VIP RIA

RB311RUO

For Informational/Research Purposes Only

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History

Summary of change:

Previous Version: **191011-1**	**Current Version:** **200224-1**
LOT	Version :
	Addition of the following sentence at the end of the English IFU: "Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/"

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en

Read entire protocol before use.

VIP RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of vasoactive intestinal polypeptide (VIP) in human plasma.
For Research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource VIP RIA

B. Catalog number : RB311RUO : 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

Vasoactive intestinal peptide (VIP) is a linear polypeptide containing 28 amino acid residues. The molecular weight is 3381. VIP is structurally related to secretin and to other members of the secretin family. The carboxyterminal amino acid of VIP (Asn) is amidated.
Immunocytochemical studies have shown that VIP is present in neurons throughout the gastrointestinal tract, the centrol and peripheral nervous systems, the salivary glands and the pancreas.
VIP stimulates water and bicarbonate secretion by the pancreas. VIP possesses the capacity to relax smooth muscle. Administration of VIP produces vasodilation and pulmonary broncho-dilation and relaxes the lower oesophageal sphincter and smooth muscle of the fundus of the stomach.
VIP is believed to play crucial roles in the regulation of intestinal motility and intestinal epithelial ion and water transport.
Increased plasma immunoreactive VIP concentrations have been reported in patients with the WDHA syndrome (water, diarrhoea, hypokalemia and achlor hydria). Increased plasma levels of VIP have also been reported in patients with cirrhosis.

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IV. PRINCIPLES OF THE METHOD

VIP is analysed by the competitive radioimmunoassay using antibodies to a VIP-albumin conjugate. VIP in standards and samples compete with ^{125}I-labelled VIP in binding to the antibodies. ^{125}I-VIP binds in a reverse proportion to the concentration of VIP in standards and samples. In order to increase the sensitivity of the assay a sequential incubation is performed. Antibody-bound ^{125}I-VIP is separated from the unbound fraction using the double antibody polyethylene glycol precipitation technique. The radioactivity of the precipitates is measured. The antiserum used in this kit is directed to the C-terminal part of the VIP molecule.
The result shall not be used for clinical diagnosis or patient management.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
ANTISERUM Rabbit antiserum raised against highly purified porcine VIP conjugated to bovine serum albumin in phosphate buffer with human serum albumin, sodium azide and aprotinin.	1 vial lyophilised	Blue	**Add** 22 ml distilled water
Ag ^{125}I Contains 1.5 µCi or 56 KBq. Produced by iodination of synthetic, human VIP. HPLC-purified, monoiodinated. Specific activity: 1700-2100 µCi/nmol (62-77 Mbq/nmol). Lyophilized phosphate buffer with human serum albumin, sodium azide, normal rabbit serum and aprotinin.	1 vial lyophilised 56 kBq	Red	**Add** 12.5 ml distilled water
Ab PEG Goat anti-rabbit-Ig antiserum in phosphate buffer with human serum albumin, NaN3. and polyethylene glycol 6000.	1 vial 50 mL	Green	**Ready** for use
DIL BUF Lyophilized human plasma for dilution of the VIP standards. Contains aprotinin (Trasylol® or equivalent) and sodium azide.	1 vial lyophilised	Black	**Add** 10 ml distilled water
CAL Synthetic human VIP. Lyophilized in human plasma. Contains aprotinin (Trasylol® or equivalent) and sodium azide.	1 vial lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on the vial label
ASS BUF Buffer for use instead of antiserum in non-specific binding controls in phosphate buffer with human serum albumin, sodium azide and aprotinin (Trasylol® or equivalent).	1 vial 5 mL	Black	**Ready** for use
CONTROL N Controls - N = 1 or 2 Contains sodium azide.(<0.1%).	2 vials lyophilised	Silver	**Add** 1.5 mL distilled water

VI. SUPPLIES NOT PROVIDED

1. Distilled water.
2. 11-13 x 55 mm disposable tubes of polystyrene.
3. Pipettes: 1 mL, 5 mL and 10 mL.
4. Pipettes with disposable tips: 100, 200 and 500 µl.
5. Vortex mixer.
6. Centrifuge, refrigerated, giving minimum 1700 x g.
7. Gamma counter.

