Diagnostic Reagents Ltd

PCA Ratio Kit

PCA Ratio in Factor V Depleted Plasma

Catalogue Number: APCV380

For *in vitro* diagnostic use only.

Intended Use

For the determination of resistance to activated Protein C, due to the Factor V Leiden mutation (FV:Q^{506}). The test is designed for use on plasma from untreated individuals or patients on oral anticoagulant therapy.

Summary and Principle

Activated Protein C (APC) is a serine protease, an important anticoagulant enzyme that is required for the regulation of blood coagulation. APC acts by inactivating pro-coagulant factors Va and VIIIa. Normally, factor Va is inactivated by an initial cleavage of the peptide bond on the carboxyl side of arginine 506 followed by a second cleavage at arginine 306. In patients with the The (FV:Q^{506}) mutation, the 506 cleavage is inhibited causing a much slower cleavage rate leading to the phenomenon of resistance to the anticoagulant activity of APC.

APC resistance can be measured by the APC ratio of an APTT with the addition of preformed APC divided by the ordinary APTT[(1)], where ratios above 2.2 indicate normality, and ratios below 2.2 indicate the Factor V Leiden mutation. Protein C (PC) in the presence of Protein S (PS) may be activated to APC *endogenously* by Protein C activator (PCA) contained in a venom fraction from the snake ***Agkistrodon contortrix contortrix***. The PCA ratio is similarly obtained by dividing the PCA.APTT by the ordinary APTT [(2)]. When the patient sample is diluted in Factor V depleted plasma, vitamin K dependent factors, intrinsic factors, PC and PS are all normalised. Thus PCA may be used instead of APC, and the PCA ratio becomes a definitive test for APC resistance [(3, 4)] because APC is generated normally by *endogenous* PC and PS in the factor V depleted plasma. In the test, plasma is diluted 1/5 in factor V depleted plasma. This is then incubated with an APTT reagent with and without added PCA and the clotting times determined by the addition of $CaCl_2$. The clotting time ratio of PCA.APTT/ordinary APTT is then determined.

Reagents

1. **Factor V depleted plasma** 4 vials
A lyophilised, buffered human plasma with a factor V activity depleted to less than 0.5%. Reconstitute with 2.0 mL of distilled water, swirl gently and allow 5 – 10 minutes for complete solution. Once reconstituted, the product is stable for 8 hours when held at 2 – 8 °C. The plasma may be frozen at -20°C and thawed once at 37°C.

2. **APTT reagent** 2 vials
A phospholipid based platelet substitute, with micronised silica used as a contact activator. Reconstitute with 2.0 mL of distilled water, allow 5 – 10 minutes for complete solution. Once reconstituted, the reagent is stable for 2 weeks when held at 2 - 8°C.

3. **PCA.APTT reagent** 2 vials
A phospholipid based platelet substitute, with micronised silica used as a contact activator and a venom fraction of ***Agkistrodon contortrix contortrix*** added as a protein C activator. Reconstitute with 2.0 mL of distilled water, allow 5 – 10 minutes for complete solution. Once reconstituted, the reagent is stable for 2 weeks when held at 2 - 8°C.

4. **APC resistant control plasma** 1 vial
A lyophilised, buffered human plasma positive for the FV:Q^{506} Leiden mutation. Reconstitute with 0.5 mL of distilled water, swirl gently and allow 5 – 10 minutes for complete solution.

Once reconstituted, the product is stable for 8 hours when held at 2 – 8 °C. The plasma may be frozen at -20°C and thawed once at 37°C.

5. **Calcium chloride in saline** 2 vials
8.0 ml of 25 mM Calcium chloride in saline. Supplied ready for use.

Warnings and precautions

POTENTIAL BIOHAZARD MATERIAL. The APC Resistant Control and Factor V depleted plasmas are of human origin. All donor units used in production of these products have been found negative for anti HIV, anti HCV, HBsAg and Syphilis by approved methods. However, all plasma of human origin should be considered as potentially infectious and handled appropriately. **Please refer to Human Plasma MSDS Sheets (provided on request) for handling and safety procedures.** Dispose of all waste materials according to the stated international, national and local regulations.

Collection of Blood Samples

Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep frozen plasma.

Procedure

Materials Provided

Materials provided with these instructions are:

Cat. No.

APCV380 – PCA Ratio Kit with Factor V depleted plasma.

Materials and equipment required, but not provided:

1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipettes delivering: 20 µL, 80 µL, 100 µL, 0.5 mL & 2.0 mL.
4. Distilled water.

Procedure

Technique (Manual)

1. Prepare duplicate tubes containing 20 µL of test sample (or control plasma) and 80 µL of factor V depleted plasma.
2. To one tube, add 100 µL of APTT reagent and to the duplicate tube 100 µL of PCA.APTT reagent.
3. Incubate both tubes for 5 min at 37°C.
4. Add 100 µL of 25 mM $CaCl_2$ / saline to both tubes and record the clotting times.
5. Calculate the PCA.APTT/APTT clotting time ratio (PCA ratio).

Notes

1. It is important to use the Calcium chloride provided, which contains saline.
2. The APTT and PCA.APTT reagents are not interchangeable between lots.
3. Our freeze dried APC resistant control plasma can be used as a control and day to day QC.

Automated Method

The method may be used with the Sysmex CA instruments, the ACL series, the MDA, Amelung, Amax & Amga (usually in the intrinsic factor mode using 10 µL of sample and 40 µL factor V depleted plasma). Whilst using the above instruments and other variations, the manufacturer's protocol should be followed at all times. Clotting times of normal and Factor V Leiden samples may vary slightly with different instruments (see calibration).

