VASOPRESSIN

RIA

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

RK-AR1-U 100 tests

Version: 02
Revision date: 2022-04-07

NovoLytiX GmbH
Benkenstrasse 254C
CH - 4108 Witterswil / Switzerland
Tel.: +41 61 721 01 00
Fax: +41 61 530 11 06
info@novolytix.ch

INTENDED USE

This double antibody radioimmuno-assay is designed for the quantitative *in vitro* diagnostic determination of immunoreactive **arginine vasopressin** ([Arg8]-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). Extracted plasma samples, controls 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-hours incubation, the solid phase second antibody is added to the mixture, and the antibody-bound fraction is finally precipitated and counted.

The procedure recommends a solid-phase extraction of the plasma samples and controls with reversed-phase phenylsilylsilica columns. 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 section "Extraction"
Phosphate Buffer	1 vial 120 mL	B-AR1-PB	Ready to use
Antiserum Lyophilized rabbit anti-vasopressin antibody	1 vial	B-AR1-AS	Reconstitute with 10 mL Phosphate Buffer
Tracer ^{125}I-Vasopressin	1 vial	B-ADH-TR	Ready to use
Calibrators[1)] Lyophilized synthetic arginine vasopressin	1 vial	B-AR1-CASET	Reconstitute with 5 mL of Phosphate Buffer
Controls Normal / High[2)] Arginine Vasopressin in a buffer matrix	2 vials	B-AR1-CONSET	Reconstitute with 5 mL of deionized water
Second Antibody Cellulose coated anti-rabbit antibody	1 vial 11 ml	B-AB2	Ready to use

Table 1

[1)] After reconstitution the Calibrators A to E contain 0.75, 2, 5, 15 and 50 pg/mL of [Arg8]-vasopressin. **Note:** DO NOT EXTRACT CALIBRATORS.

[2)] Lot specific amounts of [Arg8]-vasopressin in buffer matrix. We recommend to extract the Controls with the same procedure as the samples. Refer to the additional QC Data Sheets for exact concentrations.

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	
Tracer	
Calibrator	
Controls	
Second Antibody	

Table 2

WARNINGS AND PRECAUTIONS

Radioactive Material: This kit contains radioactive material which does not exceed 37 kBq (1 µCi) 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 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.

EQUIPMENT REQUIRED

- 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.
- 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.

MATERIALS RECOMMENDED BUT NOT PROVIDED

- 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.
- Deionized or distilled water.
- Methanol p.a. (e.g. Merck # 6009).
- 1 N HCl (hydrochloric acid) (e.g. Merck # 9057).

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, patient 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 2.5 mL of blood into an **EDTA venipuncture tube** and, whenever possible, place the sample on ice. Centrifuge at 2-8°C at 2000 x g for 15 minutes, whenever possible within 60 minutes after blood collection. Separate the plasma from the cells and freeze the specimen in a fresh tube at ≤–20°C or proceed to the extraction procedure (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, acidified 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.

Note: NovoLytiX highly recommends to use EDTA plasma ONLY to inhibit potential metalloprotease activities (and consequent degradation of [Arg8]-vasopressin in the sample). Heparinized plasma may be used. NovoLytix showed in a in a

small pilot study that Heparin plasma yields about 30% lower values than EDTA plasma.

EXTRACTION

Important Procedural Notes

NovoLytiX **highly recommends 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** (see also section PERFORMANCE CHARACTERISTICS) 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 method 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 section PERFORMANCE CHARACTERISTICS below).
- NovoLytiX recommends toextract the Controls using the same procedure as for the samples.
- 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.
- With the ethanol extraction, samples usually show a recovery between 45% and 75%. The average extraction recovery found by spiking with either ^{125}I-vasopressin or ^{3}H-vasopressin or different amounts of synthetic vasopressin resulted in approx. 60%. Therefore, we recommend:
  i) to multiply the raw data of reconstituted ethanol extracts with 1.67;
  ii) to use an internal recovery control (i.e. 1 ml of EDTA plasma is spiked with 50 µL of ^{125}I-vasopressin). The radioactivity in the reconstituted extract is then compared to the total activity of the spike, and the resulting factor may be used to calculate the recovery rate; or
  iii) to extract the two kit Controls and to compare the resulting raw data with the lot-specific target value given in the QC data sheet. The resulting factor may also be used to calculate the recovery rate.

