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VASOPRESSIN - RIA

KIPERB319

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

Previous Version: 200224-1	Current Version: 200701
	Text added: **V. REAGENTS PROVIDED** CAL and CONTROL N see exact value on vial label
X. PROCEDURE **A2: Ethanol extraction procedure** 8. Centrifuge all extraction tubes (samples and R) at 2000 g. for 15 min. at 2-8°C.	**X. PROCEDURE** **A2: Ethanol extraction procedure** 8. Centrifuge all extraction tubes (samples and R) at 2000 g. for 15 min. at 4°C.
X. PROCEDURE B. Procedure and **XVIII. SUMMARY OF THE PROTOCOL:** Incubation at 4°C	**X. PROCEDURE B. Procedure** and **XVIII. SUMMARY OF THE PROTOCOL:** Replacement by "incubation at 2-8°C"
X. PROCEDURE ***A1: Sep-pak C18 extraction procedure*** "Extraction recoveries should score values between 60-80%." ***A2: Ethanol extraction procedure*** "Extraction recoveries should score values between 40-50%."	**X. PROCEDURE** ***A1: Sep-pak C18 extraction procedure*** "Extraction recoveries should score values of, at least, 60%." ***A2: Ethanol extraction procedure*** "Extraction recoveries should score values of, at least, 40%."
	Text added: **XVI. PRECAUTIONS AND WARNINGS** For more information, see Material Safety Data sheet (MSDS).

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Read entire protocol before use.

Vasopressin RIA

I. INTENDED USE

The DIAsource vasopressin kit contains reagents and instructions for the quantitative measurement 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.
For professional use within a laboratory.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Vasopressin RIA

B. Catalog number : KIPERB319 : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. CLINICAL BACKGROUND

A. Biological activities

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.

B· Clinical application

The clinical application of a vasopressin radioimmunoassay is in 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.

IV. PRINCIPLES OF THE METHOD

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 calibration curve can be generated. The values of the extracted samples are corrected for extraction recovery.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
Ab Lyophilized anti-vasopressin	1 vial lyophilized	Blue	**Add** 22 ml distilled water
Ag | ^{125}I TRACER: ^{125}Iodine labelled vasopressin Specific activity: 62-77 MBq/nmol (1700-2100 µCi/nmol).	1 vial lyophilized 28 kBq	Red	**Add** 25 ml distilled water
DASP Double antibody solid phase Goat anti-rabbit IgG's bound to solid phase in phosphate buffer with Human serum albumin, NaCl, NaN3, EDTA and Tween 80.	1 vial 11 mL	Green	**Ready** for use
ASS BUF Assay diluent Phosphate buffer with HSA, EDTA disodium salt, NaN3 and aprotinin (Trasylol® or equivalent)	2 vials 50 mL	Black	**Ready** for use
CAL Vasopressin Calibrator **(see exact value on vial label)**	1 vial lyophilized	Yellow	**Reconstitute** with distilled water by the volume stated on the vial label
CONTROL N Controls - N = 1 or 2 **(see exact value on vial label)**	2 vials lyophilized	Silver	**Add** 2 mL distilled water

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

Pipettes (100 µL, 200 µL, 300 µL, 1 mL, 2 mL, 5 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
Acetic acid 4%
Methanol absolute (99%)
1N HCl

VII. REAGENT PREPARATION

PREPARE ALL REAGENTS 15 MINUTES BEFORE USE !

A. Anti-vasopressin : Reconstitute with 22 mL of distilled water. Mix gently. Store at -20°C for at least 3 months after reconstitution.

B. ^{125}I- vasopressin : Reconstitute with 25 mL of distilled water. Mix gently. Store at -20°C until expiry date.

C. Double antibody solid phase : Ready for use. The separation reagent should be placed on a magnetic stirrer for 10 minutes at room temperature (18-25°C). Store at 2-8°C until expiry date. It is possible to pipette the reagent with a repeating dispenser.

D. Assay buffer : Ready for use. Store at 2-8°C until expiry date.

E. Calibrator : Reconstitute with distilled water by the volume stated on vial label. Mix gently. Store at -20°C for at least 3 months after reconstitution. Refer to table in section *X. B* for calibration curve preparation.

F. Controls : Reconstitute with 2 mL of distilled water. Mix gently. Store at -20°C for at least 3 months after reconstitution. The value of the controls is found on the label of the vial (without extraction).

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

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 in section *VII. Reagent Preparation*.
The reconstituted reagents are stable according to table in section *VII. Reagent Preparation*, but no longer than the expiry date.

IX. SPECIMEN COLLECTION

Careful standardization of the patient preparation and sampling conditions is recommended.

Vasopressin in plasma
- Draw blood from fasting patient 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 aprotinin (Trasylol® or equivalent) 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.

X. PROCEDURE

A. 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

DO NOT EXTRACT CALIBRATORS AND CONTROLS.
1. Wash the column with 10 mL distilled water, 5 mL methanol and 10 mL distilled water, respectively.
2. Acidify 1 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 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 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 the 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 Total Recovery tubes (TR).

