Pancreatic Polypeptide RIA

For the quantitative determination of pancreatic polypeptide (PP) in serum

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 38-PPTHU-R100
Size: 100 Determinations
Version: 230123– ALPCO 4.1

1. Intended Use

The Pancreatic Polypeptide RIA is a radioimmunoassay for the quantitative determination of pancreatic polypeptide (PP) in human serum. For research use only. Not for use in diagnostic procedures.

2. Introduction

Pancreatic polypeptide (PP) is synthesized as an amino-terminal moiety of a precursor peptide. PP isolated from the 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, though detectable levels within the 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 show the intact C-terminal tyrosine amide is required for biological activity.

3. Principle of the Assay

The intended use of these reagents is for the assay of PP in human serum. PP in serum is assayed without extraction by a competitive radioimmunoassay using 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-polyethylene glycol (PEG) precipitation technique. The radioactivity of the precipitates is measured. Human, synthetic PP is used for standardization.

4. Reagents Provided

Reagent	Quantity	Color Code	Reconstitution
Antiserum: Rabbit antiserum raised against bovine PP. Contains phosphate buffer with human serum albumin and NaN_3.	1 vial lyophilized	Blue	Add 52 mL distilled water
Tracer: ^{125}Iodine labeled PP in phosphate buffer with human serum albumin and NaN_3. Contains normal rabbit serum.	1 vial lyophilized 28 kBq	Red	Add 12.5 mL distilled water
Double Antibody-PEG: Goat anti-rabbit Ig antiserum in phosphate buffer with human serum albumin and sodium azide (<0.1%). Contains polyethylene glycol.	1 vial 50 mL	Green	Ready for use
Calibrator diluent: PP-free human serum lyophilized. Contains aprotinin. For preparation of PP-working calibrators.	1 vial Lyophilized	Black	Add 10 mL distilled water
Assay Buffer: Phosphate buffer containing human serum albumin and sodium azide (<0.1%). To be used instead of antiserum in the non-specific binding tubes.	1 vial 5 mL	Black	Ready for use.
PP calibrator: In phosphate buffer containing human serum albumin and sodium azide (<0.1%).	1 vial lyophilized	Yellow	Reconstitute with distilled water by the volume stated on vial label.

Controls: Lyophilized controls with two different levels of PP.	2 vials lyophilized	Silver	Add 1 mL distilled water

5. Supplies Required but Not Provided

- Distilled water
- 11-13 x 55 mm disposable tubes, polystyrene
- Pipettes with disposable tips: 100 and 500 µL
- Pipettes: 1 mL, 5 mL, and 10 mL
- Measuring cylinders: 25 mL and 50 mL
- Vortex mixer
- Centrifuge, refrigerated, minimum of 1700 x g
- Gamma counter
- Timer

6. Reagent Preparation and Storage

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

b. ^{125}I-PP
Reconstitute with 12.5 mL distilled water. 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. PP-calibrator, 2000 pmol/L
Reconstitute with distilled water by the volume stated on the 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 mL distilled water. Store at -18°C or lower if reused.

7. Storage and Expiration Dating of the 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 vial labels. For lyophilized reagents the expiry dates are valid only for the lyophilized (un-reconstituted) reagents. Reconstituted reagents are stable for 10 weeks (no longer than the expiry date) when stored as directed.

8. Sample Collection

Subjects should fast 10 hours prior to sample collection. Collect venous blood in tubes without additives. Allow the sample to clot. Separate the serum 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.

9. Procedure

Handling Notes
Reconstitute the reagents as specified. Reagents should be brought to room temperature prior to use. Accuracy in all pipetting steps is essential. All testing (calibrators, controls, and samples) should be performed in duplicate.

A complete assay includes:

- **Calibrators:** 7 different concentrations; 0, 6.25, 12.5, 25.0, 50.0, 100 and 200 pmol/L
- **Controls**
- **Samples**
- Tubes for determination of the **non-specific binding (NSB-tubes)**
- Tubes for determination of the **total radioactivity** added **(TOT-tubes)**

Assay Procedure

1. Reconstitute the reagents according to the instructions.

2. Prepare the PP-working standards by diluting the PP-calibrator (2000 pmol/L) with the calibrator diluent according to the following:

a) 0.2 mL calibrator 2000 pmol/L + 1.8 mL diluent = 200 pmol/L
b) 1 mL calibrator 200 pmol/L + 1 mL diluent = 100 pmol/L
c) 1 mL calibrator 100 pmol/L + 1 mL diluent = 50 pmol/L
d) 1 mL calibrator 50 pmol/L + 1 mL diluent = 25 pmol/L
e) 1 mL calibrator 25 pmol/L + 1 mL diluent = 12.5 pmol/L
f) 1 mL calibrator 12.5 pmol/L + 1 mL diluent = 6.25 pmol/L
g) Calibrator diluent = 0 pmol/L, used as the zero calibrator.

