VASOPRESSIN

RIA

This product is for research use only
It is not intended for use in diagnostic procedures

RK-AR1-U 100 tests

Revision date: 2016.01.20

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ENGLISH

INTENDED USE

This double antibody radioimmuno-assay is designed for the quantitative *in vitro* diagnostic determination of immunoreactive **arginine vasopressin** (anti-diuretic hormone, ADH) in EDTA plasma and urine after extraction (1-3). This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

Immunoreactive arginine vasopressin is measured by a double-antibody radioimmunoassay using a modification of the method of Glick and Kagan (4). Column (or ethanol) extracted plasma samples and calibrators are pre-incubated for 24 hours with the anti-vasopressin antibody; ^{125}I-vasopressin competes then with vasopressin present in samples and calibrators for the same antibody binding sites. After a second 24 hour incubation, the solid phase second antibody is added to the mixture, the antibody-bound fraction is finally precipitated and counted.

The procedure recommends a solid-phase extraction of the plasma samples with reversed-phase columns (phenylsilylsilica). Alternatively, an extraction of plasma samples with ethanol may also be used. However, the values using the ethanol procedure are somewhat higher due to unspecific matrix effects (see Page 3).

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Extraction Column (1ml) Reversed phase columns containing 100 mg phenyl-silylsilica	10 pieces	B-AEC	For column preparation and conditioning see chapter "Extraction"
Phosphate Buffer lyophilized Buffer	1 vial	B-AR1-PB	Reconstitute with 100 ml of deionized water
Antiserum lyophilized rabbit anti-vasopressin antibody	1 vial	B-AR1-AS	Reconstitute with 10 ml of deionized water
Tracer lyophilized ^{125}I-Vasopressin	1 vial	B-ADH-TR	Reconstitute with 11 ml of Phosphate Buffer
Calibrator[1)] Lyophilized synthetic arginine vasopressin in Phosphate Buffer	1 vial	B-AR1-CA	Reconstitute with 5 ml of deionized water
Controls Normal / High[2)] Arginine Vasopressin in a buffer matrix	2 vials	B-AR1-CONSET	Reconstitute with 5 ml of Phosphate Buffer
Second Antibody Cellulose coated anti-rabbit antibody	1 vial 11 ml	B-AB2	Ready to use

Table 1

[1)] Reconstitution of the Calibrator results in a stock solution of 80 pg/ml arginine vasopressin.

[2)] Lot specific amounts of arginine vasopressin in buffer matrix. Refer to the additional QC Data Sheets for exact concentrations.

Note: DO NOT EXTRACT NEITHER CONTROLS NOR CALIBRATORS.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
The Extraction columns are stable at 18-28°C. The other kit components are stable at 2-8°C. Do not use past kit expiration date printed on the labels. Do not freeze the Second Antibody.	
Opened / Reconstituted Reagents	
Extraction columns	Used columns should be stored at 18-28°C and protected from light and dust.
Phosphate Buffer	Stable for 2 months at 2-8°C
Antiserum	Stable for 2 months at -20°C
Tracer	Store at -20°C. Aliquot if repeated use is expected
Calibrator	Stable for 2 months at -20°C Aliquot if repeated use is expected
Controls	
Second Antibody	Store at 2-8°C (do not freeze)

Table 2

WARNINGS AND PRECAUTIONS

Radioactive Material: This kit contains radioactive material which does not exceed 56 kBq of ^{125}Iodine. The receipt, acquisition, possession, use and transfer are subject to the local regulations. Concerning the proper precautions for the handling and disposal of kit reagents, radioactive material, radioactive waste and specimens, we highly recommend first to consult the special local regulations of your country.

Reagents Containing Human Source Material: All kit reagents besides the extraction columns (B-AEC) and the second antibody (B-AB2) contain components of human origin. Each serum used in the preparation of the kit components was tested by a FDA-approved method and found negative for HBV surface antigen, HCV and HIV1/2 antibodies. Although those methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. Therefore, all specimens and kit components should be handled as if capable of transmitting infections. All reagents and samples containing human source material should be handled in accordance with good laboratory practice using appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

- 100 µl, 400 µl, 500 µl, 1000 µl, and 5000 µl precision pipettes (or preferably a 100 - 1000 µl adjustable multipipette) with disposable tips.
- 10 ml volumetric pipette.
- Disposable polystyrene tubes for the preparation of the calibrator dilutions.
- Disposable conical polystyrene tubes to run the assay (e.g. Sarstedt # 57.477).
- Disposable polypropylene, polystyrene or glass tubes for the preparation of plasma extracts.
- Cylinder for the preparation of phosphate buffer.
- Deionized or distilled water.
- Methanol p.a. (e.g. Merck # 6009).
- 1 N HCl (hydrochloric acid) (e.g. Merck # 9057).
- Extraction vacuum manifold for applying the extraction columns (optional).
- Vacuum concentrator or supply of particle free nitrogen.
- Refrigerated centrifuge.
- Vortex rotator.
- Stir bar and magnetic stirrer.
- Aspiration device.
- Gamma counter.

