PancrePAP

IMMUNOENZYMATIC KIT FOR ASSAYING HUMAN PAP

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INTRODUCTION

The Pancreatitis Associated Protein (PAP) is synthesized by the exocrine pancreas in the course of acute pancreatitis. It is found in the blood within 12 hours of the onset of the pancreatitis episode. A multicenter study in the adult (Gastroenterology, 1994, 106 : 728-734) has shown that :

- Patients presenting with a normal serum PAP value at admission generally do not develop complications. The range of normal serum PAP concentrations is 0-25ng/ml (median 12.5 ng/ml)
- An increase with time of serum PAP concentration reflects a progression of the disease.
- A decrease indicates that the acute phase of the crisis is ending.

Thus, analysis of the variations of the serum PAP level in a patient provides information on the progression of acute pancreatitis.

ASSAY PRINCIPLE

The PANCREPAP kit is used to assay human PAP in samples of serum, plasma or whole blood dried on blotting paper. Its principle is based on a sandwich immunoenzymatic system.

Microtitration plates are coated with anti-PAP antibodies. In the first step, the samples to assay are deposited in the wells and PAP is allowed to bind to specific antibodies. All proteins not specifically bound are eliminated by washing. Then anti-PAP antibodies coupled to biotin are allowed to attach to the bound PAP. After washing, antigen-antibody complexes are detected by an avidin-peroxidase complex and are visualized by the addition of a chromogenic substrate. The intensity of the color reaction is proportional to the quantity of PAP bound in the first step.

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KIT COMPOSITION

REAGENTS	CHARACTERISTICS
One 96-well microtitration plate coated with anti-PAP antibodies.	Ready to use
Standard solution of recombinant PAP (rhPAP), freeze-dried in a protein buffer.	Reconstitute with the volume of sterile apyrogenic H_2O mentioned on the vial label.
Dilution buffer, freeze-dried in a protein medium.	Reconstitute with 8 ml of sterile apyrogenic H_2O. The 8 ml are then diluted in 42 ml sterile apyrogenic H_2O.
Solution of biotinylated anti-PAP antibodies freeze-dried in a protein medium.	Reconstitute carefully with 11 ml sterile H_2O.
Solution of avidin-POD freeze-dried in a protein medium.	Reconstitute carefully with 11 ml sterile H_2O.
Solution of chromogenic substrate TMB-H_2O_2.	Ready to use
H_2SO_4 solution.	Ready to use
PBS (tablet).	Dissolve the content of the sachet in 1 litre sterile apyrogenic H_2O to obtain 1 litre of PBS.
Solution of Tween 20 (10 %).	Dilute the 10 ml of solution to 1 litre with PBS to obtain the washing buffer.
Control Serum	Reconstitute with the volume of sterile H_2O mentioned on the vial label.

STORAGE AND STABILITY

The assay kit should be kept at 4°C until the printed expiration date. Once lyophilisates are reconstituted, they should be stored at -20°C, except for the avidin-POD solution witch remains stable at 4°C. The wash solution can be stored at -20°C. With these storage conditions, the assay kit can be used for 30 days after opening.

MATERIALS NOT SUPPLIED WITH THE KIT AND NEEDED FOR THE ASSAY

Two litres of distilled water.
Material :
- Vortex mixer.
- A semi-automatic or automatic plate washer
- A plate reader for measuring absorbance at 450 nm, equipped with a 630 nm reference filter
- Disposable tip micropipettes, single- and multi-channel.
- 1L bottle (washing buffer storage)

Disposable material :
- Pipettes 10 ml
- 1.5 ml microcentrifuge tubes (preparation of the range)

PRECAUTIONS FOR USE

Use limited to research.
Do not pipette by mouth.
Do not eat, drink or smoke during the test .
Reagents containing H_2O_2, the H_2SO_4 solution and the PBS tablet may be toxic and irritant. They must be handled to avoid any contact with the skin, eyes and mucosae.
In case of accidental contact, rinse the affected parts immediately with plenty of water.

RECOMMENDATIONS

Never allow the plate to dry since well drying may impair the quality of the results.
Special care should be taken to the washing steps in order to avoid non-specific signal.
Do not use reagents after their date of expiration.
Do not mix reagents from different batches.
Avoid biological and chemical contamination of reagents and samples.
Equilibrate every reagent at room temperature.
Freeze-dried reagents must be reconstituted at least 10 minutes before use in order to obtain total and homogeneous dissolution.
Shake reagents gently before use.
Respect strictly incubation times.

SAMPLE TAKING AND STORING

Blood can be drawn in dry or heparinized tubes. After sampling, serum or plasma can be stored at 2 to 8°C for 24 hours, or frozen at -20°C for longer storage. Do not exceed 3 freeze/thaw cycles. Hemolysed or hyperlipemic samples of plasma or serum may interfere with the PAP assay.

PREPARATION OF REAGENTS

The plate packaged under vacuum must be equilibrated at room temperature before removal from its wrapping..

The wash buffer (PBS/0.1% Tween) is obtained by dissolving the PBS tablet in 1L of distilled water, then adding the whole content of the Tween 20 vial.

