COATEST® APC™ Resistance V S - 82 3138 63

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Intended use

For determination of resistance to activated protein C (APC), caused by the factor V:Q ^{506} (factor V Leiden) mutation, in plasma from untreated individuals and from patients on oral anticoagulant (OAC) or heparin therapy.

Background and summary

The APC resistance phenotype [1,2] is in more than 90% of the cases due to a mutation in the factor V gene, resulting in a replacement of Arg ^{506} (R) with Gln (Q) in the factor V protein [3-5]. The selectivity for the factor V:Q ^{506}, or other mutations in the factor V gene rendering the protein resistant to inactivation by APC [6] , is increased by normalizing the concentrations of other plasma proteins involved in formation and regulation of thrombin. Hence, by performing the APTT-based APC resistance assay in the presence of an excess of factor V deficient plasma, the sensitivity and specificity for the factor V:Q ^{506} mutation is significantly increased. Further, this modification allows for the analysis of plasma from patients who are on OAC therapy [7-11].

Measurement principle

Sample plasma is prediluted in V-DEF Plasma and incubated with the APTT reagent for a standard period of time. Coagulation is triggered by the addition of $CaCl_2$ in the absence and presence of APC and the time for clot formation is recorded.

REAGENTS

1. **V-DEF Plasma** 2 vials
   Stabilized, lyophilized human plasma, with a low level of factor V activity, containing the heparin antagonist Polybrene®. Reconstitute with 4.0 mL of NCCLS type II water [12]. Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.
2. **$CaCl_2$** 2 vials
   2 mL of calcium chloride, 0.025 mol/L, in Tris buffer containing 0.5% bovine serum albumin.
3. **APTT reagent** 2 vials
   4 mL of purified phospholipids with colloidal silica as contact activator. Contains a preservative. Mix thoroughly on a Vortex mixer before use.
4. **APC/$CaCl_2$** 2 vials
   Human activated protein C colyophilized with $CaCl_2$. Reconstitute with 2.0 mL of NCCLS type II water [12]. Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.
5. **Control Plasma Level 1** 1 vial
   Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water [12]. Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.
6. **Control Plasma Level 2** 1 vial
   Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water [12]. Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

Reagents 3 and 4 are not interchangeable between lots.

V-DEF Plasma (Art. No 82 3146 63), **Control Plasma Level 1** (Art. No 82 2650 63) and **Control Plasma Level 2** (Art. No 82 2668 63) are also available separately from Chromogenix.

CAUTION: Each donor unit used in the preparation of human source reagent has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borne diseases, the handling and disposal of human source reagents from this product should be made with care.

Avoid contact with skin and eyes (S24/25).
Do not empty into drains (S29).
Wear suitable protective clothing (S36).

This product is for *in vitro* diagnostic use.

Materials required but not provided:

— Deionized water, filtered through 0.22 µm or NCCLS type II water[12]
— Calibrated pipettes.
— Automated or semi-automated coagulation instruments which, employ mechanical or optical detection, methods should be used.

NOTE: When using automated or semi-automated instruments, always refer to the operator manual from the instrument manufacturer for exact procedures.

Storage conditions and stability

The sealed reagents are stable at 2-8°C until the expiry date printed on the label. Avoid contamination of the reagents by microorganisms.

1. **V-DEF Plasma**
   Stability after reconstitution is 8 hours at 15-25°C, 24 hours at 2-8°C or 3 months at -20°C or below when stored in the original vial. *See NOTE.
2. **$CaCl_2$**
   Opened reagent in the original vial is stable for 1 week at 15-25°C or 1 month at 2-8°C.
3. **APTT reagent**
   Opened reagent in the original vial is stable for 1 week at 15-25°C or 1 month at 2-8°C. Do not freeze!
4. **APC/$CaCl_2$**
   Stability after reconstitution is 2 hours at 37°C, 8 hours at 15-25°C, 5 days at 2-8°C or 3 months at -20°C or below when stored in the original vial. *See NOTE.
5. **Control Plasma Level 1**
   Stability after reconstitution is 6 hours at 2-25°C or 3 months at -20°C or below when stored in the original vial. *See NOTE.
6. **Control Plasma Level 2**
   Stability after reconstitution is 6 hours at 2-25°C or 3 months at -20°C or below when stored in the original vial. *See NOTE.

