Diagnostic Reagents Limited

UK CA

PCA Ratio Kit

(screening test for APC resistance)

Catalogue Number: APCR390

For *in vitro* diagnostic use only.

Intended Use

Diagen PCA Ratio Kit provides a plasma screening test for resistance to activated Protein C, due to the Factor V Leiden mutation (FV:Q^{506}).

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

In the Activated Protein C (APC) resistance test, an APTT is performed with and without added **exogenous** preformed APC and the ratio of APC-APTT/Ordinary APTT obtained. Ratios below 2.0 indicate an APC cofactor defect. Exogenous APC by-passes both Protein C (PC) and Protein S (PS) and will only detect the APC cofactor. PC in the presence of PS may be activated to APC by PC activators (PCA) either by thrombin/thrombomodulin or by venom fractions from various snakes including ***Agkistrodon contortrix contortrix***. When PCA is added to plasma, the active inhibitor which destroys factors Va and VIIIa, is generated by **endogenous** PC, PS and APC cofactor. PC and PS assays are performed as part of a routine thrombophilia screen and thus **in the presence of normal PC and PS levels** it becomes unnecessary to use preformed APC and the addition of PCA to plasma will detect APC resistance.

Reagents

1. **APTT reagent** 3 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.

2. **PCA.APTT reagent** 3 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.

3. **APC resistant control plasma** 1 vial

A lyophilised, buffered human plasma positive for the FV:Q^{506} type 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.

4. **Calcium chloride in saline** 2 vials

9.0 mL of 25 mM Calcium chloride in saline.

Warnings and precautions

POTENTIAL BIOHAZARD MATERIAL. The APC Resistant Control plasma is of human origin. All donor units used in production of this product 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 the PCA Ratio Kit and APC Resistant

Control Plasma SDS 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 needed for Activated Protein C resistance screening are shown below:

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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: 100 µL, 0.5 mL & 2.0 mL.
4. Distilled water.

Procedure

Technique (Manual)

1) To duplicate **prewarmed** tubes or cuvettes at 37°C, add 100 µL of test (or control) sample.
2) To one tube add 100 µL of APTT reagent and to the duplicate tube 100 µL of PCA.APTT reagent.
3) Incubate both for 5 min. at 37°C.
4) Add 100 µL of 25 mM $CaCl_2$ / saline to both tubes and record the clotting time.
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. Omission of saline results in long normal clotting times for the PCA reagent.

2) Our freeze-dried APC resistant control plasma can be used as a control and for day-to-day QC.

Automated Method

The method may be used with the Sysmex CA range of instruments, the ACL range of Instruments, the MDA and the Amelung Amax and Amga instruments in the APTT mode. 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).

Calculation of results

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

Interpretation

The PCA ratio has been compared with the APC resistance test of Chromogenix using 74 normal samples, 107 samples from patients for thrombophilia screen, 32 samples with the APC cofactor defect and 32 samples from patients with PS and PC defects. The method gives better discrimination than the APC method, between normal samples and those with the factor V Leiden defect [1, 2] (see Figure 1). In general, the PCA ratios are higher with normal samples and lower for APC resistant samples compared with the APC method. All factor V Leiden samples gave ratios of <2.2, and in the presence of normal PC and PS levels, ratios of <2.2 indicate APC resistance. All Protein C defects, and most Protein S defects were also detected. The ratio cut-off point between normal and abnormal should be determined locally according to instrument and methodology and especially centrifugation of blood samples. In our experience, ratios <2.2 will indicate APC resistance and ratios above 3.6 will exclude APC resistance, PC and most PS defects, If in doubt PC and PS assays should be performed. The method should also serve as a simple global screening test to identify possible 'at risk' patients prior to contraceptive pill use, in obstetrical practice, or prior to surgery. It may also serve to discover any other possible factors or co-factors which may be involved in the endogenous formation of the APC inhibitor, and **which may not be revealed by the addition of preformed APC.** For APC resistance we recommend the parallel use of our definitive test with dilution of the samples in factor V depleted plasma. Any abnormality other

than Factor V Leiden, which may result in APC resistance, should give an abnormal result in the screening test and a normal result in the dilution test. **Factor V depleted plasma from Chromogenix is not suitable for our PCA ratio test, and we cannot guarantee correct results with this, or any other source of factor V depleted plasma.**

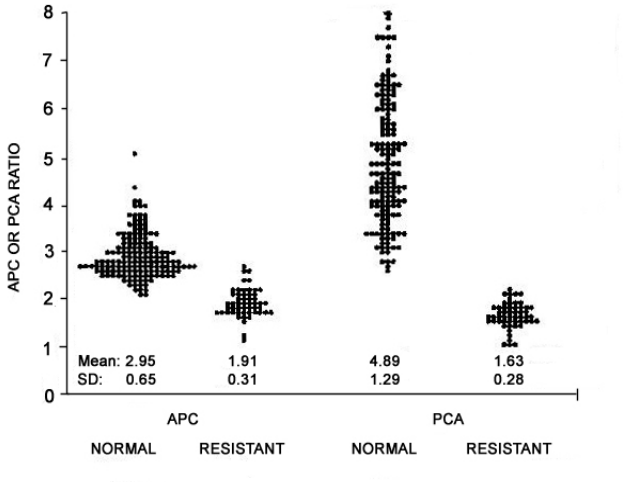

Figure 1. Graph showing the superior discrimination between normal and APC resistant samples using the PCA ratio rather than the APC ratio method.

Calibration

In our experience, with the manual method, **a ratio of below 2.2 with normal levels of PC & PS suggests 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, in particular, to define ratios above which may be useful to help identify possible PC & PS deficiency.

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.

Quality Control

The APC resistant control plasma (APCC400) provided 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.

Limitations

A prolonged APTT would suggest the possibility of coagulation factor deficiency, phospholipid antibodies such as Lupus anticoagulant or the presence of heparin (see below). For factor deficiency (or patients receiving oral anticoagulant therapy – OAT) and phospholipid antibodies, it is recommended that the test be repeated with a 1/5 dilution of the sample plasma in factor V depleted plasma.

If testing patients anticoagulated with 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.

Acquired APC resistance has been described in several hypercoagulable conditions. In the main, any effect is normalised by 1/5 dilution in factor V depleted plasma. However, occasional equivocal results have been described. In these cases, it may be beneficial to test the samples both neat and 1/10 in factor V depleted plasma to obtain more information.

Storage and stability

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

Packaging

Contents of Kit (60 manual tests, 120 automated tests)

1) 3 x 2.0 mL of APTT reagent.
2) 3 x 2.0 mL of PCA APTT reagent.
3) 1 x 0.5 mL of APC resistant plasma.
4) 2 x 9.0 mL of 25 mM $CaCl_2$ **in saline.**

References

1) 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.
2) Morse C, Standen G. Specificity of clotting tests for Factor V Leiden. Brit J Haemat 1996;95:432.

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.	+8°C +2°C	Store refrigerated between 2 - 8°C.

Manufacturer.

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