Instruction

EURIA-VIP

Vasoactive intestinal peptide radioimmunoassay

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For Research Use Only. Not for use in diagnostic procedures.

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INTRODUCTION

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 eosophagaeal 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.

PRINCIPLE OF THE METHOD

The intended use of these reagents is for the determination of vasoactive intestinal polypeptide (VIP) in human plasma. 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.

PRECAUTIONS

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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.

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.

COMPOSITION OF THE REAGENT KIT

The reagents provided in each kit are sufficient for 100 tubes.

1. Anti-VIP (Reagent A)
Rabbit antiserum raised against highly purified porcine VIP conjugated to bovine serum albumin. Lyophilized in 2.0 mL 0.5 M phosphate buffer, pH 7.4, 4.0% human serum albumin, 0.5% sodium azide and 5000 KIU Trasylol® per mL. For 100 tubes. Reconstitution in 22 mL distilled water. Colour: Yellow.

2. ^{125}I-VIP (Reagent B)
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 in 1.25 mL 0.5 M phosphate buffer, pH 7.4, 4.0% human serum albumin, 0.5% sodium azide. Contains 0.12 mL normal rabbit serum and 6500 KIU Trasylol®. Reconstitution in 12.5 mL distilled water. Colour: Blue.

3. Double antibody-PEG (Reagent C)
50 mL diluted goat anti-rabbit-Ig antiserum in 0.05 M phosphate buffer, pH 7.4, 0.4% human serum albumin and 0.05% NaN_3. Contains 5.0% (w/v) polyethylene glycol 6000. Colour: Red.

4. Standard diluent (Reagent D)
10.0 mL lyophilized human plasma for dilution of the VIP standards. Contains 500 KIU Trasylol® per mL and 0.05% sodium azide. Reconstitution in 10.0 mL distilled water.

5. VIP standard (Reagent E)
2.5 mL, 120 pmol/L, synthetic human VIP. Lyophilized in human plasma. Contains 500 KIU Trasylol® per mL and 0.05% sodium azide. Reconstitution in 2.5 mL distilled water.

6. Assay buffer (Reagent F)
5.0 mL buffer for use instead of antiserum in non-specific binding controls. 0.05 M phosphate buffer, pH 7.4, 0.4% human serum albumin, 0.05% sodium azide and 500 KIU Trasylol® per mL.

7. Controls (Reagent G-H)
Lyophilized plasma controls with low (normal) and high concentration of VIP. 1.5 mL of each control after reconstitution. The VIP concentrations are given on the labels of the vials. Contains 0.05% sodium azide.

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

Distilled water.

11-13 x 55 mm disposable tubes of polystyrene.

Pipettes glass: 1.00, 5.00 and 10.00 mL.

Pipettes with disposable tips: 100, 200 and 500 µl.

Vortex mixer.

Centrifuge, refrigerated, giving minimum 1700 x g.

Gamma counter.

REAGENT PREPARATION AND STORAGE

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of lyophilized reagents should be destilled 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 unreconstituted reagents. Reconstituted reagents are stable for 10 weeks stored correctly.

Reagent A: Anti-VIP
Reconstitute with 22 mL distilled water.
Store at 2-8° C.

Reagent B: ^{125}I-VIP
Reconstitute with 12.5 mL distilled water immediately before use.
Store at -18° C or lower if reused.

Reagent C: double antibody-PEG
Ready for use. Mix thoroughly before use.
Store at 2-8° C.

Reagent D: Standard diluent
Reconstitute with 10.0 mL distilled water.
Store at -18° C or lower if reused.

Reagent E: VIP-standard, 120 pmol/L
Reconstitute with 2.5 mL distilled water. For preparation of working standards, see radioimmunoassay procedure.
Store at -18° C or lower if reused.

Reagent F: Assay buffer
Ready to use.
Store at 2-8° C.

Reagent G-H: Controls
Reconstitute with 1.5 mL distilled water. Store at -18° C or lower if reused.

SPECIMEN COLLECTION

Blood is collected in tubes containing EDTA and Trasylol® (5000 KIU Trasylol 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.

ASSAY PROCEDURE

For an overview see page 11.
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 (St-tubes): 7 concentrations: 0, 3.8, 7.5, 15.0, 30, 60 and 120 pmol/L.

Controls (C-tubes): Controls with known concentrations of VIP for quality control.

Samples (S-tubes).