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

3. Pipette 100 µL of the calibrators (0-200 pmol/L), samples, and controls into their respective tubes. Pipette 100 µL of the zero-calibrator into the NSB-tubes.

4. Pipette 500 µL antiserum into all tubes except the NSB-tubes and TOT-tubes.

5. Add 500 µL assay buffer to the NSB-tubes.

6. Vortex-mix and incubate for 20-24 hours at 2-8°C.

7. Pipette 100 µL ^{125}I-PP into 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 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).

10. Calculation of Results

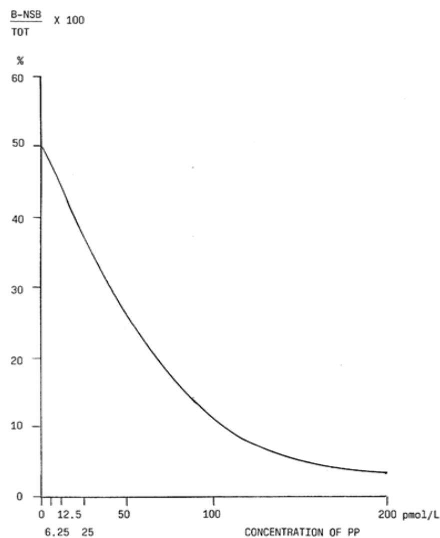
1. Subtract the average count rate (CPM) of the non-specific binding tubes from the count rates (CPM) of the replicates of calibrator, controls, and samples.

2. A calibration curve is generated by plotting the precipitated CPM, bound fraction in CPM or %B/TOT against the concentrations of the PP-calibrators.

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

4. The calibration curve and the calculations of the concentrations in samples and controls can also be done with an appropriate computer method.

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



11. Performance and Limitations

a. Analytical Sensitivity
The lowest detectable concentration is 5 pmol/L. The figure corresponds to a decrease in binding of two x SD of the bound radioactivity in the zero-concentration calibrator.

b. Precision
Intra-assay variation

Level	Coefficient of variation (%CV)	N

12.9 pmol/L	3.2%	16
62.2 pmol/L	4.3%	18

Inter-assay variation (total variation)

Level	Coefficient of variation (%CV)	N
12.5 pmol/L	16.4%	10
66.3 pmol/L	4.2%	10

c. Accuracy

A mean recovery of 98% was achieved when known amounts of hPP were added to human serum.

	Quantity Added	Observed Value	Theoretical Value	% Recovery
Sample 1		39.9		
Sample 1 + (10 pmol/L)	10	55.5	49.9	111 %
Sample 1 + (20 pmol/L)	20	62.4	59.9	104 %
Sample 1 + (50 pmol/L)	50	95.0	89.9	106 %
Sample 1 + (100 pmol/L)	100	129.7	139.9	93 %
Sample 2		37.8		
Sample 2 + (10 pmol/L)	10	42.9	47.8	90 %
Sample 2 + (20 pmol/L)	20	44.1	57.8	76 %
Sample 2 + (50 pmol/L)	50	91.3	87.8	104 %
Sample 2 + (100 pmol/L)	100	137.7	137.8	100 %

d. Analytical Specificity

The following cross-reactivity has been found:

Peptide	Cross-reactivity
Pancreatic polypeptide, human	100%
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%

e. Interference

Samples displaying cloudiness, hemolysis, gross lipemia or containing fibrin may give inaccurate results.

f. Dilution

Sera with high analyte concentrations were tested at different dilutions. Samples were diluted with diluent buffer.

Dilution	Expected (pmol/L)	Measured (pmol/L)	% Recovery
Serum 1	41.3		
1/2	20.6	19.5	94 %
1/4	10.3	9.9	96 %
Serum 2	144.7		
1/2	72.3	71.4	99 %
1/4	36.2	37.6	104 %
1/8	18.1	19.7	109 %

12. Quality Control

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

1. **Controls**
   The concentrations of the controls should fall 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 the activity reference date (counting efficiency = 80%).