The standard range is prepared from freeze-dried rhPAP. This standard solution is reconstituted with the volume of distilled water mentioned on the vial label. This gives a solution at 4 ng/ml that is further diluted in dilution buffer to prepare the standard range.

The dilution buffer is to be reconstituted by adding to the vial 8 ml of sterile apyrogenic H_2O. The 8 ml are then diluted in 42 ml sterile apyrogenic H_2O.

Biotinylated antibodies The lyophilisate is dissolved in 11 ml of distilled water.

Avidin-POD The lyophilisate is dissolved in 11 ml of distilled water.

The substrate-chromogen solution (TMB-H_2O_2) is ready to use.

The Control Serum (internal control) is reconstituted with the volume of distilled water mentioned on the vial label.

ASSAY PROCEDURE

A PAP range is prepared from the reconstituted reference solution (4 ng/ml). It will include solutions with concentrations ranging from 0.25 to 0.015 ng/ml. The dilution buffer provided with the kit will be used to prepare successive dilutions.

PREPARATION OF THE PAP RANGE

Dilute 1/2 the standard PAP solution. That solution (PAP = 2 ng/ml) will be used to prepare serial dilutions with the dilution buffer.

500 µl of solution at 2 ng/ml + 500 µl buffer = 1 ng/ml

500 µl of solution at 1 ng/ml + 500 µl buffer = 0.5 ng/ml

500 µl of solution at 0.5 ng/ml + 500 µl buffer = 0.25 ng/ml

500 µl of solution at 0.25 ng/ml + 500 µl buffer = 0.125 ng/ml

500 µl of solution at 0.125 ng/ml + 500 µl buffer = 0.06 ng/ml

500 µl of solution at 0.06ng/ml + 500 µl buffer = 0.03 ng/ml

500 µl of solution at 0.03 ng/ml + 500 µl buffer = 0.015 ng/ml

Each dilution will be assayed in duplicate (100 µl/puits). Background value will be obtained in duplicate (100 µl buffer/well)
Samples, the PAP range and the control sample are deposited in wells (100 µl/well) and incubated 3 h at room temperature (~21°C), after covering the plate with adhesive. Then, wells are washed 5 times with the wash buffer, as follows:

- Thoroughly draw up the wells
- Fill with ~300 µl wash buffer
- Repeat the first two steps 4 times
- After the last wash, eliminate residual liquid by inverting the plate and tapping it on absorbent paper.

Note: it is recommended to use an automatic or semiautomatic plate washer.

The reconstituted solution of biotinylated anti-PAP antibodies is then immediately deposited on the plate at 100 µl/well and incubated for 30 minutes at room temperature. The plate is covered with adhesive. The plate is then washed 5 times with PBS/0.1 % Tween (as described above) and then 100 µl of the reconstituted avidin-POD solution is immediately added to each well.

After 15 minutes incubation at room temperature, the plate is washed 5 times with PBS/0.1% Tween. During the last wash, washing buffer should be left 1 min in the wells. After the plate has been tapped dry, the chromogenic substrate is added at 100 µl/well. After 10 to 15 minutes of incubation in the dark, the color reaction is stopped by adding H_2SO_4, 100 µl/well. The absorbance of each well is read on a spectrophotometric plate reader at 450 nm, using a 630 nm filter as reference. **Plates should be read immediately after the reaction is stopped**

CALCULATION OF RESULTS

The concentration of PAP in each sample is determined by extrapolation from the standard curve. This curve is constructed by plotting the mean absorbance values of each point of the range versus the theoretical concentrations. The use of a computer programme to define, from range values, the parameters of the [PAP] = f(D.O.450nm) function, and use that function to calculate PAP concentrations in eluates is recommended.

EXAMPLE OF RESULTS

NB : This is only an example. A reference curve must be obtained for each plate.

PAP ng/ml	Absorbance (D.O. 450 nm)	Absorbance (- background)
0	0.039	
0.015	0.192	0.153
0.03	0.346	0.307
0.06	0.647	0.608
0.125	1.191	1.152
0.25	1.925	1.886

CHARACTERISTICS OF THE TEST

1/ Detection limit
The detection limit is estimated at 15 pg/ml, a PAP concentration corresponding to the mean absorbance of 20 measurements of the zero of the range plus two times the standard deviation.

2/ Specificity
Absence of cross-reaction with IL2, IL6, IFNγ, TNFα and E.Coli proteins.
Absence of hook effect up to a concentration of 30 ng/ml.

SUMMARY OF THE ASSAY

1 Prepare buffers and the PAP range
2 After equilibration at room temperature, open the aluminium bag and deposit the samples and the range in the plate (100 µl/well) Incubate 3h at room temperature
3 Draw up liquid, 5 washes
4 Distribute the biotinylated antibody (100 µl/well)
5 Incubate 30 min at room temperature
6 Draw up liquid, 5 washes
8 Distribute avidin-POD (100 µl/well)
9 Incubate 15 min at room temperature
10 Draw up liquid, 5 washes. Dry.
11 Distribute the substrate-chromogen mix (100 µl/well).
12 Incubate 10-15 min in the dark
13 Stop the reaction with H_2SO_4 (100 µl/well).
14 Read absorbance at 450 nm.