VII. REAGENT PREPARATION

A. **Anti-VIP :** Reconstitute with 22 mL of distilled water. Store at 2-8°C.
B. **^{125}I-VIP :** Reconstitute with 12.5 mL of distilled water immediately before use. Store at -18°C or lower if reused.
C. **Double antibody PEG** : Ready for use. Mix thoroughly before use. Store at 2-8°C.
D. **Calibrator diluent** : Reconstitute with 10 mL distilled water. Store at -18°C or lower if reused.
E. **VIP calibrator 120 pmol/L** : Reconstitute with distilled water by the volume stated on vial label. Store at -18° C or lower if reused.
F. **Assay buffer** : Ready to use. Store at 2-8°C.
G. **Controls** : Reconstitute with 1.5 mL distilled water. Store at -18°C or lower if reused.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of lyophilized reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the contents in a vial by gentle inversion and avoid foaming. The stability of the reagents is found on the label of the vials. For lyophilized reagents the expiry date is valid for the reconstituted reagents. Reconstituted reagents are stable for 10 weeks stored correctly.

IX. SPECIMEN COLLECTION

Blood is collected in tubes containing EDTA and aprotinin (Trasylol® or equivalent) (5000 KIU aprotinin (Trasylol® or equivalent) in a 10 mL vacutainer). The sample is cooled in an ice-bath immediately. Plasma is separated by centrifugation at +4° C. The plasma should be frozen within 1 hour and stored at -18° C or lower until assayed. Repeated freezing and thawing should be avoided.

X. PROCEDURE

A. Handling notes

Accuracy in all pipetting steps is essential. Reagents should be brought to room temperature prior to use. The assay is performed with duplicates (standards, controls, samples, control tubes for non-specific binding and total activity).
A complete assay includes:
Standard: 7 concentrations: 0, 3.8, 7.5, 15, 30, 60 and 120 pmol/L.
Controls: Controls with known concentrations of VIP for quality control.
Samples.
Tubes for determination of the non-specific binding for standards and samples (NSB-tubes).
Tubes for determination of the total radioactivity added (TOT-tubes).

B. Procedure

1. Reconstitute the reagents according to the instructions.
2. Prepare the VIP working standards by dilution of the VIP standard 120 pmol/L with the Standard diluent according to the following:
   a/ 1.00 mL standard 120 pmol/L + 1.00 mL diluent = 60 pmol/L.
   b/ 1.00 mL standard 60 pmol/L + 1.00 mL diluent = 30 pmol/L.
   c/ 1.00 mL standard 30 pmol/L + 1.00 mL diluent = 15 pmol/L.
   d/ 1.00 mL standard 15 pmol/L + 1.00 mL diluent = 7.5 pmol/L.
   e/ 1.00 mL standard 7.5 pmol/L + 1.00 mL diluent = 3.8 pmol/L
   f/ Standard diluent = 0 pmol/L.
   Store the standard solutions at -18° C or lower if reused.
3. Pipette 200 µl of standards (0-120 pmol/L), samples and controls in their respective tubes. Pipette 200 µl of the zero-standard in the NSB-tubes.
4. Add 200 µl anti-VIP to all tubes except the NSB- and TOT-tubes.
5. Add 200 µl assay diluent to the NSB-tubes.
6. Vortex-mix and incubate for 24 hours at 2-8° C.
7. Add 100 µl ^{125}I-VIP to all tubes. The TOT-tubes are sealed and kept aside.
8. Vortex-mix and incubate for 24 hours at 2-8° C.
9. Add 500 µl double antibody-PEG to all tubes except the TOT-tubes (mix this reagent before pipetting).
10. Vortex-mix and incubate for 30-60 minutes at 2-8° C
11. Centrifuge the tubes for 15 minutes at +4° C (1700 x g).
12. Decant the supernatants immediately after centrifugation.
13. Count the radioactivity of the precipitates in a gamma counter (counting time: 2minutes).

XI. CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the non-specific binding from the count rate (CPM) of the replicates of standards, controls and samples.
2. A standard curve is generated by plotting the precipitated CPM, bound fraction (in CPM or %B/TOT) against the concentrations of the VIP standards.

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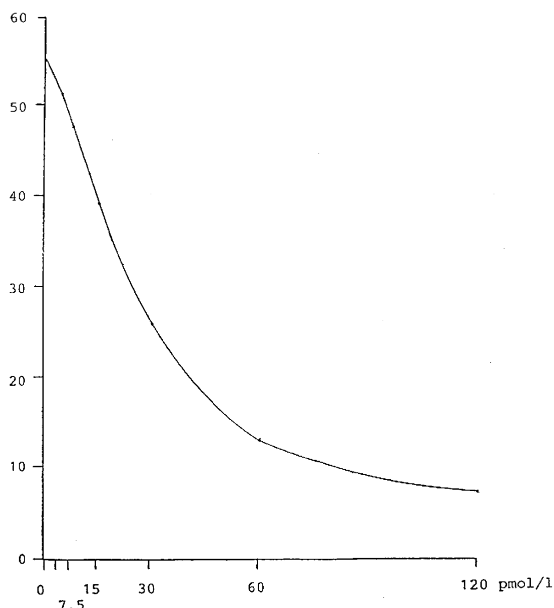
3. To obtain the VIP concentrations in the samples and controls read the corresponding concentrations to their precipitated CPM or %B/TOT from the generated standard curve.
4. The standard curve and the calculation of the concentrations in the samples can also be done by a computer method. A spline method may be used.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.