Tubes for determination of the ***non-specific binding*** for standards and samples ***(NSB-tubes).***
Tubes for determination of the ***total radioactivity*** added ***(TOT-tubes).***

PERFORMANCE

1. Reconstitute the reagents according to the instructions.

2. Prepare the VIP working standards by dilution of the VIP standard 120 pmol/L (Reagent E) with the Standard diluent (Reagent D) 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 (Reagent A) to all tubes except the NSB- and TOT-tubes.

5. Add 200 µl assay diluent (Reagent F) to the NSB-tubes.

6. Vortex-mix and incubate for 24 hours at 2-8° C.

7. Add 100 µl ^{125}I-VIP (Reagent B) 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 (Reagent C) 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).

CALCULATIONS 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. An example of a standard curve is given on page 12.

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.

ASSAY CHARACTERISTICS

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

Precision
Intra assay variation

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

Inter assay variation (total variation)

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

Specificity
The following cross reactions have been found:

Polypeptide	Cross reaction
VIP 1-28 (whole sequence)	100 %
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%

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

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.

QUALITY CONTROL

In order for the laboratory to completely monitor the consistent performance of the radioimmunoassay there are some important factors which must be checked.

1. The found concentrations of the controls
(Reagent G and H) are 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 18 000-25 500 CPM at the reference date (counter efficiency = 80%).

3. Maximum binding (Bo/TOT)
Calculate for each assay the % bound radioactivity in the zero-standard: $\frac{Bo}{TOT} \times 100$.

$\frac{Bo}{TOT} \times 100$ is generally 35-55% at the reference date.

4. Non-specific binding (NSB/TOT)
Calculate for each assay the % non-specific binding $\frac{NSB}{TOT} \times 100$.

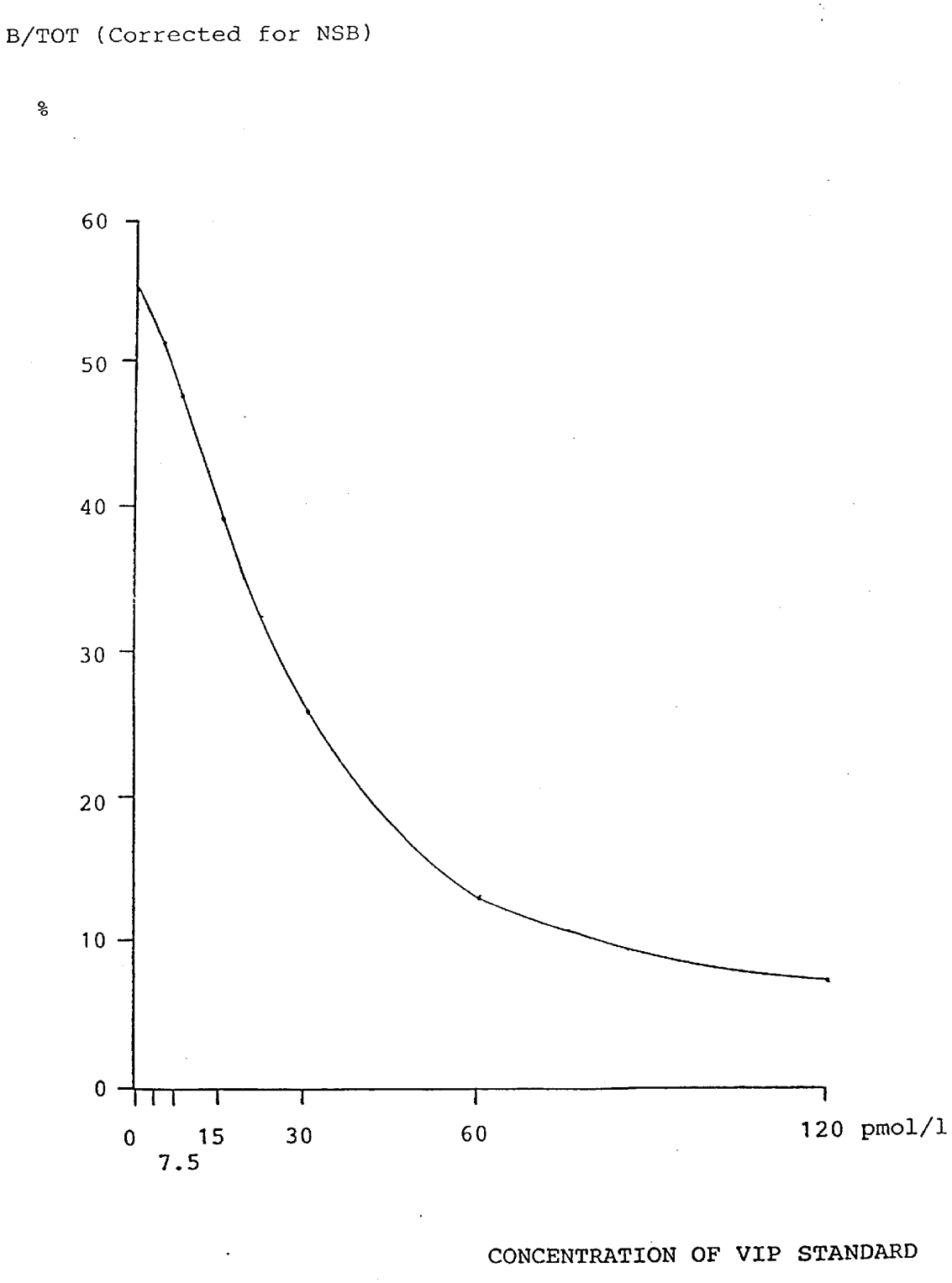
$\frac{NSB}{TOT} \times 100$ is less than 7%.

