VASOPRESSIN Direct

RIA

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

RK-VPD-U 100 tests

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ENGLISH

INTENDED USE

This double antibody radioimmuno-assay is designed for the quantitative direct measurement of **arginine vasopressin** (anti-diuretic hormone, ADH) in EDTA plasma (1-3).

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

PRINCIPLE OF THE ASSAY

Immunoreactive vasopressin is measured with a double antibody radioimmunoassay according to a modified method of Glick and Kagan (4). Samples and calibrators are at first pre-incubated with the anti-vasopressin antibody for 24 hours. ^{125}I-vasopressin then competes with vasopressin present in samples and calibrators for the same antibody binding sites. After a second incubation of 24 hours incubation, the solid-phase second antibody is added to the mixture, and the antibody-bound fraction is finally precipitated and counted.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Phosphate Buffer lyophilized Buffer	1 vial	B-VPD-PB	Reconstitute with 50 ml of deionized water
Antibody Dilution Buffer	1 vial 6 ml	B-VPD-DB	Ready to use
Antiserum lyophilized anti-vasopressin antibody	1 vial	B-VPD-AS	Reconstitute with 5 ml of Antibody Dilution Buffer
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-VPD-CA	Reconstitute with 5 ml of deionized water
Controls Normal / High[2)] Arginine Vasopressin in a buffer matrix	2 vials	B-VPD-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)] Reconstitution of the Calibrator results in a stock solution of 80 pg/ml arginine vasopressin [Arg 8]-vasopressin.

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

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
The kit components are stable at 2-8°C. Do not use the kit beyond the expiration date printed on the labels. Do not freeze the Secondary Antibody.	
Opened / Reconstituted Reagents	
Phosphate Buffer	Stable for 2 months at 2-8°C
Antibody Dilution Buffer	Stable for 2 months at 2-8°C
Antiserum	Stable for 2 months at -20°C
Tracer	Stable for 2 months at -20°C Aliquot if repeated use is expected
Calibrator	Stable for 2 months at -20°C
Controls	Aliquot if repeated use is expected
Secondary Antibody	Stable for 2 months at 2-8°C (do not freeze)

Table 2

PRECAUTIONS

Safety 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. Unused solutions and radioactive waste should be disposed of according to local State and Federal regulations.
- All kit reagents except of the second antibody (B-AB2) **contain** components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with good laboratory practices using appropriate precautions.

Technical Precautions

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected if reagents are incorrectly diluted, modified or stored under conditions other than those as detailed in this instruction for use.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Let the reagents adjust to reach room temperature. Reconstitute the lyophilized reagents as indicated. Mix well (vortex) the reagents before use.
- Counting time should be selected in order to keep statistical counting error small: e.g., at 2000 cpm the counting error is at 5%; at 10000 cpm it is only 1%.
- If the initial concentration of an unknown sample reads above the highest calibrator, the sample should be diluted with phosphate buffer and tested again according to the assay procedure.

MATERIALS REQUIRED BUT NOT PROVIDED

- 50 µl, 100 µl, 250 µl, 400 µl and 1000 µl precision pipettes with disposable tips.
- 5.0 ml and 10 ml volumetric pipettes.
- Beaker and cylinder necessary for the reconstitution of the phosphate buffer.
- Disposable polystyrene tubes for the preparation of the calibrator dilutions.
- Disposable conical polystyrene tubes to run the assay (e.g. Sarstedt # 57.477).
- Distilled or deionized water.
- Vortex mixer.
- Stir bar and magnetic stirrer.
- Centrifuge.
- Aspiration device.
- Gamma counter.

SPECIMEN COLLECTION AND STORAGE

Appropriate sample collection is essential to ensure accurate results of the vasopressin analysis. The procedure calls for true basal levels, Subject`s 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 blood (at least 2 ml) into an **EDTA venipuncture tube** (see below) and immediately place the sample on ice. Centrifuge at 2-8°C at 2000 g for 15 minutes within 10 minutes after blood collection, separate the plasma from the cells and freeze the specimen immediately in a plastic tube at ≤–20°C if it will not be used immediately. The procedure calls for 400 µl of plasma per assay tube.

ASSAY PROCEDURE

Calibrator Dilution: In order to obtain an entire standard curve, serial dilutions of the Calibrator are prepared as follows:

- Label seven tubes A through G and Pipet 1.0 ml of phosphate buffer into tubes B through G.
- 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 are:

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)		

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

1. Label 10 polystyrene tubes in duplicate: A to G (Calibrator), NSB (blank), MB (maximum binding) and T (total activity). Label additional polystyrene tubes in duplicate for samples and controls.

2a. Pipet 700 µl of phosphate buffer into the NSB tubes and 650 µl into the MB tubes.

2b. Add 250 µl of phosphate buffer to all remaining tubes, except the T tubes.

3a. Pipet 400 µl of each Calibrator (from A to G) into the corresponding tubes.

3b. Pipet 400 µl of the samples and Controls into the corresponding tubes.

