



# **VASOPRESSIN-RIA**

***RB319RUO***

For Information/Research Purposes Only

# History

## Summary of change:

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

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 Research use only. Not for use in diagnostic procedures.**

## II. GENERAL INFORMATION

- A. **Proprietary name :** DIAsource Vasopressin RIA
- B. **Catalog number :** RB319RUO : 100 tests
- C. **Manufactured by :** DIAsource ImmunoAssays S.A.  
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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## III. BACKGROUND

- A. **Purpose of research product**  
After solid phase extraction (SPE) or ethanol extraction the plasma vasopressin concentrations are measured by radioimmunoassay (RIA). Urine vasopressin concentrations can be measured directly.  
The result shall not be used for clinical diagnosis or patient management.
- B. **Application**  
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 axonal flow to the posterior pituitary for storage and eventual release. Vasopressin determination is useful in the study of 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 [<sup>125</sup>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   |
|---|---------------------------------|-------------|--|
| <b>Ab</b><br>Lyophilized anti-vasopressin   | 1 vial<br>lyophilized           | Blue        | Add 22 ml<br>distilled water   |
| <b>Ag</b> <sup>125</sup> I<br>TRACER: <sup>125</sup> Iodine labelled<br>vasopressin<br>Specific activity: 62-77 MBq/nmol<br>(1700-2100 μCi/nmol).   | 1 vial<br>lyophilized<br>28 kBq | Red         | Add 25 ml<br>distilled water   |
| <b>DASP</b><br>Double antibody solid phase.<br>Goat anti-rabbit IgG's bound to<br>solid phase in phosphate buffer with<br>Human serum albumin, NaCl,<br>NaN <sub>3</sub> , EDTA and Tween 80. | 1 vial<br>11 mL                 | Green       | Ready for use  |
| <b>ASS BUF</b><br>Assay diluent<br>Phosphate buffer with HSA, EDTA<br>disodium salt, NaN <sub>3</sub> and aprotinin<br>(Trasylol® or equivalent)  | 2 vials<br>50 mL                | Black       | Ready for use  |
| <b>CAL</b><br>Vasopressin calibrator<br>(see exact value on vial label)   | 1 vial<br>lyophilized           | Yellow      | Reconstitute<br>with distilled<br>water by the<br>volume stated on<br>the vial label |
| <b>CONTROL N</b><br>Controls - N = 1 or 2<br>(see exact value on vial label)  | 2 vials<br>lyophilized          | Silver      | Add 2 mL<br>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. <sup>125</sup>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 sample preparation and sampling conditions is recommended.

##### Vasopressin in plasma

- Draw blood from fasting subject 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) <sup>125</sup>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 <sup>125</sup>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 <sup>125</sup>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 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 <sup>125</sup>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 <sup>125</sup>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 tubes (R) 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 500 µL 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 <sup>125</sup>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 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 samples and controls, their cpm, % B/Bo or % B/T of precipitated bound fractions are interpolated now 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%

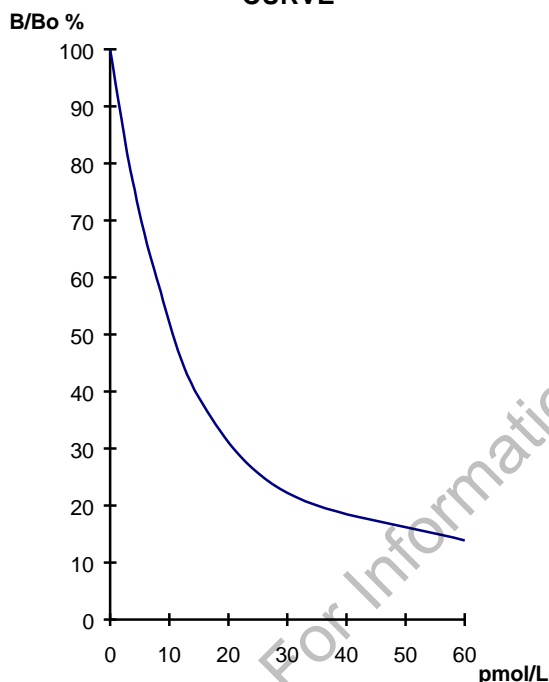
Patient sample concentration corrected :  $\frac{10 \times 100}{45} = 22.2$  pmol/L

45

**XI. 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             |

**VASOPRESSIN STANDARD CURVE**



**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  | 9.2                     | 9.8                     | 106        |
| A2  | 14.3                    | 14.4                    | 101        |
| B1  | 9.6                     | 10.0                    | 104        |
| B2  | 15.3                    | 15.1                    | 98         |

**Specificity**

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

| Peptide                       | % Cross reactivity |
|-------------------------------|--------------------|
| Arg <sup>8</sup> Vasopressin  | 100                |
| Oxytocin                      | <0.1               |
| Lys <sup>8</sup> -Vasopressin | <0.1               |
| Desmopressin                  | <0.1               |
| Arg <sup>8</sup> 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 Quality Control sheet and 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. NORMAL RANGE**

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**

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

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 <sup>125</sup>I (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.

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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            |          |         |
| <b>Vortex and incubate for 18-24 hours at 2-8°C.</b>                            |             |        |                   |          |         |
| <sup>125</sup> I Tracer   | 200 µl      |        |                   |          |         |
| <b>Vortex and incubate for 18-24 hours at 2-8°C.</b>                            |             |        |                   |          |         |
| Double antibody solid phase   | -           | 100 µl |                   |          |         |
| <b>Vortex and incubate for 30-60 min at 2-8°C.</b>                              |             |        |                   |          |         |
| <b>Centrifuge 15 min ( 1700 g; 4°C )</b>  |             |        |                   |          |         |
| <b>Aspirate or decant the supernatant and count the residue for 2-4 minutes</b> |             |        |                   |          |         |

|                                      |                         |
|--------------------------------------|-------------------------|
| DIAsource Catalogue Nr :<br>RB319RUO | Revision nr :<br>200701 |
|--------------------------------------|-------------------------|

Revision date : 01/07/2020

Distributed by :



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