3. **Maximum binding (B_0/TOT)**
   Calculate for each assay the % bound radioactivity in the zero-calibrator: $\frac{B_0}{TOT} \times 100$

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 calibration curve**
   For example, monitor the 80, 50, and 20% points of the calibration curve for run to run reproducibility.

13. Precautions and Warnings

Safety

- This kit is for Research Use Only. It is 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 is familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this assay.

- 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) radiation. 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. If the reagents are disposed of in a sink, 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.

14. Summary of the Protocol

<table>
<tr><th></th><th>Total count</th><th>NSB</th><th>Calibrators (0-6)</th><th>Controls</th><th>Samples</th></tr>
<tr><td>Calibrator 0 (=calibrator diluent)</td><td></td><td>100 µL</td><td></td><td></td><td></td></tr>
<tr><td>Calibrators</td><td></td><td></td><td>100 µL</td><td></td><td></td></tr>
<tr><td>Controls</td><td></td><td></td><td></td><td>100 µL</td><td></td></tr>
<tr><td>Samples</td><td></td><td></td><td></td><td></td><td>100 µL</td></tr>
<tr><td>Antiserum</td><td></td><td></td><td colspan="3">500 µL</td></tr>
<tr><td>Assay Buffer</td><td></td><td>500 µL</td><td></td><td></td><td></td></tr>
<tr><td colspan="6">Vortex and incubate for 20-24 hours at 2-8°C</td></tr>
<tr><td>^{125}I Tracer</td><td colspan="5">100 µL</td></tr>
<tr><td colspan="6">Vortex and incubate for 20-24 hours at 2-8°C</td></tr>
<tr><td>Double Antibody-PEG</td><td></td><td colspan="4">500 µL</td></tr>
<tr><td colspan="6">Vortex and incubate for 30-60 minutes at 2-8°C</td></tr>
<tr><td colspan="6">Centrifuge 15 min (1700 x g at 4°C)</td></tr>
<tr><td colspan="6">Decant and count the radioactivity of the precipitates</td></tr>
</table>

References

1. Schwartz, T.W., Gingerich, R.L. and Tager, H.S. Biosynthesis of pancreatic polypeptide: identification of precursor and cosynthesized product. J Biol Chem 225:11494-11498, 1980.
2. Greider, M.H., Gersell, D.J. and Gingerich, R.L. Ultrastructural localization of pancreatic polypeptide in the F cell of the dog pancreas. J Histo Chem Society 26:1103-1108, 1978.
3. Gersell, R.J., Gingerich, R.L. and Greider, M.H. Regional distribution and concentration of pancreatic polypeptide in human and canine pancreas. Diabetes 28:11-15, 1979.
4. Chance, R.E., Moon, N.E. and Johnson, M.C. Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). In B.M. Jaffe and H.R. Behlman (Eds). Methods of hormone radioimmunoassay. Academic Press, New York, 1979, 657-672.
5. Kimmel, J.R., Hayden, L.J. and Pollock, H.G. Isolation and characterization of a new pancreatic polypeptide hormone. J Biol Chem 250:9369-9376, 1975.
6. Adrian, R.E., Bloom, S.R., Bryant, M.G., Polak, J.M., Heitz, P.H. and Barnes, A. Distribution and release of human pancreatic polypeptide. Gut 17:940-944, 1976.
7. Hazelwood, R.L. Synthesis, storage, secretion and significance of pancreatic polypeptide in vertebrates. In S.J. Cooperstien and D. Watkins (Eds). The islets of Langerhans, Academic Press, New York, 1981, p.p. 275-283.
8. Gingerich, R.L., Akpan, J.O., Leith, K.M. and Gilbert, W.R. Patterns of immunoreactive pancreatic polypeptide in human plasma. Regulatory Peptides 33:275-285,1991.
9. Sun, Y.S., Brunicardi, F.C., Duck, P., Walfisch, S., Berlin, S.A., Chance, R.E.,Gingerich, R.L., Elahi, D. and Andersen, D.K. Reversal of abnormal glucose metabolism in chronic pancreatitis by administration of pancreatic polypeptide. Am J Surgery 151:130-140, 1986.
10. Seymour, N.E., Brunicardi, F.C., Chaiken, R.L., Lebovitz, H.E., Chance, R.E.,Gingerich, R.L., Elahi, D. and Andersen, D.K. Reversal of abnormal glucose production after pancreatic resection by pancreatic polypeptide administration in man. Surgery 104:119-129, 1988.