Instruction

EURIA-PP

Pancreatic Polypeptide radioimmunoassay

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

REF **RB 316 RUO**

Σ 100

INTRODUCTION

Pancreatic polypeptide (PP) is synthesized as an amino-terminal moiety of a precursor peptide. PP isolated from pancreas has 36 amino acid residues with an amidated C-terminal tyrosine. PP is secreted by F-cells of the islets of Langerhans. PP is localized almost entirely in the pancreas although detectable levels throughout gastrointestinal tract have been reported. PP in human plasma is reported to exist in at least four different forms:
PP 1-36, PP 3-36 and two unidentified forms.

PP is released into plasma during stimulation while eating. The physiological role of PP includes inhibition of stimulated gastric and pancreatic exocrine secretions and augmentation of insulin inhibited hepatic glucose production. These actions of PP are mediated by specific receptors. Receptor binding studies have shown that the intact C-terminal tyrosine amide is necessary for biological activity.

PRINCIPLE OF THE METHOD

The intended use of these reagents is for assay of PP in human serum. PP in serum is assayed without extraction by a competetive radioimmunoassay using a rabbit antiserum raised against bovine PP. PP in standards and samples compete with ^{125}I-labelled human PP in binding to the antibodies. ^{125}I-PP binds in a reverse proportion to the concentration of PP in standards and samples. Antibody-bound ^{125}I-PP is separated from the unbound fraction using the double antibody-polyethyleneglycol precipitation technique. The radioactivity of the precipitates is measured. Human, synthetic PP is used for standardization.
The result shall not be used for clinical diagnosis or patient management.

PHYSIOLOGICAL CONSIDERATIONS

The secretion of PP is stimulated by eating especially protein and fat. PP is also produced by endocrine active tumours in the pancreas and the gastrointestinal tract. These tumours often produce several peptide hormones in the combinations PP-VIP, PP-glucagon or PP-gastrin. Tumours with only PP-secretion have been reported. These tumours may occur at the WDHA or Verner-Morrison syndrome.

Elevated fasting levels of PP in serum are found at the occurrence of PP-producing tumours and endocrine tumours in the pancreas and in the gastrointestinal tract.

Normal level of PP in human serum: <100 pmol/L (fasting level obtained with this procedure).

PRECAUTIONS

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 by immunoassay 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 is sufficient for 100 tubes.

1. Anti-PP (Reagent A)
Rabbit antiserum raised against bovine PP. For 100 tubes. Lyophilized in 5.0 mL 0.5M phosphate buffer, pH 7.4, 2.5% human serum albumin and 0.5% NaN_3.
Reconstitution in 52 mL distilled water.

2. ^{125}I-PP (Reagent B)
Contains 28 KBq or 0.75 μCi of ^{125}I-hPP at the activity reference date. Produced by iodination of synthetic human PP. HPLC-purified, monoiodinated.
Specific activity: 1700-2100 μCi/nmol (62-77 MBq/nmol).
Lyophilized in 1.25 mL 0.5M phosphate buffer, pH 7.4, 2.5% human serum albumin, 0.5% NaN_3. Contains 0.12 mL normal rabbit serum.
Reconstitution in 12.5 mL distilled water.

3. Double antibody-PEG (Reagent C)
50 mL diluted goat anti-rabbit-Ig antiserum. Diluent: 0.05M phosphate buffer, pH 7.4, 0.25% human serum albumin and 0.05% NaN_3. Contains 7.5% polyethylene glycol 6000 (w/v).

4. Standard diluent (Reagent D)
10.0 mL PP-free human serum, lyophilized. Contains 500 KIU aprotinin (Trasylol® or equivalent) /mL. Reconstitution in 10.0 mL distilled water. For preparation of PP-working standards.

5. PP-standard, 2 000 pmol/L (8370 pg/mL) (Reagent E)
2.00 mL, 2 000 pmol/L synthetic human PP-standard. Lyophilized in 0.05M phosphate buffer, pH 7.4, 0.25% human serum albumin, 0.05% NaN_3. Reconstitution in 2.00 mL distilled water.