Extraction Procedure using Centrifugation

Sample Pretreatment

- Mark one polypropylene, polystyrene or glass tube for each sample and Control 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 and Control 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 controls and sample onto the correspondingly marked column and centrifuge for 1 minute at 200 x g. Repeat centrifugation step for 1 minute at 500 x g, if sample does not entirely run through the extraction column.

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 extraction 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 (and Controls)

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 a 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.

PROCEDURAL NOTES

- Use of conical polystyrene tubes for the radioimmunoassay is strongly recommended, as in 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.

- 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.

ASSAY PROCEDURE

Note: Allow all reagents for steps 2 to 4 to come to room temperature (18-28°C) prior to use.

1. Label 8 polystyrene tubes in duplicate: A to E (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 E calibrators into the corresponding tubes.
2c. Pipet 400 µl of the extracted samples and controls 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 ± 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 ± 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 ± 1 minutes at 18-28°C.
9. Add 1 ml of deionized water to each tube (except T tubes). **Do not resuspend the precipitate**.
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-E 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-logistics (4-PL) fitting algorithm.

Determine [Arg8]-vasopressin concentrations for the samples and Controls from this standard curve. Alternative data reduction methods are equally acceptable. Refer to Table 3 and Figure 1 for examples of results and standard curve.

If the initial concentration of an 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.

To get the pmol/L concentrations of the results, multiply the pg/mL values with a factor of 0.92.

Standardization: The [Arg8]-vasopressin Calibrators consist of weighed-in material which was calibrated against the United States Pharmacopeia (USP) Reference Standard (Merck #1711100; CAS 113-79-1).

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 assured by comparison against the United States Pharmacopeia (USP) Reference Standard (Merck #1711100; CAS 113-79-1).

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.

PERFORMANCE LIMITATIONS

- NovoLytiX highly recommends using EDTA plasma ONLY to inhibit potential metalloprotease activities (and consequent degradation of [Arg8]-vasopressin in the sample). Heparinized plasma may be used. NovoLytiX showed in a in a small pilot study that Heparin plasma yields about 30% lower values than EDTA plasma.
- Samples that are not properly collected and handled may cause inaccurate [Arg8]-vasopressin results. EDTA plasma samples should be frozen immediately after collection in order to ensure correct results at the time of measurement (see also section SPECIMEN COLLECTION AND STORAGE).
- Transportation of EDTA plasma samples should be carried out at -20°C or lower.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Blank, LoB): 0.36 pg/mL (0.33 pmol/L). Twenty Zero Calibrator (Phosphate Buffer) duplicates 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. This test was repeated twice at different time points. The sensitivity was calculated to be 0.39, 0.34 and 0.36 pg/mL.

Functional Sensitivity (Limit of Quantitation, LoQ): 1.4 pg/mL (1.3 pmol/L). Eleven different plasma samples with values between 1.3 and 20.0 pg/mL were each tested 10 to 20 times in duplicates in a single run (intra-assay). The CVs and the mean values were calculated for each sample. The Limit of Quantitation (LoQ) was determined at 10% CV.

Intra-Assay Precision (Within-Run): 6.8%. The intra-assay precision was calculated from the results of 20 pairs of results from 6 plasma samples (partially spiked with different amounts of arginine vasopressin), extracted with phenylsilylsilica columns (B-AEC) and subsequently assayed in a single run. The values are presented in Table 4.

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 plasma samples and Normal and High Control (not extracted), respectively, assayed in 20 consecutive runs. The values are presented in Table 5.