- Add 100 µL assay buffer and mix.
- Cap and set aside these tubes to be counted for recovery calculation.

c/ Reconstitute the dried Recovery sample (R) by adding 1 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:

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

Extraction recoveries should score values of, at least, 60%.

A2: Ethanol extraction procedure

DO NOT EXTRACT CALIBRATORS AND CONTROLS.

1. Label one extraction tube for each patient sample. Label one additional tube 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.
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 Recovery 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 Total Recovery tubes (TR).
   - Add 100 µL assay buffer and mix.
   - Cap and set aside these tubes to be counted for recovery calculation.
6. Add 4 mL chilled ethanol to each sample and Recovery tube (R).
7. Mix and vortex for 2 minutes.
8. Centrifuge all extraction tubes (samples and R) at 2000 g. for 15 min. at 4°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 of, at least, 40%.

B. Procedure

Preparation of Calibrator solutions

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

1. After preparation of the Calibrator solutions, pipette 300 µL of each Calibrator, control, each extract from plasma, or diluted urine into the correspondingly labelled tubes.
2. Add 300 µL assay buffer to the max binding (0 pmol/L binding) tubes and 500µL assay buffer to the blank tubes (NSB).
3. Add 200 µL vasopressin antiserum to all tubes, except blank (NSB) and Total counts tubes.
4. Vortex all tubes and incubate at 2-8°C for 18-24 hours.
5. Add 200 µL ^{125}I-vasopressin to all tubes.
6. Vortex all tubes and incubate at 2-8°C for 18-24 hours.
7. While stirring continuously add 100 µL double antibody solid phase. to all tubes, except Total count tubes.
8. Vortex and incubate 30-60 minutes at 2-8°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.

XI. CALCULATION OF RESULTS

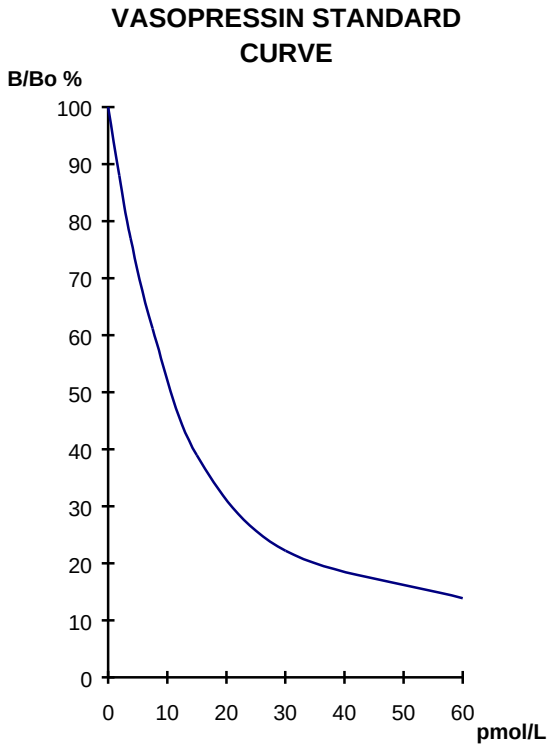
- Subtract the average count rate (cpm) of the NSB from the average count rate (cpm) of the replicates of calibrators, controls and patient samples.
- A calibration curve can be generated by plotting cpm, % B/Bo or % B/T of precipitated bound fraction against the concentration of the vasopressin calibrators.
- To obtain the vasopressin concentration in the extracted patient samples and controls, their cpm, % B/Bo or % B/T of precipitated bound fractions are interpolated from the generated calibration curve.
- The calibration curve can also be constructed by computer methods. For automated data reduction, both logit/log and Spline methods can be used.

- Correct the urine values according to the dilution factor applied.
  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.

- Correct the plasma values for % extraction Recovery
  For example:
  Patient sample concentration measured from the curve: 10 pmol/L
  Extraction recovery measured after Ethanol extraction : 45%
  Patient sample concentration corrected : $\frac{10 \times 100}{45}$ = 22.2 pmol/L

XII. TYPICAL DATA

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



XIII. PERFORMANCE AND LIMITATIONS

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 Calibrator

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

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

Peptide	% Cross reactivity
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

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

XIV. INTERNAL 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 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.

XV. REFERENCE INTERVALS

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

XVI. PRECAUTIONS AND WARNINGS

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 125I (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.
For more information, see Material Safety Data sheet (MSDS).

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrators (0-6)	Controls	Samples
Assay buffer		**500 µl**			
Calibrators	**-**	**-**	**300 µl**	**-**	**-**
Controls	**-**	**-**	**-**	**300 µl**	**-**
Samples	**-**	**-**	**-**	**-**	**300 µl**
Anti-vasopressin	**-**	**-**	**200 µl**		
Vortex and incubate for 18-24 hours at 2-8°C.					
^{125}I Tracer	**200 µl**				
Vortex and incubate for 18-24 hours at 2-8°C.					
Double antibody solid phase	**-**	**100 µl**			
Vortex and incubate for 30-60 min at 2-8°C.					
Centrifuge 15 min (1700 g; 4°C)					
Aspirate or decant the supernatant and count the residue for 2-4 minutes					

Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/

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