6. Assay buffer (Reagent F)
5.0 mL 0.05 M phosphate buffer, pH 7.4, 0.25% human serum albumin and 0.05% NaN_3.
To be used instead of antiserum in the non-specific binding test tubes.

7. Controls (Reagent G-H)
Lyophilized serum controls with low (G) and high (H) concentration of PP.
1.00 mL of each control after reconstitution. The PP concentrations of the controls are given on the label of the vials. Contains 0.05% NaN_3.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Distilled water

11-13x55 mm disposable tubes, polystyrene

Pipettes with disposable tips: 100 and 500 µl

Pipettes (glass): 1.00, 5.00 and 10.00 mL

Measuring cylinders: 25 mL and 50 mL

Vortex mixer

Centrifuge, refrigerated giving a minimum of 1700 x g

Gamma counter

SPECIMEN COLLECTION

Subjects should be fasting 10 hours prior to sample collection.

Veinous blood is collected in tubes without additives. The sample is allowed to clot.
The serum is separated by centrifugation at +4° C. The serum should be frozen within 4 hours and stored at -18° C or lower until assayed. Repeated thawing and freezing should be avoided.

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 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 labels of the vials. For lyophilized reagents the expiry dates are valid for the unreconstituted reagents. Reconstituted reagents are stable for 10 weeks (no longer than to the expiry date) stored correctly.

Reagent A: Anti-PP
Reconstitute with 52 mL distilled water.
Store at 2-8° C.

Reagent B: ^{125}I-PP
Reconstitute with 12.5 mL distilled water.
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: PP-standard, 2 000 pmol/L
Reconstitute with 2.00 mL distilled water.
Store at -18° C or lower if reused.
For preparation of PP-working standards, see radioimmunoassay procedure.

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

Ragent G-H: Controls
Reconstitute with 1.00 mL distilled water. Store at -18° C or lower if reused.

RADIOIMMUNOASSAY PROCEDURE

Reconstitute the reagents as specified. Reagents should be brought to room temperature prior to use. Accuracy in all pipetting steps is essential. All tests (standards, controls and samples) should be performed in duplicate.
A complete assay includes:
Standards (St-tubes): 7 different concentrations; 0, 6.25, 12.5, 25.0, 50.0, 100 and 200 pmol/L.
Controls (C-tubes).
Samples (P-tubes).
Tubes for determination of the ***non-specific binding (NSB-tubes).***
Tubes for determination of the ***total radioactivity*** added ***(TOT-tubes).***
For an overview se table 1 on page 10.

PERFORMANCE

1. Reconstitute the reagents according to the instructions.
2. Prepare the PP-working standards by dilution of the PP-standard 2000 pmol/L (Reagent E) with the standard diluent (Reagent D) according to the following:

   a/ 0.200 mL standard 2000 pmol/L + 1.800 mL diluent = 200 pmol/L
   b/ 1.00 mL standard 200 pmol/L + 1.00 mL diluent = 100 pmol/L
   c/ 1.00 mL standard 100 pmol/L + 1.00 mL diluent = 50 pmol/L
   d/ 1.00 mL standard 50 pmol/L + 1.00 mL diluent = 25 pmol/L
   e/ 1.00 mL standard 25 pmol/L + 1.00 mL diluent = 12.5 pmol/L
   f/ 1.00 mL standard 12.5 pmol/L + 1.00 mL diluent = 6.25 pmol/L
   g/ Standard diluent = 0 pmol/L.

   Store the standard solutions at -18° C or lower if reused.

