VASOPRESSIN Direct

RIA

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

RK-VPD-U 100 tests

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INTENDED USE

This double antibody radioimmunoassay is designed for the quantitative direct measurement of arginine-vasopressin ([Arg8]-vasopressin, anti-diuretic hormone, ADH) in EDTA plasma (1-3) and urine.

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	1 vial 100 mL	B-VPD-PB	Ready to use
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 ^{125}I-Vasopressin	1 vial 11 mL	B-ADH-TR	Ready to use
Calibrator A - E[1)] Lyophilized synthetic arginine vasopressin	1 set of 5 vials	B-VPD-CASET	Reconstitute with 5 mL of Phosphate Buffer
Controls Normal / High[2)] Arginine Vasopressin in a buffer matrix	1 set of 2 vials	B-VPD-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)] After reconstitution the Calibrators A to E contain 1.5, 4, 10, 30 and 100 pg/mL of [Arg8]-vasopressin.

[2)] Lot specific amounts of [Arg8]-vasopressin in buffer matrix. Refer to the additional QC Data Sheet for the exact concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
The kit components are stable at 2-8°C until the expiration date indicated on the reagent labels. Do not use the kit beyond the expiration date printed on the labels. **Do not freeze the Second Antibody**.	
Opened / Reconstituted Reagents	
Phosphate Buffer	Stable for 2 months at 2-8°C
Antibody Dilution Buffer	
Antiserum	
Tracer	
Second Antibody	
Calibrators	
Controls	

Table 2

PRECAUTIONS

Safety 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. Unused solutions and radioactive waste should be disposed of according to local State and Federal regulations.
- All kit reagents except the second antibody (B-AB2) and the antibody dilution buffer (B-CPD-DB) 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 may 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 prior to use. Reconstitute the lyophilized reagents as indicated. Mix well (vortex) the reagents before use.

EQUIPMENT REQUIRED

- 50 µl, 100 µl, 250 µl, 400 µl and 1000 µl precision pipettes with disposable tips.
- 5.0 mL volumetric pipette.
- Vortex mixer.
- Stir bar and magnetic stirrer.
- Centrifuge.
- Aspiration device.
- Gamma counter.

MATERIALS RECOMMENDED BUT NOT PROVIDED

- Disposable conical polystyrene tubes to run the assay (e.g. Sarstedt # 57.477).

SPECIMEN COLLECTION AND STORAGE

Appropriate sample collection is essential to ensure accurate results of the vasopressin analysis. 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.

EDTA plasma: Collect blood (at least 2 mL) into an **EDTA venipuncture tube** and, whenever possible, place the sample on ice until centrifugation. 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, if it will not be assayed immediately. The procedure calls for 400 µl of EDTA plasma per assay tube.

Note: 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.

Urine: Collect urine (at least 2 mL) into a plain tube and, whenever possible, place the sample on ice until centrifugation. Centrifuge at 2-8°C at 2000 x g for 5 minutes, whenever possible within 60 minutes after urine collection, aliquot (if needed) and freeze the specimen in fresh tubes at ≤–20°C if not assayed the same day. The procedure calls for 400 µl of urine per assay tube.

Note: The concentration of urine, and consequently the content of arginine-vasopressin in urine, may vary considerably between subjects, sampling time/schedule, drinking behavior and disease conditions. NovoLytiX GmbH tested some exemplary normal donor samples and found that normal daytime values range between <1.7 pg/mL and approx. 15 pg/mL, while first morning urine samples usually measure above 10 pg/mL up to 40 pg/mL. If higher values are expected, a five-fold dilution of urine samples with Phosphate Buffer (B-VPD-PB) is recommended prior to their use in the RK-VPD-U.

PROCEDURAL NOTES

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

Allow all reagents for steps 2-4 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 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 E) 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 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.

10. Add 1 mL of deionized water to each tube (except the T tubes). **Do not resuspend the precipitate**.

11. Centrifuge for 5 minutes 1000 x g at 18-28°C. Aspirate the supernatant **(except the T tubes)** and retain the precipitates for counting.

12. Count all tubes for 2 minutes in a gamma counter.

CALCULATION OF RESULTS & STANDARDIZATION

- 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:

  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 or a four-parameter-logistics (4-PL) fitting algorithm.
- Determine the [Arg8]-vasopressin concentrations in 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 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.

PERFORMANCE LIMITATIONS

- 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.
- The 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 [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*).
- Transportation of EDTA plasma and urine samples should be carried out at -20°C or lower.

PERFORMANCE CHARACTERISTICS

The assay performance characteristics have been validated in duplicates, unless otherwise stated.

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 results are presented in Table 4.

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 results are presented in Table 5.

Analytical Sensitivity (Limit of Blank, LoB): 0.75 pg/mL (0.82 pmol/L). The limit of blank of the Vasopressin Direct RIA was calculated by subtracting two standard deviations of averaged Zero Calibrator duplicates from the counts at maximum binding and intersecting this value with the standard curve run in the same assay.