4. Add 50 µl of the reconstituted vasopressin antiserum to all tubes except the NSB and T tubes. Vortex.
5. Incubate all tubes for 24± 3 hours at 18-28°C.
6. Add 100 µl of the reconstituted vasopressin tracer to all tubes. Vortex. Remove the T tubes for counting at step 12, they will require no further processing.
7. Incubate for 24± 3 hours at 18-28°C.

8a. Invert the solid phase second antibody bottle several times, add a stir bar and place the bottle on a magnetic stirrer.

8b. While stirring the second antibody suspension continuously, add 100 µl of the suspension to all assay tubes (except the T tubes). Vortex.

9. Incubate for 20±1 minutes at 18-28°C. **Do not resuspend the precipitate**.
10. Add 1 ml of deionized water to each tube (except the T tubes).
11. Centrifuge for 5 minutes 1000 x g. Aspirate the supernatant **(except the T tubes)** and retain the precipitates for counting.
12. Count all tubes for 1 minute in a gamma counter.

CALCULATION OF RESULTS &STANDARDIZATION

- Record the cpm for all tubes (T, NSB, MB, Calibrators A-G 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:

  Net cpm = $cpm_{Average}$ - $cpm_{Average\ NSB}$

- 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%.

$$B/B_0(\%) = \text{percent bound} = \frac{\text{net cpm}}{\text{net MB cpm}} \times 100$$

- 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 spline smoothed fitting algorithm.
- Determine Vasopressin concentrations in 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 [Arg 8]-vasopressin Calibrator is weighed in material which was calibrated against WHO reference preparation 77/501.

Refer to Table 11 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 calibrated against 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.

PERFORMANCE LIMITATIONS

- It is mandatory using EDTA plasma ONLY in order to inhibit metalloprotease activities.
- Use of conical polystyrene tubes is strongly recommended. During step 11 of the assay procedure, a more solid pellet will be achieved and the following aspiration of the supernatant can be done much easier.
- Samples that are not properly collected and handled may cause inaccurate arginine vasopressin results. Samples must be frozen immediately in order to ensure correct results at the time of measurement (see Specimen Collection).
- Vasopressin is extremely unstable. It is crucial to freeze the samples immediately. Transportation has to be carried out at -20 °C.

PERFORMANCE CHARACTERISTICS

The assay performance characteristics have been validated in duplicates.

Intra-Assay Precision (Within-Run): 6.0%. The intra-assay precision was calculated from the results of 10 pairs of values from each sample in a single run. The values are presented in Table 12.

Inter-Assay Precision (Run-to-Run): 9.9%. The inter-assay precision was calculated from the results of 20 pairs of values from two EDTA plasma samples in 20 different runs. The values are presented in Table 13.

Detection limit (LoB): 0.75 pg/ml (0.82 pmol/l). The limit of blank of Vasopressin Direct RIA was calculated by subtracting two standard deviations of averaged Zero Calibrator duplicates from the counts at maximum binding.

Detection limit (LoQ): 1.3 pg/ml (1.2 pmol/l). The least detectable dose (limit of quantification) of the assay was calculated to be at the intra assay CV of 10%.

Spiking Recovery: 101.8%. EDTA plasma was spiked with increasing amounts of synthetic arginine-vasopressin and analyzed according to the assay procedure. The values are presented in Table 14.

Specificity: The following cross-reactions of the vasopressin antiserum were determined at 50% binding:

Arginine vasopressin	100.0	%
Lysine vasopressin*	**0.25**	**%**
Desmopressin (DDAVP)	0.0085	%
Oxytocin	0.001	%
Vasotocin	0.001	%

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

Method comparison: The VASOPRESSIN-DIRECT RIA has been compared with the Bühlmann VASOPRESSIN RIA (RK-AR1-U; column extraction). Results obtained with 28 blood donors (EDTA plasma) show an excellent correlation (see Figure 2).

Table 3: **Example of Results**

Incubation temperature at BÜHLMANN: 20°C.
Data reduction: Multicalc Software (PerkinElmer), spline smoothed option.