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

OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	Anti-VIP (A)	Assay diluent (F)		^{125}I-VIP (B)		Double antibody PEG (C)	
TOT	1- 2	-	-	-	Vortex-mix and incubate for 24 hours at 2-8° C.	100 µL	Vortex-mix and incubate for 24 hours at 2-8° C.	-	Vortex-mix and incubate for 30-60 min. at 2-8° C. Centrifuge 15 min. at 1700 x g at +4° C. Decant the supernatants and count the radioactivity of the precipitates.
NSB, St 0	3- 4	200 µL	-	200 µL		100 µL		500 µL	
Stand 0	5- 6	200 µL	200 µL	-		100 µL		500 µL	
Stand 3.8	7- 8	200 µL	200 µL	-		100 µL		500 µL	
Stand 7.5	9-10	200 µL	200 µL	-		100 µL		500 µL	
Stand 15	11-12	200 µL	200 µL	-		100 µL		500 µL	
Stand 30	13-14	200 µL	200 µL	-		100 µL		500 µL	
Stand 60	15-16	200 µL	200 µL	-		100 µL		500 µL	
Stand 120	17-18	200 µL	200 µL	-		100 µL		500 µL	
Control low	19-20	200 µL	200 µL	-		100 µL		500 µL	
Control high	21-22	200 µL	200 µL	-		100 µL		500 µL	
Sample 1	23-24	200 µL	200 µL	-		100 µL		500 µL	
Sample 2 etc.	25-26	200 µL	200 µL	-		100 µL		500 µL	

EXAMPLE OF VIP STANDARD CURVE



Literature

1. Fahrenkrug, J. and Schffalitzky de Muckadell, O.
   Radioimmunoassay of vasoactive intestinal polypeptide (VIP) in plasma.
   J. Lab Clin Med 89:1379 (1977).

2. Lundberg, J.M., Fahrenkrug, J., Hökfelt, T., Martling, C.-R., Larsson, O., Tatemoto, K. and Änggård, A.
   Coexistence of peptide HI (PHI) and VIP in nerves regulating blood flow and bronchial smooth muscle tone in various mammals including man.
   Peptides 5:593-606 (1984).

3. Fahrenkrug, J.
   Evidence for common precursors but differential processing of VIP and PHM in VIP-producing tumors.
   Peptides 6:357-361 (1985).

4. Bloom, S.R., and Polak, J.M. Vipomas.
   In: Vasoactive intestinal peptide. Editor: Said, S.I.
   Raven Press, New York, 457-468 (1982).

5. Bloom, S.R., Christofides, N.D., Delawarter, J., Buell, G., Kawashima, E. and Polak, J.M.
   Diarrhoea in VIPoma patients associated with cosecretion of a second active peptide (peptide histidine isoleucine) explained by a single loding gene.
   Lancet ii: 1163-1165 (1983).

SYMBOLS USED ON LABELS

	Batch code.
	Catalogue number.
	Use by date.
	Temperature limit.
	Date of manufacture.
	Contains radioactive substances.
	Biological risks.
	Consult instructions for use.
	Manufacturer.
 **100**	Contains sufficient for 100 tests.

REAG A Ab	Anti-VIP.
REAG B Ag ^{125}I	^{125}I-VIP.
REAG C DAB	Double antibody-PEG.
REAG D DIL CAL	Standard diluent.
REAG E CAL 120	VIP-standard, 120 pmol/L
REAG F BUF AS	Assay buffer.
REAG G CONTROL	Control, level 1 (low).
REAG H CONTROL	Control, level 2 (high).

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