Instruction

EURIA-Vasopressin

Vasopressin radioimmunoassay

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For Research Use Only. Not for use in diagnostic procedures.

REF **RB 319 RUO**

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PURPOSE OF RESEARCH PRODUCT

The vasopressin kit contains reagents and instructions for the determination of vasopressin in plasma or urine. After solid phase extraction (SPE) or ethanol extraction the plasma vasopressin concentrations are measured by radioimmunoassay (RIA). Urine vasopressin concentrations can be measured directly.
The result shall not be used for clinical diagnosis or patient management.

APPLICATION

Vasopressin, or Antidiuretic Hormone (ADH) is a cyclic nanopeptide with a molecular weight of 1083. Its structure is very similar to that of oxytocin, differing in only two amino acids. Endogenous ADH has antidiuretic and pressor activity, both approaching 400 units per mg, with an antidiuretic-to-vasopressin ratio of 1, and a biphasic plasma half-life of 2.5 and 14.5 minutes. ADH is synthesized in the hypothalamic supraoptic nucleus and paraventricular nucleus of primates and transported via exonal flow to the posterior pituitary for storage and eventual release. Vasopressin determination is useful in the study of diabetes insipidus, psychogenic water intoxication, hyponatraemia, stress conditions, ADH as a neurotransmitter and hypertension studies. ADH values can be influenced by cigarettes, tea, coffee, alcohol and some drugs.

PRINCIPLE OF THE TEST

After solid phase extraction (SPE) or ethanol extraction of the plasma samples, vasopressin is assayed by a competitive radioimmunoassay. Urinary vasopressin can be measured directly. This assay uses a rabbit anti-vasopressin antiserum and a radioiodinated vasopressin [^{125}I] tracer. Bound and free phases are separated by a second antibody bound to solid phase particles, followed by a centrifugation step. The radioactivity in the bound fractions is measured and a typical standard curve can be generated. The values of the extracted samples are corrected for extraction recovery.

PRECAUTIONS

For research use only. Not for use in diagnostic procedures.

Materials derived from human blood and used in the preparation of this kit were tested and found negative for hepatitis B surface antigen (HBsAg), antibodies to HCV and for antibodies to HIV-1 and HIV-2. However, handle all components as a possible source of infection.

This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. The radioactive material included may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals for in-vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulation of each country.

Adherence to the basic rules of radiation safety should provide adequate protection.

- Do not eat, drink, smoke or apply cosmetics where radioactive materials are used.
- Do not pipette radioactive solutions by mouth.
- Avoid direct contact with all radioactive materials by using protective articles such as lab coats and disposable gloves.
- All radiological work should be done in a designated area.
- Radioactive materials should be stored in original containers in a designated area.
- Laboratory equipment and glassware, which are subject to contamination, should be segregated to prevent cross-contamination of different radioisotopes.
- Any radioactive spills should be taken care of immediately in accordance with established procedures.
- All radioactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies jurisdiction over the laboratory.

The reagents in this kit contain sodium azide (0.05%). Contact with copper or lead drain pipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbing suspected of being contaminated with these explosive deposits should be rinsed thoroughly with 10% sodium hydroxide solution.

SPECIMEN COLLECTION

Careful standardization of sample preparation and sampling conditions is recommended.

Vasopressin in plasma
- Draw blood from fasting subject into a chilled tube, containing EDTA or Heparin.
- Centrifuge at 4° C to separate the plasma.
- Freeze the sample in plastic tubes at -20° C until assayed.

NOTE: Vasopressin (ADH) in plasma is stable at -20° C only for 4 weeks, or stable up to 3 months after addition of 500 KIU Trasylol (Bayer) per mL blood; after extraction, Vasopressin is stable at -20° C for 6 months.

Vasopressin in urine
Vasopressin can be determined directly, in unextracted human urine.
- Collect 24 hours urine sample.
- Register urine volume.
- Measure urinary osmolarity.
- If the sample is not assayed immediately, keep an aliquot at -20° C.
- Measure urine samples undiluted and in dilutions of 1:2, 1:4 or higher.