SPECIMEN COLLECTION AND STORAGE

Appropriate sample collection is essential to ensure accurate results of the vasopressin analysis. Hemolyzed, highly icteric or lipemic samples may adversely affect results. If the procedure calls for true basal levels, the must be fasting for at least 12 hours and must stay recumbent, without any stress and in a quiet environment, for at least 1 hour prior to blood collection.

Collect at least 5 ml blood into an **EDTA venipuncture tube** and immediately place the sample on ice. Centrifuge at 2-8°C at 2000 g for 15 minutes within 10 minutes of blood collection. Separate the plasma from the cells and freeze the specimen immediately at –20°C or precede to the extraction procedures (see below).

Vasopressin **in urine** remains stable for at least 2 months at –20°C, if such samples have been acidified with 10% (v/v) of 1 N HCl (hydrochloric acid) immediately after collection (6). After a short centrifugation step, these samples may be added directly to the conditioned extraction columns (see below). The procedure calls for 1 ml of EDTA plasma or urine for duplicate determinations.

EXTRACTION

We recommend an extraction procedure using a reversed-phase column extraction which is highly specific for the adsorption and the subsequent elution of arginine vasopressin. Alternatively, an ethanol extraction method may also be used.

- The extraction columns provided with this kit can **each be utilized up to five times** if used according to the extraction procedures described in this protocol. Used columns should be stored at 18-28°C and protected from light and dust.
- The column extraction methods described below result in **recoveries** of greater than 90% with ^{125}I-vasopressin or ^{3}H-vasopressin, respectively, spiked in human EDTA plasma samples.
- If samples have to be measured containing very low concentrations of arginine vasopressin the sample application volume may be increased up to 4 ml without a notable change in extraction recoveries (see Extractive Concentration in the assay performance below).
- The column extraction procedures were **tested and validated for human EDTA plasma** and **urine** samples. If other specimen such as **animal plasma** are used, it is recommended to validate the extraction recovery using a spike of ^{125}I-vasopressin in the specimen. The high ionic strength in **urine** due to high salt concentrations might interfere with the radioimmunoassay for vasopressin. Therefore, column extraction of urine samples after acidification with HCl is highly recommended (6). However, Panzali *et al.* (8) have assayed 17 urine samples without column extraction and found results close to those obtained with extracted samples.

Extraction Procedure using Centrifugation

Sample Pretreatment

- Mark one polypropylene, polystyrene or glass tube for each sample to be extracted.
- Add 1 ml of corresponding plasma sample.
- Add 100 µl of 1 N HCl, vortex and centrifuge for 5 minutes at 2000 x g.
- Use acidified plasma supernatant in step Sample application

Column Preparation and Conditioning

- Mark one extraction column for each sample to be extracted and place into polypropylene, polystyrene or glass centrifugation tubes.
- Add 1 ml of methanol to columns and centrifuge for 1 minute at 200 x g. Repeat this step once.
- Add 1 ml of distilled or deionized water to columns, centrifuge for 1 minute at 200 x g. Repeat this step once.
- Empty tubes to avoid tips of extraction columns from contacting eluates.

Sample Application

- Load 1 ml of acidified sample onto the correspondingly marked column and centrifuge for 1 minute at 200 x g.

Washing

- Add 1 ml of distilled or deionized water to columns, centrifuge for 1 minute at 500 x g. Repeat this step once.

Elution of Extract

- Place each extraction column into a clean correspondingly marked polypropylene, polystyrene or glass tube.
- Add 1 ml of methanol containing 0.5 % (v/v) of 1 N HCl solution to columns and centrifuge for 1 minute at 200 x g.
- Use column for extracting the next sample (up to 5 times) or store column at 18-28°C and protected from light and dust.