*NOTE: Frozen reagent should be rapidly thawed at 37°C and gently mixed before use. Do not refreeze.

Quality controls

Control Plasma Level 1 and Level 2 should be used for validation of the assay series. Level 1 shows a normal response to APC whereas Level 2 shows a response consistent with presence of the factor V:Q ^{506} mutation. Ranges of expected APC-V ratios are provided with each batch. If values outside the specified range are obtained, a complete check of reagents and instrument performance should be made and the analysis should be repeated. (See Calibration section for QC use of Control Plasma Level 1 and 2).

Traceability of calibrators and control materials

The reported values were determined over multiple runs on ACL Futura using a specific lot of reagent and against an internal House Standard. As an International Standard is not still availablefor the APCR-V assay, the values have been assigned against a House Standard which is traceable to frozen plasma samples which have been determined to be homozygous or heterozygous respectively for factor V dependent APC Resistance.

Specimen collection

The patient should be at rest for 10 min. before sampling. Collect blood (9 volumes) in 0.1 mol/L sodium citrate (1 volume) and centrifuge within 24 hours at 2000 x g for 20 min. at room temperature. Take care to avoid contamination from the platelet layer into the plasma when the plasma is separated from the cells. Analyse the plasma within 25 hours from blood sampling [13]. Alternatively, freeze rapidly at -70°C in aliquots of 1 mL or less and store for not more than 3 years at -70°C. Specimens should not be stored in a self defrosting freezer and not be thawed and refrozen before assay. Treat specimens as potentially infectious. For more infor-mation see NCCLS document H21-A3 [14].

Procedure

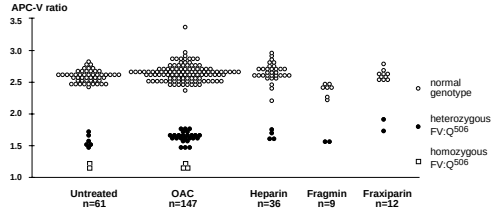
1 All reagents must be brought to room temperature before use. Frozen plasma samples should be rapidly thawed at 37°C in a standardized way ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate.
2 Pre-warm a sufficient volume of $CaCl_2$ and APC/$CaCl_2$ at 37±0.5°C.
3. Pre-dilute one volume of sample plasma or Control Plasma with four volumes of V-DEF Plasma. Pre-diluted plasma should be analyzed within 45 minutes.
4. Add one volume of plasma to a test tube or cuvette, then add an equal volume of the APTT reagent. Incubate at 37°C for 5 minutes. An instrument with a different, preset, incubation time may be used provided it is at least 3 minutes.
5. Add one volume of $CaCl_2$ and simultaneously begin timing of clot formation. Record the time for clot formation.
6. Perform a second analysis on the plasma exchanging $CaCl_2$ with APC/$CaCl_2$ and record the time for clot formation.

Results

Calculate the factor V related APC ratio for the samples and controls:

$$\text{APC ratio} = \frac{\text{Clot time APC/CaCl}_2}{\text{Clot time CaCl}_2}$$

APC resistance due to a factor V mutation is indicated when the APC-V ratio is below or equal to the cut-off value (see Calibration). The following results illustrate the applicability of the Coatest APC Resistance V assay on the analysis of plasma from untreated individuals (Untreated), patients on OAC therapy, INR 1.3 - 6.0 (OAC), and patients receiving unfractionated heparin (Heparin) or low molecular weight heparins (shown here are Fragmin® and Fraxiparin®).



Sample APC-V ratios are distributed according to the factor V genotype. For a given genotype, similar APC-V ratios are obtained regardless of patient group. Typical median values are 2.6 (normal), 1.7 (heterozygous FV:Q ^{506}) and 1.2 (homozygous FV:Q ^{506}). Basal APT times for untreated individuals may differ moderately as compared to the original Coatest APC Resistance assay.