Dilution Linearity/Parallelism: 95.9%. A human plasma sample was spiked with synthetic [Arg8]-vasopressin, diluted serially with Phosphate Buffer before and after column extraction and subsequently assayed according to the assay procedure. The values are presented in Table 6.

Spiking Recovery: 92.3%. Seven plasma samples were spiked with increasing amounts of [Arg8]-arginine vasopressin, extracted with the column extraction method and analyzed according to the assay procedure. The values are presented in Table 7.

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 page 3). 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 8.

Re-usability of Phenylsilylsilica Extraction Colums (B-AEC): Two EDTA plasma samples were applied onto extraction columns and extracted according to the protocol (see page 3). The procedure was repeated four times using the same extraction column again. The **average CV** between extractions was **7.0%** with no loss of extraction recovery over time. The results are summarized in Table 9.

Specificity: Cross-reactions of the vasopressin antiserum were determined at 50% binding and are presented in Table 10.

Method comparison: The Vasopressin RIA (RK-AR1; employing phenylsilylsilica column extraction) has been compared with the Vasopressin Direct RIA (RK-VPD). Results obtained with 67 EDTA plasma samples yield an excellent correlation coefficient $R^2 = 0.954$ (see Figure 2).

Freeze/Thaw Cycles: Three EDTA plasma samples containing different amounts of arginine-vasopressin were frozen at -20°C and thawed at 20-30°C up to three times. Then, the EDTA plasma samples were applied onto extraction columns, extracted according to the protocol (see page 3) and assayed in the RK-AR1-U. The stability data are shown in Figure 3. There is no effect on the measured arginine-vasopressin concentration after up to three freeze-thaw cycles.

NORMAL VALUES, REFERENCE RANGE

In an evaluation with 72 apparently healthy blood donors collected by the Swiss Red Cross blood donation center Basel, a normal reference range (5-95 percentile) between <1.4 pg/mL and 5.0 pg/mL was established with a median value of 2.0 pg/mL. However, each laboratory may generate its own reference values.

Values for above blood donors may be slightly elevated due to unknown fasting conditions and unknown position before donation. The osmolality has not been determined. Therefore, correctly collected basal values should be in the lower range or even undetectable (<1.4 pg/mL).

Vasopressin is mainly determined after dynamic testing by stimulation or suppression of vasopressin release.

Vasopressin values should be used as supplementary data available to the physician in developing a diagnosis.

Change Log

Date	Version	Reason for change
2022-03-30	01	1st NovoLytiX version; ready-to-use Phosphate Buffer, ready-to-use Tracer; ready-to-dilute Calibrators and Controls; all reagents can be kept at 2-8°C, unopened and reconstituted; freshly calibrated against United States Pharmacopeia (USP) Reference Standard (Merck #1711100; CAS 113-79-1); a new chapter Important Procedural Notes added to the section EXTRACTION; performance data for re-usability of extraction columns, B-AEC, are given; normal reference range determined with 28 apparently healthy blood donors is given; additional method comparison data; slightly adapted layout, correction of typos.
2022-04-07	02	More data points for the normal reference range (n=72); more data points for method comparison to RK-VPD (n=67); up to 3 freeze/thaw cycles of ETDA plasma samples possible (new Figure 3).