MATERIALS AND EQUIPMENT REQUIRED

Pipettes (100 µL, 200 µL, 300 µL, 1.00 mL, 2.00 mL, 5.00 mL)
Repeating dispensers (100 µL, 200 µL)
Measuring cylinder 25 mL
Polystyrene RIA tubes (12 x 75 mm)
Ethanol absolute (99%)
Vortex
Centrifuge
Icebath
Vac-concentrator
Nitrogen gas
Polystyrene or glass tubes for extraction (16 x 100 mm)
Sep-pak C18 (Waters Ass Inc. art. 051910)
Acetic acid 4%
Methanol absolute (99%)
1N HCl

QUALITY CONTROL

Controls should be carried out in each assay run. Two controls are included in the kit, the value (without extraction procedure) is indicated on the Quality Control sheet and on the label of the vials. Use also controls as recommended by the control plasma manufacturer and in accordance with reference laboratories practice to monitor the accuracy and precision of reagents and techniques. Each laboratory should establish its own extraction recovery under their own experimental conditions.

SHELFLIFE AND STORAGE

This kit is stable until the stated expiry date if stored as specified.
Upon receipt of the kit, all reagents should be stored at 2-8° C.
The reconstituted reagents should be stored according to table on page 6.
The reconstituted reagents are stable according to table on page 6, but no longer than to the expiry date.

CONTENTS OF THE KIT

Item	**Nr. of Vials**	**Containing**
Anti-vasopressin (Reagent A)	1	Lyophilized anti-vasopressin (Rabbit) for 100 tubes. Colour: Yellow.
^{125}I-vasopressin (Reagent B)	1	28 KBq or 0.75 μCi. Lyophilized. Specific activity: 62-77 MBq/nmol (1700-2100 μCi/nmol). Colour: Blue.
Double antibody solid phase (Reagent C)	1	Goat anti-rabbit IgG's bound to solid phase in 0.01 M phosphate buffer pH 6.8 with 0.25% Human serum albumin, 0.045% NaCl, 0.05% NaN3, 0.185% EDTA and 0.05% Tween 80. 11 mL suspension.
Assay buffer (Reagent D)	2	0.05 M phosphate buffer with 0.25% HSA, 0.25% EDTA disodium salt, 0.05% NaN_3, 500 KIU Trasylol/mL. 2 x 50 mL (liquid).
Vasopressin standard 60 pmol/L (Reagent E)	1	5.00 mL vasopressin standard, 60 pmol/L. Lyophilized in assay buffer.
Vasopressin, low control (Reagent F)	1	2.00 mL lyophilized vasopressin control, low level.
Vasopressin, high control (Reagent G)	1	2.00 mL lyophilized vasopressin control, high level.

PREPARATION OF REAGENTS

Item	**Reconstitute each vial with**		**Stable at**	**Special remarks**
Anti-vasopressin (Reagent A)	22 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	
^{125}I-vasopressin (Reagent B)	25 mL distilled water	Mix gently	-20° C until expiry date	
Double antibody solid phase (Reagent C)	Ready for use. The separation reagent should be placed on a magnetic stirrer for 10 minutes at room temp		2-8° C until expiry date	It is possible to pipette the reagent with a repeating dispenser
Assay buffer (Reagent D)	Ready for use		2-8° C until expiry date	
Vasopressin standard 60 pmol/L (Reagent E)	5.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	Refer to table for standard curve preparation
Vasopressin low control (Reagent F)	2.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	The value of the control is found on the lable of the vial and in the QC sheet (without extraction)
Vasopressin high control (Reagent G)	2.00 mL distilled water	Mix gently	-20° C for at least 3 months after reconstitution	The value of the control is found on the lable of the vial and in the QC sheet (without extraction)

P R E P A R E A L L R E A G E N T S 1 5 M I N U T E S B E F O R E U S E !

SAMPLE PREPARATION

Before proceeding in the RIA procedure two different sample preparation methods can be used:

A1 : Sep-pak C18 extraction
A2 : Ethanol extraction

A1: Sep-pak C18 extraction procedure

Column: Sep-pak C18 cartridge (Waters Ass. Inc. art. 051910)

Procedure: DO NOT EXTRACT STANDARDS AND CONTROLS.