XIII. PERFORMANCE AND LIMITATIONS

A. Sensitivity
The sensitivity calculated from a decrease in binding of 2 SD in the zerostandard is 3 pmol/L.

B. Precision

Intra assay variation:

Level	Coefficient of variation (%CV)
14.5 pmol/L	5.9
61.0 pmol/L	3.3

Inter assay variation:

Level	Coefficient of variation (%CV)
12.6 pmol/L	7.7
26.4 pmol/L	6.7
51.4 pmol/L	6.1

C. Specificity
The following cross reactions have been found:

Peptide	Cross reaction
VIP 1-28 (whole sequence)	100.0%
VIP 1-6	<2.5%
VIP 1-18	<2.5%
VIP 1-22	<2.5%
VIP 11-28	83.3%
VIP 7-28	90.9%
VIP 18-28	71.4%
Secretin, porcine	<0.01%
Gastric inhibitory peptide, porcine	<0.01%
Pancreatic glucagon, porcine	<0.01%
Enteroglucagon, porcine	<0.01%
Pancreatic polypeptide, human	<0.01%
Substance P	<0.01%
Somatostatine, ovine	<0.01%

D. Interference
Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

XIV. VIP CONCENTRATION IN HUMAN PLASMA

The VIP concentration in plasma after 12 hours fasting were assayed in normal subjects. The range was <3 pmol/L to 30.0 pmol/L.

XV. INTERNAL QUALITY CONTROL

In order to enable the laboratory to completely monitor the consistent performance of the assay, the following important factors should be checked.

1. The found concentrations of the controls
The found concentrations of the controls should be within the limits given on the labels of the vials.

2. Total counts
Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I-VIP in this kit will give 19000-32000 CPM at the reference date (counting efficiency: 80%).

3. Maximum binding (Bo/TOT)
Calculate for each assay the % bound radioactivity in the zero-standard:

$$\frac{Bo}{TOT} \times 100$$

4. Non-specific binding (NSB/TOT)
Calculate for each assay the % non-specific binding:

$$\frac{NSB}{TOT} \times 100$$

The non-specific binding should be less than 7%.

5. Slope of standard curve
For example, monitor the 80, 50 and 20% points of the standard curve for run to run reproducibility.

XVI. PRECAUTIONS AND WARNINGS

Safety

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

As the regulations may vary from one country to another, it is essential that the person responsible for the laboratory are familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this test.
This kit contains components of human origin. They have been tested for hepatitis B surface antigen, antibodies to HCV and for antibodies to HIV-1 and HIV-2 and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives, should be observed.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. Steps should be taken to ensure the proper handling of the radioactive material, according to local and/or national regulations. Only authorized personnel should have access to the reagents.
The following precautions should be observed when handling radioactive materials:
- Radioactive material should be stored in specially designated areas, not normally accessible to unauthorized personnel.
- Handling of radioactive material should be conducted in authorized areas only.
- Care should be exercised to prevent ingestion and contact with the skin and clothing. Do not pipette radioactive solutions by mouth.
- Drinking, eating or smoking should be prohibited where radioactive material is being used.
- Hands should be protected by gloves and washed after using radioactive materials.
- Work should be carried out on a surface covered by disposable absorbing material.
- Spills of radioactive material should be removed immediately, and all contaminated materials disposed as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

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The reagents in this kit contain sodium azide. 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.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrator (0-6)	Controls	Samples
Calibrator	-	-	200 µl	-	-
Controls	-	-	-	200 µl	-
Samples	-	-	-	-	200 µl
Anti-VIP	-	-	200 µl		
Assay diluent	-	200 µl	-	-	-
Vortex-mix and incubate for 24 hours at 2-8°C.					
^{125}I Tracer	100 µl				
Vortex-mix and incubate for 24 hours at 2-8°C.					
Double antibody PEG	-	500 µl			
Vortex-mix and incubate for 30-60 min at 2-8°C.					
Centrifuge 15 min (1700 g; 4°C)					
Decant and count the radioactivity of the precipitates					

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