Evaporation and Reconstitution of Extract

- Evaporate the methanol solution to dryness using a vacuum concentrator with a cold trap. Alternatively, use a 37°C heating block or water bath and evaporate the methanol to dryness with a stream of particle free nitrogen.
- Reconstitute the samples with 1 ml of phosphate buffer, vortex.
- Equilibrate the extracts for 30 minutes at 2-8°C and vortex them from time to time. Store reconstituted extracts capped and frozen at -20°C if not assayed immediately.

Extraction Procedure Using Vacuum Manifold

With the help of negative pressure the fluids will be passed through the column. The procedure is the same as described in the Extraction procedure using centrifugation.

The following flow rates should be used:

- Sample application and elution should be done with a flow rate of 2 ml/min
- All the other fluids can pass the column with a flow rate of 5 ml/min.

Ethanol Extraction of Samples

Alternatively, samples may be extracted using “ethanol precipitation”.

- Mix 1 ml of each sample with 5 ml of chilled 98 % (v/v) ethanol. Vortex for 1 minute.
- Centrifuge for 20 minutes at 2-8°C and 1000 x g. Decant into another clean polypropylene, polystyrene or glass tube.
- Dry the supernatant in a vacuum concentrator. Alternatively, use a 37°C heating block or water bath and evaporate the ethanol to dryness with a stream of compressed and particle free nitrogen or air.
- Reconstitute the samples with 1 ml of phosphate buffer, vortex and store them frozen at –20°C if not assayed immediately.

With the ethanol extraction, samples show usual recoveries of 65-80%. Therefore, we recommend using as an internal recovery control 1 ml of EDTA Plasma spiked with 50 µl ^{125}I-Vasopressin. Compared to the total activity this factor can be used to calculate the recovery rate.

PROCEDURAL NOTES

- We recommend the use of EDTA plasma in order to inhibit metalloprotease activities.
- Use of **conical polystyrene tubes** are strongly recommended. During step 10 of the assay procedure, a more solid pellet will be achieved and the following aspiration of the supernatant can be done much easier.
- In order to obtain an entire standard curve, serial dilutions of the Calibrator are prepared as follows:
  - Label eight tubes A through H and pipet 1.0 ml of phosphate buffer into tubes B through H .
  - Pipet 1.0 ml of the reconstituted Calibrator stock solution (80 pg/ml) into tubes A and B, vortex.
  - Transfer 1.0 ml from tube B to tube C, vortex.
  - Continue to transfer 1.0 ml from each tube until dilution series is completed. The corresponding concentrations of arginine vasopressin will be:

A	80 pg/ml (73.6 pmol/L)	E	5.0 pg/ml (4.6 pmol/L)
B	40 pg/ml (36.8 pmol/L)	F	2.5 pg/ml (2.3 pmol/L)
C	20 pg/ml (18.4 pmol/L)	G	1.25 pg/ml (1.1 pmol/L)
D	10 pg/ml (9.2 pmol/L)	H	0.63 pg/ml (0.6 pmol/L)

ASSAY PROCEDURE

Note: Allow all reagents for steps 1 to 4 to come to room temperature (18-28°C) prior to use.

1. Label 11 polystyrene tubes in duplicate: A to H (Calibrators), NSB (blank), MB (maximum binding) and T (total activity). Label additional polystyrene tubes in duplicate for samples and controls.
2a. Pipet 500 µl of phosphate buffer into the NSB tubes and 400 µl into the MB tubes.
2b. Pipet 400 µl of each of the A to H Calibrators into the corresponding tubes.
2c. Pipet 400 µl of the extracted samples and controls (without extraction) into each of the corresponding tubes.
3. Add 100 µl of the vasopressin antiserum to all tubes except the NSB and T tubes. Vortex.
4. Incubate for 24 hours ± 3 hours at 2-8°C.
5. Add 100 µl of vasopressin tracer to all tubes. Vortex. Cap and remove the T tubes, they will require no further processing until counting step 12.
6. Incubate for 24 hours ± 3 hours at 2-8°C.
7a. Invert several times the bottle containing the solid phase second antibody, add a stir bar and place the bottle on a magnetic stirrer.
7b. While stirring continuously, add 100 µl of the suspension to each assay tube (except T tubes). Vortex.
8. Incubate for 20 minutes ± 1 minute at 18-28°C.
9. Add 1 ml of deionized water to each tube (except T tubes) **without resuspending the precipitates**.
10. Centrifuge for 5 minutes at 2-8°C and 1000 x g.
11. Aspirate all supernatants **(except the T tubes)** and retain the precipitates for counting.
12. Count all tubes for 1 minute in a gamma counter.