3. Pipette 100 µL of the standards (0-200 pmol/L), samples and controls in their respective tubes. Pipette 100 µL of the zero-standard in the NSB-tubes.
4. Pipette 500 µL anti-PP (Reagent A) to all tubes except the NSB- and TOT-tubes.
5. Add 500 µL assay buffer (Reagent F) to the NSB-tubes.
6. Vortex-mix and incubate for 20-24 hours at 2-8° C.
7. Pipette 100 µL ^{125}I-PP (Reagent B) to all tubes. The TOT-tubes are sealed and kept aside.
8. Vortex-mix and incubate for 20-24 hours at 2-8° C.
9. Pipette 500 µL double antibody-PEG (Reagent C) to all tubes except the TOT-tubes. Mix this reagent before pipetting.
10. Vortex-mix carefully and incubate for 30-60 minutes at 2-8° C.
11. Centrifuge the tubes for 15 minutes at +4° C (minimum 1700 x g).
12. Decant the supernatants immediately after centrifugation.
13. Count the radioactivity of the precipitates in a gamma counter (counting time: 2-4 minutes).

CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the non-specific binding tubes from the count rates (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 PP-standards. An example of a standard curve is given on page 11.

3. Interpolate the PP concentrations of the samples and controls from the generated standard curve.

4. The standard curve and the calculations of the concentrations in samples and controls can also be done by a computer method.

QUALITY CONTROL

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

1. The found concentrations of the control sera
PP levels 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-PP in this kit will give 10 500 CPM (-5, +20%) at activity reference date (counter efficiency = 80%).

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

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 line for run to run reproducibility.

ASSAY CHARACTERISTICS

Sensitivity
The lowest detectable concentration is 3 pmol/L. The figure corresponds to a decrease in binding of two x SD of the bound radioactivity in the zero-concentration standard.

Accuracy
A mean recovery of 104% (95-113%) was achieved when known amounts of hPP were added to human serum.

Precision
Intra assay variation

Level	Coefficient of variation (%CV)	N
28.8 pmol/L	2.6	10
108.5 pmol/L	1.8	10

Inter assay variation (total variation)

Level	Coefficient of variation (%CV)	N
38.8 pmol/L	2.0	10
99.3 pmol/L	3.5	10

Specificity
The following cross-reactions have been found

Peptide	Cross-reaction
Pancreatic polypeptide, human	100.0 %
Pancreatic polypeptide, bovine	120 %
Gastric inhibitory peptide, porcine	0.02 %
Cholecystokinin 39, porcine	0.02 %
Secretin, porcine	0.02 %
Gastrin 34, human	<0.01 %
Gastrin 17, human	<0.01 %
Glucagon, human, porcine	0.03 %
Insulin, porcine	<0.01 %
ACTH 1-39, porcine	<0.003%
Neuropeptide Y, human	<0.8 %
Peptide YY, human	<1.0 %