Functional Sensitivity (Limit of Quantitation, LoQ): 1.7 pg/mL (1.6 pmol/L). The Limit of Quantitation (LoQ) of the assay was determined a precision profile and calculated to be at the concentration at which the profile crosses the intra-assay CV value of 10%.

Spiking Recovery: 100.9%. Three EDTA plasma and one urine samples were spiked with increasing amounts of synthetic arginine-vasopressin and analyzed according to the assay procedure. The results are presented in Table 6.

Dilution Linearity of Urine: 106.3%. Four urine samples were diluted with Phosphate Buffer (B-VPD-PB) by factors of 3 and 10 and then measured according to the assay procedure. The results are presented in Table 7.

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

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

* In pigs and hippopotamus the arginine at position 8 is replaced by lysine.

Method comparison: The Vasopressin Direct RIA (RK-VPD) has been compared with the Vasopressin RIA (RK-AR1; employing phenylsilylsilica column extraction). Results obtained with 72 EDTA plasma samples yield an excellent correlation coefficient $R^2 = 0.954$ (see Figure 3).

Freeze/Thaw Cycles: Three EDTA plasma and six urine samples containing different amounts of arginine-vasopressin were frozen at -20°C and thawed at 20-30°C up to three times. The stability data are shown in Figure 3. There might be a slight increase of the measured arginine-vasopressin levels after the third freeze-thaw cycle for the EDTA plasma samples.

NORMAL VALUES, REFERENCE RANGE

In several evaluations with a total of 180 apparently healthy blood donors collected by the Swiss Red Cross blood donation center Basel, a normal reference range (5-95 percentile) between <1.7 pg/mL and 6.3 pg/mL was established with a median value of 1.9 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 (<1.7 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.

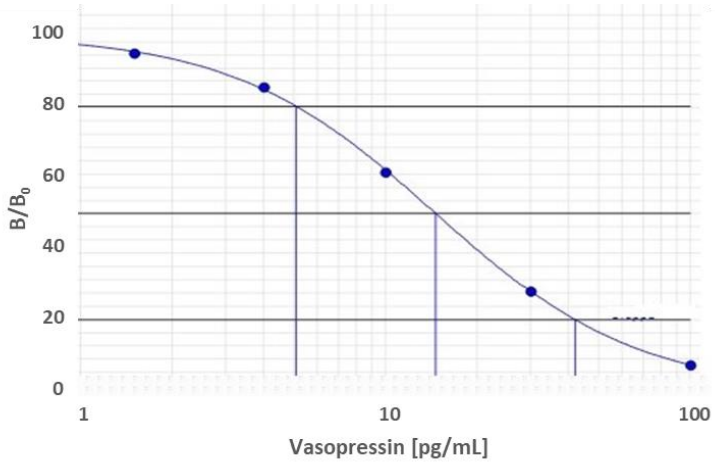
Table 3: **Example of Results**

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

	cpm	B/T [%]	B/B_0 [%]	Conc [pg/mL]	CV [%]
Total	13693	99.4			
Total	13855	100.6			
Total Avg.	**13774**	**100.0**			0.8
NSB	408	3.0			
NSB	448	3.3			
NSB Avg.	**428**	**3.1**			6.6
MB	4843	35.2	98.0		
MB	4917	35.7	99.7		
MB	4918	35.7	99.7		
MB	5047	36.6	102.6		
MB Avg.	**4931**	**35.8**	**100.0**		1.7
Calibrator A	4482	32.5	89.9		
Calibrator A	4680	34.0	94.4		
Calibrator A Avg.	**9443**	**33.3**	**92.1**	**1.5**	3.1
Calibrator B	4184	30.4	83.2		
Calibrator B	4218	30.6	84.0		
Calibrator B Avg.	**4201**	**30.5**	**83.6**	**4.0**	0.6
Calibrator C	3151	22.9	60.0		
Calibrator C	3312	24.0	63.6		
Calibrator C Avg.	**3232**	**23.5**	**61.8**	**10.0**	3.5
Calibrator D	1858	13.5	30.9		
Calibrator D	1881	13.7	31.4		
Calibrator D Avg.	**1870**	**13.6**	**31.2**	**30.0**	0.9
Calibrator E	1038	7.5	12.5		
Calibrator E	1000	7.3	11.6		
Calibrator E Avg.	**5651**	**7.4**	**12.0**	**100.0**	2.6
Control Normal	4196		83.5	3.8	
Control Normal	4176		83.0	3.9	
Control NORM. Avg	**4186**		**83.2**	**3.9**	2.1
Control High	3252		62.2	10.0	
Control High	3185		60.7	10.6	
Control High Avg.	**6780**		**61.5**	**10.3**	3.7