Table 3 **Example of Results**

Data reduction: SoftMaxPro 7.1 (Molecular Devices) employing 4-PL algorithm

	cpm	B/T [%]	B/B_0 [%]	Conc [pg/mL]	CV [%]
Total	16690	99.4			
Total	16672	100.6			
Total Avg.	**16681**	**100.0**			0.1
NSB	507	3.0			
NSB	533	3.2			
NSB Avg.	**520**	**3.1**			3.5
MB	6475	38.9	100.2		
MB	6462	38.7	99.8		
MB Avg.	**6469**	**38.8**	**100.0**		0.3
Calibrator A	5858	35.1	89.7		
Calibrator A	5868	35.2	89.9		
Calibrator A Avg.	**5863**	**35.1**	**92.1**	**0.75**	0.1
Calibrator B	5462	32.7	83.1		
Calibrator B	5252	31.5	79.5		
Calibrator B Avg.	**3850**	**32.1**	**81.3**	**2.0**	2.8
Calibrator C	3892	23.3	56.7		
Calibrator C	3808	22.8	55.3		
Calibrator C Avg.	**3850**	**23.1**	**56.0**	**5.0**	1.5
Calibrator D	1711	10.3	20.0		
Calibrator D	1573	9.4	17.7		
Calibrator D Avg.	**1870**	**9.8**	**18.9**	**15.0**	5.9
Calibrator E	1015	6.1	8.3		
Calibrator E	1094	6.6	9.6		
Calibrator E Avg.	**5651**	**6.3**	**9.0**	**30.0**	5.3
Control Normal	5479		83.4	1.8	
Control Normal	5424		82.4	1.9	
Control NORM. Avg	**5452**		**82.9**	**1.9**	4.6
Control High	1890		23.0	12.5	
Control High	2220		28.6	10.2	
Control High Avg.	**2055**		**25.8**	**11.3**	14.1

ED-20 = 16.1 pg/mL ED-50 = 5.6 pg/mL ED-80 = 2.1 pg/mL

These results and standard curve are for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

Figure 1 **Example of a Standard Curve**

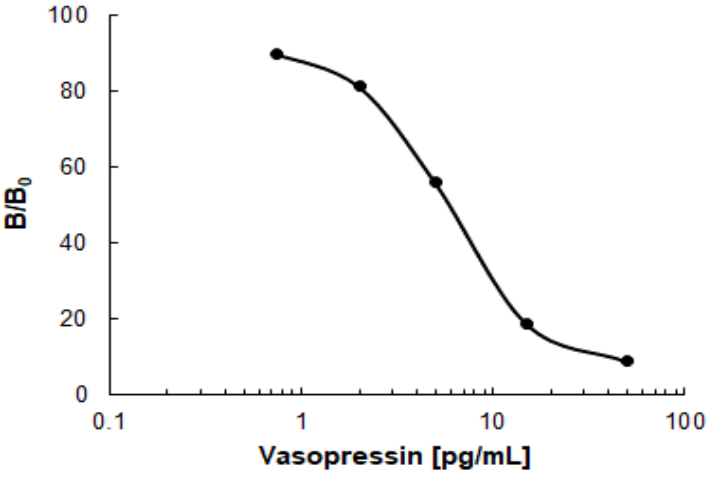


Table 4 **Intra-Assay Precision (Within-Run)**

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
Plasma 4	20.0	0.9	4.7
Plasma 5	1.7	0.2	11.1
Plasma 6	6.2	0.2	2.5
Mean			**6.8 %**

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

Sample	Mean Value [pg/ml]	SD [pg/ml]	CV [%]
Plasma 7	2.3	0.2	11.0
Plasma 8	10.2	0.9	9.1
Normal Control*	2.6	0.3	11.4
High Control*	16.5	1.4	8.6
Mean			**10.0%**

*not extracted

Table 6 **Dilution Linearity/Parallelism**

Sample	Dilution	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Plasma 9, diluted 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 9, diluted 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 [pg/mL]	Observed Value [pg/ml]	Expected Value [pg/ml]	Recovery [%]
EDTA Pool 1	-	0.8	-	
	5	5.0	5.8	85.2
	25	24.7	25.8	95.6
	50	47.6	50.8	93.6
EDTA Pool 2	-	1.2	-	
	5	5.4	6.2	86.9
	25	24.4	26.2	98.9
	50	47.3	51.2	95.3
EDTA Pool 3	-	1.1	-	
	5	5.1	6.1	87.5
	25	25.0	26.1	94.3
	50	47.2	51.1	93.3
EDTA Pool 4	-	0.8	-	
	5	5.0	5.8	85.2
	25	24.7	25.8	95.6
	50	47.6	50.8	93.6
EDTA Pool 5	-	1.2	-	
	5	5.4	6.2	86.9
	25	24.4	26.2	98.9
	50	47.3	51.2	95.3
EDTA Pool 6	-	1.1	-	
	5	5.1	6.1	87.5
	25	25.0	26.1	94.3
	50	47.2	51.1	93.3
EDTA Pool 7	-	0.9	-	
	2	3.5	2.9	120.6
	5	5.6	4.9	114.3
	10	10.1	10.9	92.7
	20	17.0	20.9	81.3
	40	33.5	40.9	81.9
Mean				**92.3%**