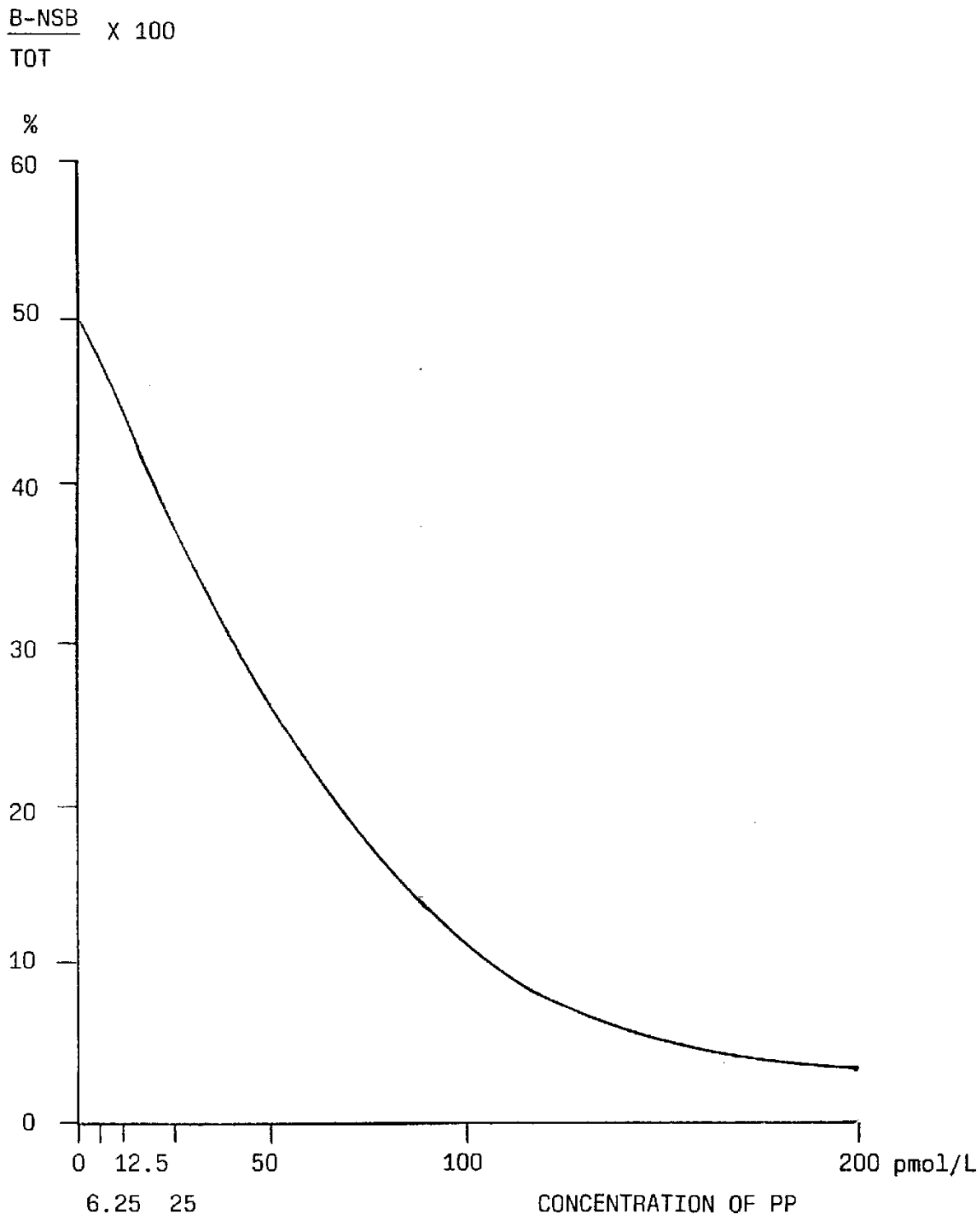
Interference
Samples displaying cloudiness, hemolysis, hyperlipemia or containing fibrin may give inaccurate results.

OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	Anti-PP (A)	Assay buffer (F)		^{125}I-PP (B)		Double antibody-PEG (C)	
TOT	1-2	-	-	-	Vortex-mix and incubate for 20-24 hours at 2-8° C.	100 µL	Vortex-mix and incubate for 20-24 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 and count the radioactivity of the precipitates.
NSB	3-4	100 µL	-	500		100 µL		500 µL	
Stand 0	5-6	100 µL	500 µL	-		100 µL		500 µL	
Stand 6.25	7-8	100 µL	500 µL	-		100 µL		500 µL	
Stand 12.5	9-10	100 µL	500 µL	-		100 µL		500 µL	
Stand 25	11-12	100 µL	500 µL	-		100 µL		500 µL	
Stand 50	13-14	100 µL	500 µL	-		100 µL		500 µL	
Stand 100	15-16	100 µL	500 µL	-		100 µL		500 µL	
Stand 200	17-18	100 µL	500 µL	-		100 µL		500 µL	
Control (G)	19-20	100 µL	500 µL	-		100 µL		500 µL	
Control (H)	21-22	100 µL	500 µL	-		100 µL		500 µL	
Sample 1	23-24	100 µL	500 µL	-		100 µL		500 µL	
Sample 2 etc.	25-26	100 µL	500 µL	-		100 µL		500 µL	

Table 1

EXAMPLE OF PP STANDARD CURVE



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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-PP.
REAG B Ag ^{125}I	^{125}I-PP.
REAG C DAB	Double antibody-PEG.
REAG D DIL CAL	Standard diluent.
REAG E CAL 2000	PP standard 2000 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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