1. Wash the column with 10 mL distilled water, 5 mL methanol and 10 mL distilled water, respectively.
2. Acidify 1.0 mL plasma sample with 150 µL 1N HCl.
3. Bring this acidified sample into the column.
4. Wash the column with 20 mL 4% acetic acid.
5. Elute with 4 mL methanol.
6. Dry the methanol under a stream of nitrogen or air.
7. Reconstitute the residue with 1.0 mL of assay buffer.
8. Follow the regular RIA manual.

To estimate recovery, add an aliquot (200 µL) ^{125}I-vasopressin tracer to a random plasma sample and submit the recovery sample for the same extraction procedure.

Recovery calculation

a/. Prepare a recovery estimation tube (R).
- Pipette 1.0 mL of a random plasma sample into the recovery tube (R). The sample used for this recovery assay should have a protein matrix similar to the samples being tested.
- Add 200 µL ^{125}I-vasopressin tracer into tube R and mix.
- Extract this sample along with samples in the above procedure.

b/. Prepare a Total Recovery tube (TR).
- Pipette 200 µL ^{125}I-vasopressin tracer into two TR tubes.
- Add 100 µL assay buffer and mix.
- Cap and set aside this tube to be counted for recovery calculation.

c/. Reconstitute the dried recovery sample (R) by adding 1.0 mL assay buffer and vortex thoroughly.

d/. Pipette 300 µL of the reconstituted recovery sample tube (R) into two assay tubes.

e/. Count the total recovery (TR) and recovery (R) tubes for at least two minutes in a gamma counter.

Calculate % recovery by dividing the cpm in the recovery tubes (R) by cpm in the total recovery tubes (TR) and multiply by 3.33:

$$\% \text{ Recovery} : \frac{\text{cpm recovery tube (R)}}{\text{cpm total recovery tube}} \times 3.33 \times 100\%$$

Extraction recoveries should score values between 60-80%.

A2: Ethanol extraction procedure

DO NOT EXTRACT STANDARDS AND CONTROLS.

1. Label one extraction tube (E) for each sample. Label one additional tube (R) in order to estimate the extraction recovery.
2. Place the extraction tubes and ethanol on ice.
3. Pipette 0.8 mL of each sample into the appropriately labelled extraction tubes (E).
4. Prepare a recovery estimation tube (R).
   - Pipette 0.8 mL of a random plasma sample into the recovery tube (R). The sample used for this recovery assay should have a protein matrix similar to the samples being tested.
   - Add 200 µL ^{125}I-vasopressin tracer into tube R and mix.
   - Extract this sample along with samples in step 6.
5. Prepare a Total Recovery tube (TR).
   - Pipette 200 µL ^{125}I-vasopressin tracer into two TR tubes.
   - Add 100 µL assay buffer and mix.
   - Cap and set aside this tube to be counted for recovery calculation.
6. Add 4 mL chilled ethanol to each sample (tubes E) and Recovery Tube (R).
7. Mix and vortex for 2 minutes.
8. Centrifuge all extraction tubes (E and R) at 2000 g. for 15 min. at 2-8° C.
9. Decant supernatant from each extraction tube into previous prepared clean, appropriately labelled 16 x 100 mm tubes.
10. Evaporate the supernatants under a stream of nitrogen to dryness (at max. 37° C), or evaporate by using a Vac-concentrator.
11. Reconstitute the dried samples by adding 0.8 mL assay buffer and vortex thoroughly.
12. Proceed RIA procedure immediately or store the extracted samples at -20° C up to two weeks before using in the assay.
13. Reconstitute the dried recovery sample (R) by adding 0.8 mL assay buffer and vortex thoroughly.
14. Pipette 300 µL of the reconstituted recovery sample tube (R) into two assay tubes.
15. Count the total recovery (TR) and recovery (R) tubes for at least two minutes in a gamma counter.

Recovery calculation

Calculate % recovery by dividing the cpm in the recovery tubes (R) by cpm in the total recovery tubes (TR) and multiply by 2.67:

$$\% \text{ Recovery} : \frac{\text{cpm recovery tube (R)}}{\text{cpm total recovery tube (TR)}} \times 2.67 \times 100\%$$

Extraction recoveries should score values between 40-50%.