Table 8 **Extractive Concentration**

Sample	Sample Load	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Plasma 10	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 **Re-usability of Extraction Columns**

Sample	Dilution	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Plasma 11, diluted 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
Plasma11, diluted 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 10 **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-AR1 vs. RK-VPD**

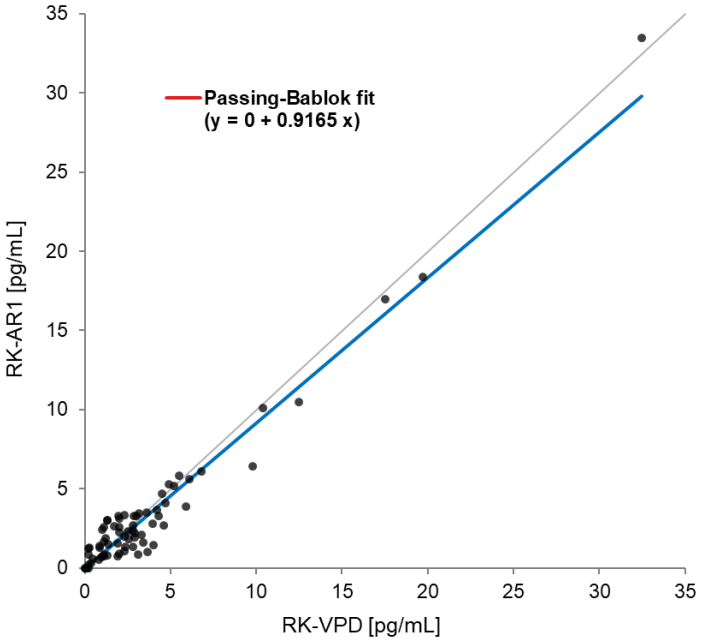
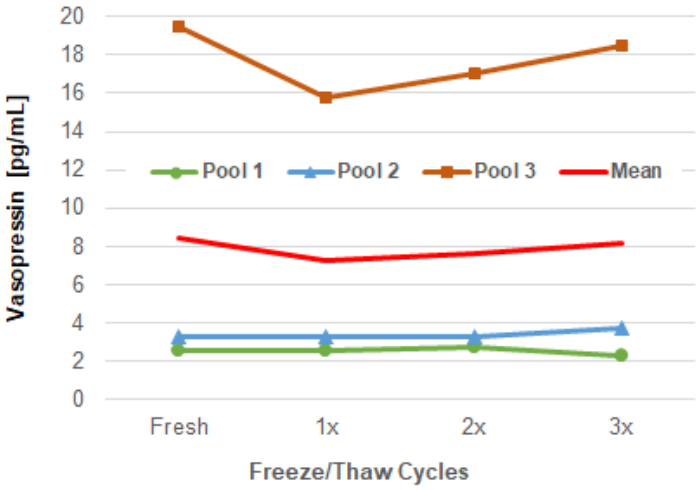


Figure 3: **Freeze/Thaw Cycles**



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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 0.75 pg/mL	--	400	100		100		100	
Std B 2 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 5 pg/mL	--	400	100		100		100	
Std D 15 pg/mL	--	400	100		100		100	
Std E 50 pg/mL	--	400	100		100		100	
	--				100		100	
Control NORMAL	--	400	100		100		100	
Control ELEVATED	--	400	100		100		100	
Sample		400	100		100		100	**Aspirate supernatant (except t tubes) and count for 1 minute**
					100		100	
					100		100	

APENDIX 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

Printing date:
2022-04-12