	cpm	B/T [%]	B/B_0 [%]	Conc [pg/ml]	CV [%]
Total	31445	100.0			
Total	31261	100.0			
Total Avg.	**31353**	100.0			0.4
NSB	768	2.45			
NSB	701	2.25			
NSB Avg.	**736**	**2.35**			6.1
MB	9863	31.46	100		
MB	9921	31.65	100		
MB	10428	33.26	100		
MB	10083	32.16	100		
MB Avg.	**10074**	**32.13**	**100**		2.5
Calibrator G	9442	30.12	93.2	1.25	
Calibrator G	9444	30.12	93.3	1.25	
Calibrator G Avg.	**9443**	**30.12**	**93.2**	**1.25**	0.0
Calibrator F	9187	29.3	90.5	2.5	
Calibrator F	9215	29.39	90.8	2.5	
Calibrator F Avg.	**9201**	**29.35**	**90.7**	**2.5**	0.2
Calibrator E	8474	27.03	82.9	5.0	
Calibrator E	8440	26.92	82.5	5.0	
Calibrator E Avg.	**8457**	**26.97**	**82.7**	**5.0**	0.3
Calibrator D	6789	21.65	64.8	10.0	
Calibrator D	6819	21.75	65.1	10.0	
Calibrator D Avg.	**6804**	**21.70**	**65.0**	**10.0**	0.3
Calibrator C	5621	17.93	52.3	20.0	
Calibrator C	5681	18.12	53.0	20.0	
Calibrator C Avg.	**5651**	**18.02**	**52.6**	**20.0**	0.8
Calibrator B	4173	13.31	36.8	40.0	
Calibrator B	4319	13.78	38.4	40.0	
Calibrator B Avg.	**4246**	**13.54**	**37.6**	**40.0**	2.4
Calibrator A	2877	9.18	22.9	80.0	
Calibrator A	2909	9.28	23.3	80.0	
Calibrator A Avg.	**2893**	**9.23**	**23.1**	**80.0**	0.8
Control NORMAL	9036		88.9	2.94	
Control NORMAL	9009		88.6	3.02	
Control NORM. Avg	**9023**		**88.7**	**2.98**	2.0
Control **High**	6743		64.3	11.5	
Control High	6817		65.1	11.1	
Control High Avg.	**6780**		**64.7**	**11.3**	2.6

ED-20 = 92.7 pg/ml ED-50 = 22.4 pg/ml ED-80 = 5.42 pg/ml

Figure 1: **Example of a standard curve**

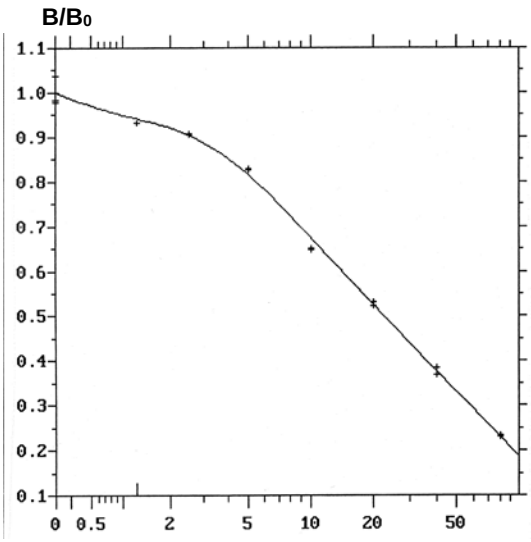

Table 4: **Intra-Assay Precision (Within-Run)**

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Plasma 1	4.0	0.39	9.5
Plasma 2	6.7	0.42	6.2
Plasma 3	19.1	0.44	2.3
Mean			6.0

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

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Plasma 4	1.78	0.23	13.0
Plasma 5	12.27	0.83	6.8
Mean			9.9

Table 6: **Spiking Recovery**

Sample	basic value [pg/ml]	spiked with [pg/ml]	Calculated [pg/ml]	Observed [pg/ml]	Recovery [%]
Plasma 6	2.0	2.6	4.6	5.0	109
	2.0	7.8	9.8	10.1	103
	2.0	23.3	25.3	26.4	104
	2.0	70.0	72.0	65.7	91
Mean					101.8

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

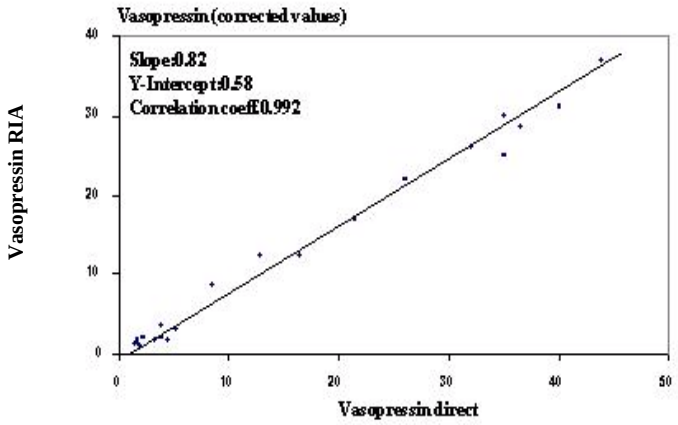

Table description: cf. “Results”, “Performance Characteristics” (page 4) and “Reference Intervals” (page **Fehler! Textmarke nicht definiert.**).

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 minute) at 18-28°C
NSB	700	--	--		100		100	
MB	650	--	50	Vortex and incubate at 18-28°C for 24 hours (± 3 h)	100	Vortex and incubate at 18-28°C for 24 hours (± 3 h)	100	
Std A 80 pg/ml	250	400	50		100		100	
Std B 40 pg/ml	250	400	50		100		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	250	400	50		100		100	
Std D 10 pg/ml	250	400	50		100		100	
Std E 5 pg/ml	250	400	50		100		100	
Std F 2.5 pg/ml	250	400	50		100		100	
Std G 1.25 pg/ml	250	400	50		100		100	
Control NORMAL	250	400	50		100		100	Aspirate supernatant (except T tubes)
Control ELEVATED	250	400	50		100		100	

APPENDIX IV

SYMBOLS

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

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