, blue coloured

Volume: 1 x 5.25 ml/vial, blue cap

BA R-8510 AS NMN **Normetanephrine Antiserum** - Ready to use

Content: Rabbit anti-normetanephrine antibody, yellow coloured

Volume: 1 x 5.25 ml/vial, yellow cap

Standards and Controls - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml		Concentration nmol/l		Volume/Vial
			MN	NMN	MN	NMN	
BA R-8601	STANDARD A	white	0	0	0	0	12 ml
BA R-8602	STANDARD B	light yellow	20	30	101	164	4 ml
BA R-8603	STANDARD C	orange	60	90	304	491	4 ml
BA R-8604	STANDARD D	dark blue	200	300	1 014	1 638	4 ml
BA R-8605	STANDARD E	light grey	600	900	3 042	4 914	4 ml
BA R-8606	STANDARD F	black	2 000	3 000	10 140	16 380	4 ml
BA R-8651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!				4 ml
BA R-8652	CONTROL 2	dark red					4 ml

Conversion: Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)
 Normetanephrine (ng/ml) x 5.46 = Normetanephrine (nmol/l)

Content: Acidic buffer with non-mercury preservatives, spiked with defined quantity of metanephrine and normetanephrine

BA R-8611 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: TRIS buffer

Volume: 1 x 30 ml/vial, white cap

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


- Calibrated precision pipettes to dispense volumes between 10 - 3000 µl
- Polystyrene tubes and suitable rack
- Centrifuge capable of at least 3 000 x g
- Temperature controlled water bath (37°C, 90°C) or similar heating device
- Suitable device for aspirating or decanting the tubes
- Gamma counter
- Vortex mixer
- Water (deionized, distilled, or ultra-pure)

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 500 x g 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 μ l	20 μ l	25 μ l	50 μ l
Acylation-Diluent	600 μ l	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 μ l** of **standards, controls** and **urine samples** into the respective **Reaction Tubes**.
2. Add **250 μ l 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 metanephrine and free normetanephrine only, leave away steps 3 and 4.***

Acylation

1. Pipette **250 μ l** of **Acylation Buffer** into **all tubes**.
2. Add **25 μ l** of **Acylation Solution** (*refer to 6.1*) to **all tubes**.
3. Mix thoroughly (vortex) and acylate for **15 min** at **RT** (20 - 25 °C).
4. Add **1 ml water** (deionized, distilled, or ultra-pure) to **all tubes**.

 The following volumes of the eluates are needed for the subsequent RIA:

Metanephrine **25 μ l**

Normetanephrine **25 μ l**

6.3 Metanephrine RIA

1. Pipette **25 μ l** of the **acylated Standard A** into polystyrene **tubes** for the **NSB**.
2. Pipette **25 μ l** of the **acylated standards, controls** and **samples** into the respective **tubes**.
3. Pipette **50 μ l** of the **¹²⁵I Metanephrine** into **all tubes**.
4. Pipette **50 μ l** of **Metanephrine Antiserum** into **all tubes** (*except totals and NSB*); mix thoroughly.
5. Cover **tubes**. Incubate for **60 min** at **37 °C**.
6. Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette each **500 μ l** into **all tubes** (*except totals*), and mix on a vortex.
7. Incubate for **15 min** at **2 - 8 °C**.
8. Centrifuge for **15 min** 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 down for 2 minutes.
10. **Count** all tubes for **1 min** in a gamma counter.

6.4 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 tubes .
3.	Pipette 50 µl of the ¹²⁵I 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 min 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 min at 2 - 8 °C .
8.	Centrifuge for 15 min 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 down for 2 minutes.
10.	Count all tubes for 1 min in a gamma counter.

7. Calculation of results

Measuring range	Metanephrine	Normetanephrine
	8 – 2 000 ng/ml	22 – 3 000 ng/ml

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.

The standard 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).



This assay is a competitive assay. This means: the counts are decreasing with increasing concentrations of the analyte. Counts found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.

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

The total amount of Metanephrine and Normetanephrine excreted per day (µg/day) is calculated according to:

concentration read from the standard curve (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:

$$125 \mu\text{g/l} \times 1.3 \text{ l/day} = 162.5 \mu\text{g/day}$$

Conversion

Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)

Normetanephrine (ng/ml) x 5.46 = Normetanephrine (nmol/l)

Expected reference values


It is strongly recommended that each laboratory should determine its own reference values.