Quality Control

The APC resistant control plasma (APCC400) included in the kit should be used as a reference control to validate the assay. It is positive for the FV:Q^{506} (Leiden) mutation and should give a ratio in the defined range for APC resistant samples, which in our hands is less than 2.2. If the value falls outside the defined range for APC resistant plasma, the test system requires investigation.

Calculation of results

$$\frac{\text{PCA.APTT}}{\text{APTT}} = \text{PCA Ratio}$$

Interpretation

In a study by the tilt-tube manual method, the PCA ratio was shown to have a much greater discrimination between normal plasma samples, factor V Leiden samples and samples from patients on oral anticoagulant therapy than the method employing exogenous

APC[2]. Similar results were obtained in the study by Morse and Standen[5] and in the MDA evaluation study[6]. The method can be used for patients on oral anticoagulant therapy, in patients with high factor VIII levels (during pregnancy); in stored samples with low factor VIII levels and in patients with PC and PS defects. It will also dilute out the effect of lupus anticoagulants and heparin. The main advantage of PCA over preformed APC is the much greater discrimination observed between normal and APC resistant samples (see Figure 1). PCA is also much more stable than APC.
We cannot guarantee results with factor V depleted plasma from Chromogenix or other sources.

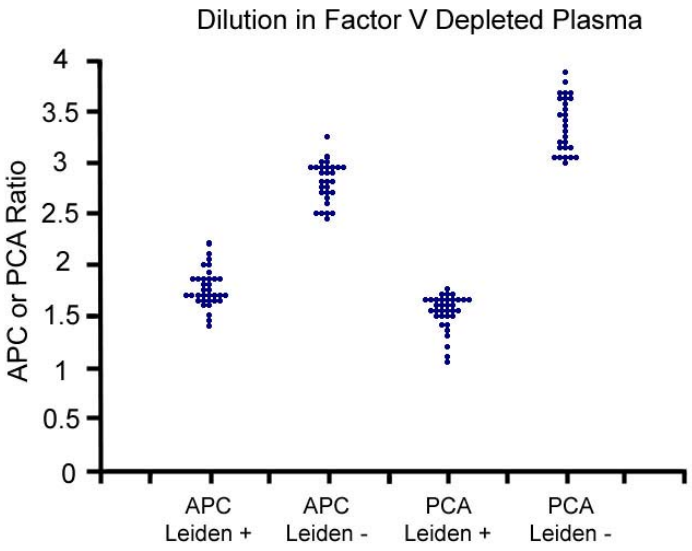

Figure 1. Graph showing the superior discrimination between normal and APC resistant samples, using the PCA ratio rather than the APC ratio method, when diluted in factor V depleted plasma.

Calibration

In our experience, with the manual method, **a ratio of below 2.2 indicates the presence of the factor V Leiden mutation (FV:Q^{506}).** It is however recommended that each laboratory perform its own calibration, establishing instrument performance characteristics with defined normal & abnormal ranges. Because of the superior discrimination of the PCA method over the APC method, our original study correctly identified the factor V Leiden positive samples in 100% of cases, with the highest PCA ratio being 1.8 (n=26). In normal plasma samples and warfarinised patient samples not exhibiting the factor V Leiden mutation (n=40), the lowest PCA ratio obtained was 2.8.

Subsequent much larger studies have substantiated our original results with the highest PCA ratio for a confirmed factor V Leiden positive sample being below 2.2

Studies on the Sysmex CA1500, CA6000, CA7000, ACL TOP, ACL Futura & MDA180/18011 have given very similar results and ranges to the manual method, with equally good discrimination between Leiden positive and Leiden negative plasma samples.

Limitations and Interferences

If testing patients on Heparin, unfractionated or low molecular weight, the addition of a neutralizing agent such as Polybrene or Protamine sulphate will allow the test to be performed accurately without interference.

Acquired APC resistance has been described in several hypercoagulable conditions. In the main, any effect is normalised by the dilution in factor V depleted plasma. However, occasional equivocal results have been described. In these cases it may be beneficial to test the samples neat and 1/10 in factor V depleted plasma to obtain more information.

Storage

The unopened kit may be stored for up to 3 years at 2 - 8°C without deterioration.

Packaging

Contents of kit (40 manual, 80 automated tests)

1) 4 x 2.0 mL of Factor V depleted plasma.
2) 2 x 2.0 mL of APTT reagent.
3) 2 x 2.0 mL of PCA.APTT reagent.
4) 1 x 0.5 mL of APC resistant plasma.
5) 2 x 8.0 mL $CaCl_2$ **in saline.**

References

1) Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognised mechanism characterised by a poor anticoagulant response to activated Protein C.
Proc Natl Acad Sci 1993; 90: 1104-08.
2) Denson KWE, Reed SV, Haddon ME, Davidson S, Littlewood TJ. A more discriminating test for APC resistance and a possible screening test to include Protein C and Protein S. Thromb Res 1996; 81: 151-156.
3) Jorquera JL, Montoro JM, Fernandez MA, Aznar J. Modified test for activated Protein C resistance. Lancet 1994; 344: 1162-3.
4) Denson KWE, Reed SV, Haddon ME. The modified APC resistance test. Thromb Haemost 1995; 74: 995.
5) Morse C, Standen G. Specificity of clotting tests for factor V Leiden. Brit J Haemat 1996;95:432.
6) Gardiner C, Mackie IJ, Machin SJ, Cooper P, Malia RG, Makris M. 1999. MDA Evaluation Report No. 00064 HMSO.

Key guide to symbols

REF	Manufacturers catalogue number.		Consult instructions for use.
LOT	Manufacturers batch number.	Recon.	Requires reconstitution.
IVD	For *in vitro* diagnostic use only.		Product expiry date.
	Biological risks.		Keep refridgerated between 2-8°C.

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