ASSAY PROTOCOL

A. Preparation of standard solutions

Dilution	**Reagent E (=standard a)**	**Vasopressin concentration 60 pmol/L**
1000 µL of Reagent E + 1000 µL assay buffer vortex	standard b	30 pmol/L
1000 µL of standard b + 1000 µL assay buffer vortex	standard c	15 pmol/L
1000 µL of standard c + 1000 µL assay buffer vortex	standard d	7.5 pmol/L
1000 µL of standard d + 1000 µL assay buffer vortex	standard e	3.8 pmol/L
1000 µL of standard e + 1000 µL assay buffer vortex	standard f	1.9 pmol/L
	assay buffer	0 pmol/L

B. Assay Procedure

1. After preparation of the standard solutions pipette 300 µL of each standard, controls, each extract from plasma, or diluted urine into the correspondingly labelled tubes
2. Add 300 µL assay buffer (Reagent D) to the max binding (0 pmol/L binding) tubes and 500µL assay buffer to the blank tubes (NSB).
3. Add 200 µL vasopressin antiserum (Reagent A) to all tubes, except blank (NSB) and total counts (TC) tubes
4. Vortex all tubes and incubate at 4° C for 18-24 hours
5. Add 200 µL ^{125}I-vasopressin (Reagent B) to all tubes
6. Vortex all tubes and incubate at 4° C for 18-24 hours
7. While stirring continuously add 100 µL double antibody solid phase (Reagent C) to all tubes, except TC tubes
8. Vortex and incubate 30-60 minutes at 4° C
9. Centrifuge all tubes for 15 min. at 1700 g at 4° C
10. Decant or aspirate supernatant
11. Count residue for 2-4 min.

	Tube No.	Assay buffer (D)	Standard or sample or contr.	Anti-Vasopressin (A)		^{125}I-Vasopressin (B)		Double antibody solid phase (C)		
TOT	1-2	-	-	-	Vortex and incubate for 18-24 hrs at +4° C.	200 µL	Vortex and incubate for 18-24 hrs at +4° C	-	Vortex and incubate for 30-60 minutes at 4° C	Centrifuge for 15 minutes at 1700 g at 4° C. Aspirate or decant the supernatant. Count the residue for 2-4 minutes.
Blank (NSB)	3-4	500 µL	-	-		200 µL		100 µL		
St. 0 pmol/L	5-6	300 µL	-	200 µL		200 µL		100 µL		
St. 1.9 pmol/L	7-8	-	300 µL	200 µL		200 µL		100 µL		
St. 3.8 pmol/L	9-10	-	300 µL	200 µL		200 µL		100 µL		
St. 7.5 pmol/L	11-12	-	300 µL	200 µL		200 µL		100 µL		
St. 15 pmol/L	13-14	-	300 µL	200 µL		200 µL		100 µL		
St. 30 pmol/L	15-16	-	300 µL	200 µL		200 µL		100 µL		
St. 60 pmol/L	17-18	-	300 µL	200 µL		200 µL		100 µL		
Control (F)	19-20	-	300 µL	200 µL		200 µL		100 µL		
Control (G)	21-22	-	300 µL	200 µL		200 µL		100 µL		
Unknown sample	23-24									