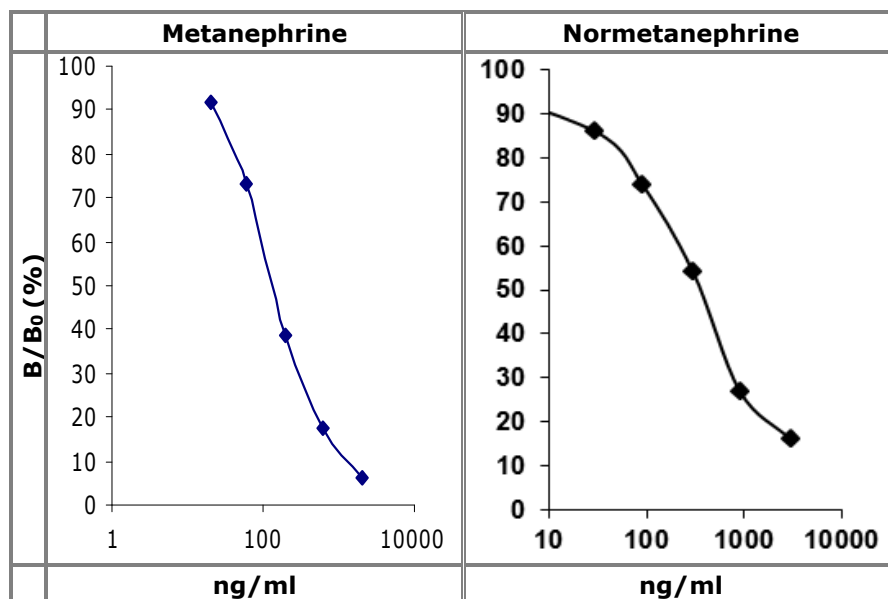
	Metanephrine	Normetanephrine
24-hour urine	< 350 µg/day	< 600 µg/day

7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and elevated 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 standard curves

 Examples, do not use for calculation!



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	Metanephrine	Normetanephrine
	8 ng/ml	22 ng/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
	Derivatized Metanephrine	100	0.06
	Derivatized Normetanephrine	0.33	100
	Derivatized 3-methoxytyramine	< 0.02	0.08
	Adrenaline	0.03	< 0.01
	Noradrenaline	< 0.02	1.07
	Dopamine	< 0.02	0.01
	Vanillic mandelic acid, Homovanillic acid, L-Dopa, L-Tyrosin, Tyramin	< 0.02	< 0.01

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Metanephrine	1	56 ± 5.9	10.6	Metanephrine	1	43.6 ± 3.6	6.2
	2	304 ± 23	7.6		2	279 ± 26	9.3
Normetanephrine	1	230 ± 36	15.8	Normetanephrine	1	372 ± 78	20.8
	2	1 095 ± 73	6.6		2	1142 ± 179	15.7

Linearity		Range (ng/ml)	Serial dilution up to	Mean (%)
	Metanephrine	42 - 803	1:16	109
	Normetanephrine	105 - 2 528	1:16	90

Recovery		Sample	Range (%)	Mean (%)
	Metanephrine	1	73 - 121	94
		2	85 - 122	103
Normetanephrine	1	86 - 111	100	

Method comparison versus HPLC*	Metanephrine	HPLC = 1.02 RIA - 0.3	r = 0.99; n = 21
	Normetanephrine	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.			

9. References/Literature













- (1) Parrott et al. Urinary corticosterone and normetanephrine levels after voluntary wheel and forced treadmill running in the db/db mouse. *Journal of Diabetes Mellitus*, 1(4):71-78 (2011)
- (2) Petramala et al. Multiple Catecholamine-Secreting Paragangliomas: Diagnosis after Hemorrhagic Stroke in a Young Woman. *Endocrine Practice*, 14(3):340-346 (2008)
- (3) Sato et al. Central control of bone remodeling by neuromedin U. *Nature Medicine*, 13:1234-1240 (2007)

For orders, please contact:

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 **For updated literature or any other information please contact your local supplier.**

Symbols:

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