RESULTS

Record the cpm for all tubes (T, NSB, MB, Calibrators A-H samples and Controls) and calculate the mean cpm for each pair of tubes. Subtract the mean assay blank (NSB tubes) from the respective mean of each pair of tubes:

$$\text{Net cpm} = \text{cpm}_{\text{Average}} - \text{cpm}_{\text{Average NSB}}$$

Calculate the binding of each pair of tubes as a percent of maximum binding (MB tubes), with the NSB-corrected cpm of the MB tubes taken as 100%.

$$\text{percent bound} = \frac{\text{net cpm}}{\text{net MB cpm}} \times 100$$

Prepare a lin/log graph paper and plot the percent bound on the vertical axis against the Vasopressin concentration (pg/ml) on the horizontal axis for each of the Calibrators. Draw the best fitting curve or calculate the standard curve using a four parameter algorithm. Determine Vasopressin concentrations for the samples and Controls from this standard curve. Alternative data reduction methods are equally acceptable.

To get the pmol/l concentrations of the results, multiply the pg/ml values by a factor of 0.92.

Standardization: The BÜHLMANN arginine vasopressin Calibrator is weighed in material which was calibrated against WHO reference preparation 77/501.

See Table 3 and Figure 1 for examples of results and standard curves. *These results and standard curves are for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.*

QUALITY CONTROL

A thorough understanding of this instruction for use is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this instruction for use.

The accuracy of each actual calibrator lot is controlled every three months by comparing with WHO reference preparation 77/501.

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. The confidence limits for the Controls are lot-specific and printed on the QC Data Sheet added to the kit.

If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) expiration dates of reagents iii) storage and incubation conditions iv) purity of water.

LIMITATIONS AND TROUBLE SHOOTING

- Vasopressin values should be used as supplementary data available to the physician in developing a diagnosis.
- samples that are not properly collected and handled may cause inaccurate arginine vasopressin results (see Specimen Collection). Freezing plasma samples immediately or extracting plasma samples without delay will preserve the integrity of the vasopressin concentration at the time of sampling.
- If the initial concentration of a unknown sample reads greater than the highest calibrator, the extracted sample should be further diluted with phosphate buffer and assayed again according to the assay procedure.
- Counting time should be sufficient to prevent statistical counting error: e.g., accumulation of 2000 cpm will yield 5% counting error; 10000 cpm will yield 1% counting error.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): 7.6%. The intra-assay precision was calculated from the results of 20 pairs of values from 3 column-extracted plasma samples spiked with different amount of arginine vasopressin in a single run. The values are presented in Table .

Inter-Assay Precision (Run-to-Run): 10.0%. The inter-assay precision was determined from the results of 20 pairs of values from 2 column-extracted and 2 non-extracted plasma samples assayed in 20 consecutive runs. The values are presented in Table .

Dilution Linearity/Parallelism: 95.9%. A human plasma sample was diluted serially with phosphate buffer before and after column extraction and subsequently assayed according to the assay procedure. The values are presented in Table .

Spiking Recovery: 93.4%. Three plasma samples were spiked with increasing amounts of synthetic arginine vasopressin, extracted with the column extraction method and analyzed according to the assay procedure. The values are presented in Table .

Extractive Concentration: 96.7%. Increasing volumes of a human plasma sample containing a low concentration of arginine vasopressin were applied onto extraction columns and extracted according to the protocol (see pages 3ff.). Each of the resulting extract was reconstituted in **1 ml** of phosphate buffer and subsequently assayed according to the assay procedure. The values are presented in Table .

Analytical Sensitivity: 0.39 pg/ml (0.36 pmol/l). Twenty Zero Calibrator duplicates (phosphate buffer) were assayed in a single run. The analytical sensitivity of vasopressin was calculated by subtracting two standard deviations from the mean cpm of the maximum binding and intersecting this value with the standard curve obtained in the same run.

Functional Sensitivity: 1.9 pg/ml (1.75 pmol/l). Five different serum samples with values between 1.3 and 4.1 pg/ml were each tested 10 times in duplicates as an intra-assay. The CV and the mean values were calculated for each sample. The functional least detectable dose (FLDD) was observed at 10% CV.

Specificity: Cross-reactions of the vasopressin antiserum were determined at 50% binding and are presented in Table .

Method comparison: The Vasopressin RIA has been compared with the VASOPRESSIN direct RIA (RK-VPD, without column extraction). Results obtained with 28 individuals (EDTA plasma) show an excellent correlation (see Figure 2).