C. Calculation

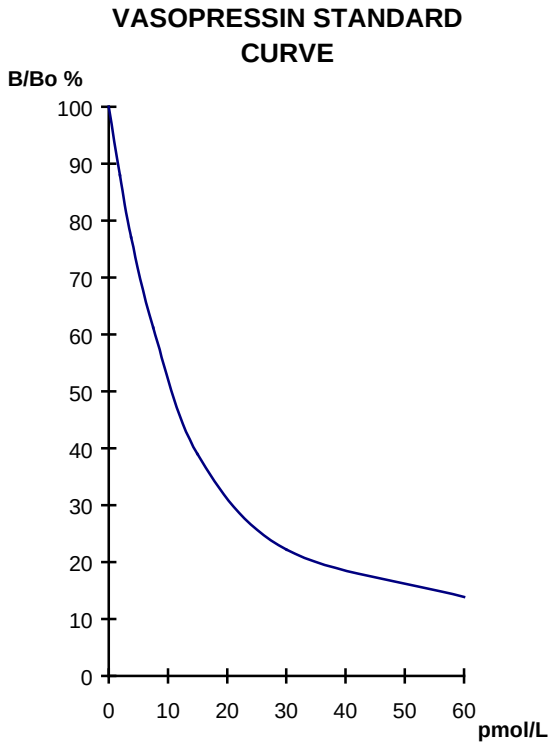
- Substract the average count rate (cpm) of the NSB from the average count rate (cpm) of the replicates of standards, controls and samples.
- A standardcurve can be generated by plotting cpm, % B/Bo or % B/T of precipitated bound fraction against the concentration of the vasopressin calibrators.
- To obtain the vasopressin concentration in the extracted samples and controls, their cpm, % B/Bo or % B/T of precipitated bound fractions are interpolated now from the generated standardcurve.
- The standard curve can also be constructed by computer methods. For automated data reduction, both logit/log and Spline methods can be used.
- Correct plasma and urine values for % extraction recovery.

Urine: calculate the 24 hour vasopressin excretion:
Vasopressin pmol/L x dilution x 24 hours urine volume in L.
This calculation provides vasopressin concentration in pmol/24 hours.

D. Standard Curve Data

	Average cpm	Corrected cpm	% B/Bo	Results (pmol/L)
Total counts	11107			
NSB	555			
Standard 0 pmol/L	4770	4215	100	
Standard f 1.9 pmol/L	4340	3785	89.8	
Standard e 3.8 pmol/L	3739	3184	75.5	
Standard d 7.5 pmol/L	3135	2580	61.2	
Standard c 15 pmol/L	2199	1644	39.0	
Standard b 30 pmol/L	1490	935	22.2	
Standard a 60 pmol/L	1142	587	13.9	
Control low	3741	3186	75.6	4.0
Control high	1769	1214	28.8	21.1

EXAMPLE OF STANDARD CURVE



ASSAY CHARACTERISTICS

Precision									
Within-run						**Between-run**			
	n	**mean pmol/L**	**SD**	**% c.v.**			**n**	**mean pmol/L**	**SD**	**% c.v.**
sample A	18	4.17	0.27	6.5		sample A	6	4.38	0.26	6.0
sample B	16	20.2	1.00	4.9		sample B	6	21.4	1.48	6.9

Calibration
This assay is calibrated against the first international WHO standard 77/501

Recovery

Two different samples are spiked with different amounts of vasopressin standard

Sample	Expected conc. (pmol/L)	Observed conc. (pmol/L)	% Recovery
A1	9.2	9.8	106
A2	14.3	14.4	101
B1	9.6	10.0	104
B2	15.3	15.1	98

Specificity
Vasopressin antiserum is raised in rabbits.
The following cross reactivities were measured at 50% binding (B/Bo)

Peptide	% Crossreactivity
Arg^8 Vasopressin	100
Oxytocin	<0.1
Lys^8-Vasopressin	<0.1
Desmopressin	<0.1
Arg^8 Vasotocin	80

Sensitivity
The sensitivity judged as 3 standard deviations change from zero calibrator is 0.5 pmol/L

Normal Range
Each laboratory should establish its own normal range of expected values.
Plasma: up to 13 pmol/L
Urine: 57 ± 22 pmol/24 hours urine

Interference
Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

LITERATURE

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SYMBOLS USED ON LABELS

	Batch code.
	Catalogue number.
	Use by date.
	Temperature limit.
	Date of manufacture.
	Contains radioactive substances.
	Biological risks.
	Consult instructions for use.
	Manufacturer.
 **100**	Contains sufficient for 100 tests.

REAG A Ab	Anti-Vasopressin.
REAG B Ag ^{125}I	^{125}I-Vasopressin.
REAG C DASP	Double antibody solid phase.
REAG D BUF AS	Assay diluent.
REAG E CAL 60	Vasopressin standard 60 pmol/L.
REAG F CONTROL	Control, level 1 (low).
REAG G CONTROL	Control, level 2 (high).

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