ED-20 = 42.1 pg/mL ED-50 = 14.6 pg/mL ED-80 = 5.1 pg/mL

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



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

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	1.8	0.23	13.0
Plasma 5	12.3	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
		7.8	9.8	10.1	103
		23.3	25.3	26.4	104
		70.0	72.0	65.7	91
Plasma 7	1.3	2	3.3	3.6	109
		5	6.3	6.1	97
		10	11.3	10.4	92
		20	21.3	17.5	82
Plasma 8	2.1	2	4.1	4.7	116
		5	7.1	6.8	97
		10	12.1	12.5	104
		20	22.1	19.7	89
		40	42.1	34.9	83
Urine 5	3.0	2	5.0	5.1	102
		5	8.0	8.9	111
		10	13.0	15.4	118
		20	23.0	22.8	99
		40	43.0	41.3	96
Mean					**100.9%**

Table 7: **Dilution Linearity (urine samples)**

Sample	Undiluted [pg/mL]	Dilution factor	Calculated [pg/mL]	Observed [pg/mL]	Recovery [%]
Urine 1	58.1	3	19.4	19.5	101
		10	5.8	6.6	114
Urine 2	49.9	3	18.2	16.6	109
		10	5.0	5.3	106
Urine 3	26.3	3	8.8	9.4	107
		10	2.6	3.0	114
Urine 4	4.7	3	1.6	1.4	93
Mean					**106.3%**

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

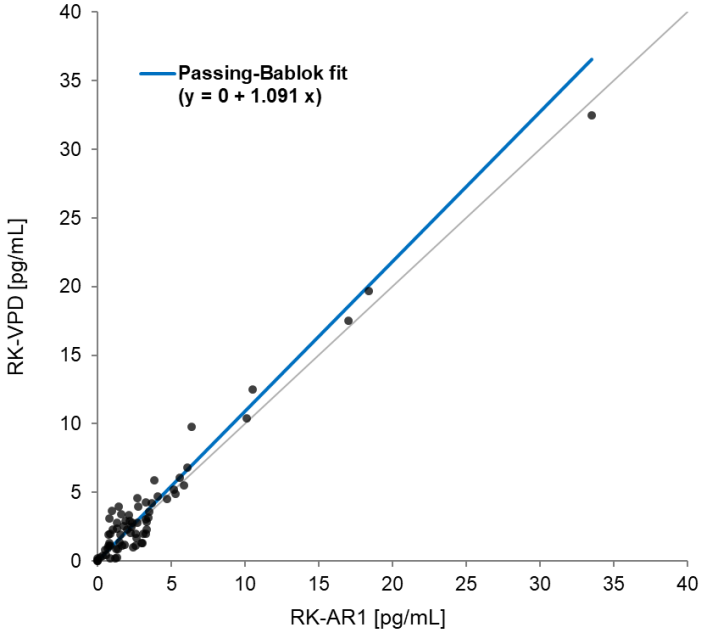
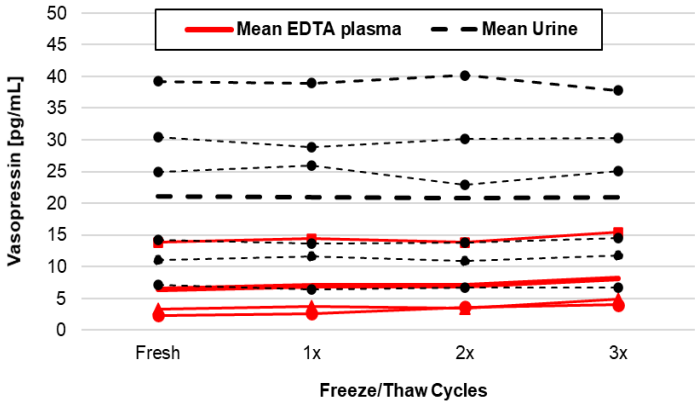


Figure 3: **Freeze/Thaw Cycles**



APPENDIX II

REFERENCES

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Change Log

Date	Version	Reason for change
2022-03-27	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); more performance data, slightly adapted normal reference range determined with 180 apparently healthy blood donors, revised method comparison data; slightly adapted layout, correction of typos.
2022-04-12	02	Extension of sections *INTENDED USE* and *SPECIMEN COLLECTION AND STORAGE* to the use of urine samples; more data points for the comparison with RK-AR1 (n=72; see Figure 2); up to 3 freeze/thaw cycles possible with EDTA plasma and urine samples (new Figure 3); correction of section *Spiking Recovery* and more spiking recovery data added including for urine (see Table 6); new data on the *Dilution Linearity* of urine samples available in the section *PERFROMANCE CHARACTERISTICS* (new Table 7).

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		100		100	
Std A 1.5 pg/mL	250	400	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	**Add 1mL of deionized water (except T tubes) and centrifuge for 5 minutes at 18-28°C and 1000 x g**
Std B 4 pg/mL	250	400	50		100		100	
Std C 10 pg/mL	250	400	50		100		100	
Std D 30 pg/mL	250	400	50		100		100	
Std E 100 pg/mL	250	400	50		100		100	
Control NORMAL	250	400	50		100		100	**Aspirate supernatant (except T tubes) and count for 2 minutes**
Control HIGH	250	400	50		100		100	
Sample	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	Second Antibody

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