APPENDIX I
TABLES

Table 3 **Example of Results** (Incubation at 28°C)

	cpm	B/T [%]	B/B_0 [%]	Conc [pg/ml]	CV [%]
Total	18212	100.0			
Total	18187	100.0			
Total Avg.	18200	100.0			
NSB	216	1.2			
NSB	210	1.2			
NSB Avg.	213	1.2			
MB	5743	31.6	100.0	0	
MB	6054	33.3	100.0	0	
MB Avg.	5898	32.4	100.0	0	
Calibrator H	5524	30.4	93.4	0.63	
Calibrator H	5490	30.2	92.8	0.63	
Calibrator H Avg.	5507	30.3	93.1	0.63	4.8
Calibrator G	5081	27.9	85.6	1.25	
Calibrator G	5208	28.6	87.8	1.25	
Calibrator G Avg.	5144	28.3	86.7	1.25	11.2
Calibrator F	4365	23.9	74.0	2.5	
Calibrator F	4359	23.9	73.9	2.5	
Calibrator F Avg.	4362	23.9	73.9	2.5	0.5
Calibrator E	3548	19.5	58.7	5	
Calibrator E	3520	19.3	58.1	5	
Calibrator E Avg.	3533	19.4	58.4	5	3.9
Calibrator D	2165	11.9	34.3	10	
Calibrator D	2082	11.4	32.9	10	
Calibrator D Avg.	2124	11.7	33.6	10	3.9
Calibrator C	1509	8.3	22.8	20	
Calibrator C	1348	7.4	20.0	20	
Calibrator C Avg.	1428	7.9	21.4	20	11.4
Calibrator B	826	4.5	10.8	40	
Calibrator B	819	4.5	10.7	40	
Calibrator B Avg.	823	4.5	10.7	40	1.1
Calibrator A	642	3.5	7.5	80	
Calibrator A	596	3.3	6.7	80	
Calibrator A Avg.	619	3.4	7.1	80	16.1
Control Normal	3340		55.0	5.1	
Control Normal	3335		54.9	5.1	
Control Norm Avg.	3337		55.0	5.1	0.2
Control High	1633		25.0	15.4	
Control High	1517		22.9	17.2	
Control High Avg.	1575		24.0	16.3	7.6

ED-20 = 20.3 pg/ml ED-50 = 6.1 ng/ml ED-80 = 1.8 pg/ml

Figure 1 **Example of a Standard Curve**

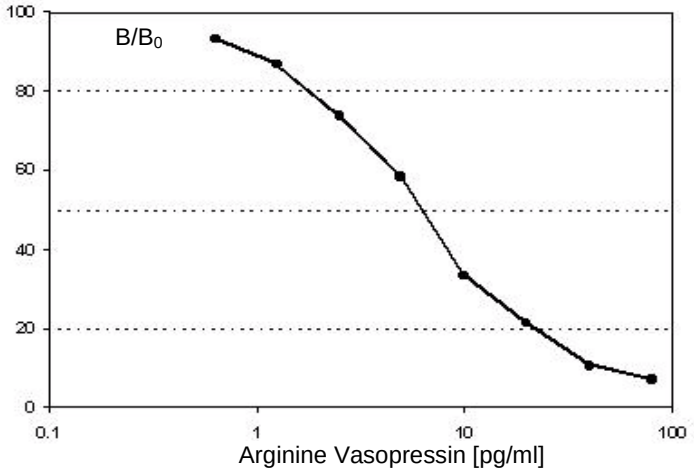


Table 4 **Intra-Assay Precision (Within-Run)**

Sample	Mean Value [pg/ml]	SD [pg/ml]	CV [%]
Plasma 1	1.9	0.2	8.5
Plasma 2	4.6	0.3	7.6
Plasma 3	10.1	0.7	6.6
Mean			7.6

Table 5 **Inter-Assay Precision (Run-to-Run)**

Sample	Mean Value [pg/ml]	SD [pg/ml]	CV [%]
Plasma 4	2.3	0.2	11.0
Plasma 5	10.2	0.9	9.1
Plasma 6*	2.6	0.3	11.4
Plasma 7*	16.5	1.4	8.6
Mean			10.0

*non-extracted samples

Table 6 **Dilution Linearity/Parallelism**

Sample	Dilution	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Plasma 6 before extraction	1:1	18.9	--	--
	1:2	9.2	9.5	97
	1:4	4.6	4.7	98
	1:8	2.2	2.4	92
	1:16	1.2	1.2	98
Plasma 6 after extraction	1:1	17.6	--	--
	1:2	8.9	8.8	101
	1:4	4.3	4.4	98
	1:8	2.1	2.2	95
	1:16	1.0	1.1	88
Mean				95.9

Table 7 **Spiking Recovery**

Sample	Spiked with Vasopressin	Observed Value [pg/ml]	Expected Value [pg/ml]	Recovery [%]
EDTA Pool1	5 pg/ml	4.9	5.8	84.9
	25 pg/ml	26.0	25.8	100.5
	50 pg/ml	51.7	50.8	101.7
EDTA Pool 2	5 pg/ml	5.3	6.0	86.9
	25 pg/ml	25.8	26.0	98.9
	50 pg/ml	48.6	51.0	95.3
EDTA Pool 2	5 pg/ml	5.1	6.0	84.0
	25 pg/ml	25.0	26.0	96.0
	50 pg/ml	47.2	51.0	92.4
Mean				93.4

Table 8 **Extractive Concentration**

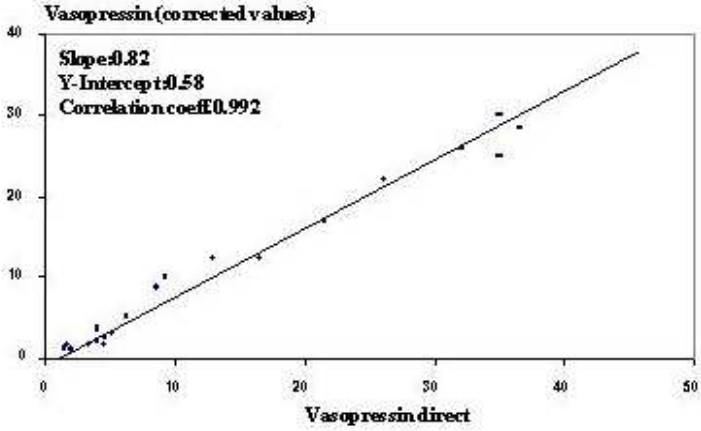
Sample	Sample Load	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Plasma 9	1 ml	1.8	---	---
	2 ml	3.6	3.6	100
	3 ml	5.4	5.4	100
	4 ml	6.5	7.2	90
Mean				96.7

Table 9 **Specificity**

Peptide	Cross-Reaction [%]
Arginine vasopressin	100
Arg8-Vasotocin	<0.001
Lysine vasopressin*	**0.016**
Desmopressin (DDAVP)	0.056
Oxytocin	<0.001

*In Pigs and Hippopotamus the arginine at position eight is replaced by lysine

Figure 2 **Method Comparison RK-VPD vs. RK-AR1**



PPENDIX III
REFERENCES

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RADIOIMMUNOASSAY PROCEDURE

Polystyrene tubes in duplicate	Phosph. Buffer (µl)	Standard, Control, Sample (µl)	Antiserum (µl)		Tracer (µl)		Second Antibody (µl)	
Total	--	--	--		100		--	Vortex and incubate for 20 minutes (± 1 min) at 18-28°C
NSB	500	--	--		100		100	
MB	400	--	100		100		100	
Std A 80 pg/ml	--	400	100		100		100	
Std B 40 pg/ml	--	400	100	Vortex and incubate at 2-8°C for 24 hours (± 3hrs)	100	Vortex and incubate at 2-8°C for 24 hours (± 3hrs)	100	Add 1ml of deionized water (except T tubes) and centrifuge for 5 minutes at 2-8°C and 1000 x g
Std C 20 pg/ml	--	400	100		100		100	
Std D 10 pg/ml	--	400	100		100		100	
Std E 5 pg/ml	--	400	100		100		100	
Std F 2.5 pg/ml	--	400	100		100		100	
Std G 1.25 pg/ml	--	400	100		100		100	
Std H 0.63 pg/ml	--	400	100		100		100	
Control NORMAL	--	400	100		100		100	Aspirate supernatant (except t tubes) and count for 1 minute
Control ELEVATED	--	400	100		100		100	
Sample	--	400	100		100		100	

PENDIX IV
SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Temperature limitation
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Radioactive Material

Symbol	Explanation
AEC	Extraction Columns
BUF H3PO4	Phosphate Buffer
Ab	Antiserum
TR	Tracer
CAL	Calibrator
CONTROL N	Control Normal
CONTROL H	Control High
Ab2	2nd Antibody