Performance Characteristics

PRECISION

APC-V ratios were calculated from 22 single replicate analyses of Control Plasma Level 1 on an ACL instrument using 11 different reagent combinations on 11 different occasions.

		CV% (Within series)	CV% (Between series)
Control Plasma Level 1	APC-V ratio 2.4	≤4 %	≤4 %

Duplicate analyses of plasma from altogether 630 individuals on three different types of instruments (ST-4, Thrombolyzer and MLA/Electra 900) resulted in within series CV ≤ 3% and ≤ 5% for the clotting times obtained in the absence and presence of APC respectively. NCCLS document EP5-T2[15].

Calibration

APC-V ratios obtained with the Coatest APC Resistance 5 method are lower than the APC ratios obtained with the original Coatest APC Resistance method, independent of instrumentation used. It is recommended that each user establishes the performance of his own instrument and determines the factor V related APC resistance cut-off value through the following procedure:

1. Perform five independent determinations of the APC-V ratio, using at least triplicates in each series, of a plasma sample with normal APC response. Confirm that the inter and intra assay variation of the APC-V ratio is below 7%. In case a satisfactory performance already has been established with the original Coatest APC Resistance method, this step may be omitted.
2. Determine the APC-V ratios for at least 30 plasma samples from healthy individuals in the age range 20 - 65 years. Include Control Plasma Level 1 and Level 2 for assay validation.
3. Verify that the APC-V ratios for the Control Plasmas are within their specified ranges.
4. Calculate the median APC-V ratio.
5. Calculate the factor V related APC resistance cut-off value as 0.8 times the median APC-V ratio when below 2.8 and as 0.75 times the median APC-V ratio when 2.8 or higher.
6. The APC-V ratio for Control Plasma Level 1 should be within the normal range. The APC-V ratio for Control Plasma Level 2 should be below the cut-off value.

Sensitivity

Coatest APC Resistance V provides 100% sensitivity for FV:Q 506 as determined on Thrombolyzer (n = 447), ACL (n = 295), ST-4 (n = 248) and MLA/Electra (n = 50).

Limitation/interfering factors

No significant differences are obtained between fresh and frozen samples.
The sensitivity and specificity for the factor V:Q ^{506} mutation on analysis of plasma from OAC patients is not affected by the INR value. The prescribed assay procedure allows for the analysis of plasma from heparinized patients at heparin levels < 1 IU/mL plasma (unfractionated and low molecular weight heparins). Although the 1+4 predilution strongly decreases interferences, it can not be excluded that analysis of plasma from patients with high inhibitor activity (e.g. phospholipid antibodies) may give an abnormal APT time and thus possibly misleading results. In such cases, increasing the dilution factor (e.g. 1+9 or 1+19) may correct the test result. As for any APTT-based assay, care should be taken to avoid contact activation of samples since this may lead to activation of FVIII and FV.

Reference values

The APC-V ratios obtained from analysis of plasmas from 61 healthy individuals on ACL and ST-4 and from 390 healthy individuals on Thrombolyzer were in the range 2 - 3.5. No difference is found between sexes.

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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

IVD	LOT				CONTROL				EC REP
In vitro diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Control	Biological risks	Manufacturer	Authorised representative
In-vitro Diagnostikum	Chargen-Bezeichnung	Verwendbar bis	Festgelegte Temperatur	Beilage beachten	Kontrollen	Biologisches Risiko	Hergestellt von	Bevollmächtigter
De uso diagnóstico *in vitro*	Identificación número de lote	Caducidad	Temperatura de Almacenamiento	Consultar la metódica	Control	Riesgo biológico	Fabricado por	Representante autorizado
Dispositif mèdical de diagnostic *in vitro*	Désignation du lot	Utilisable jusqu'à	Températures limites de conservation	Lire le mode d'emploi	Contrôle	Risque biologique	Fabricant	Mandataire
Per uso diagnostico *in vitro*	Numero del lotto	Da utilizzare prima del	Limiti di temperatura	Vedere istruzioni per l'uso	Controllo	Rischio biologico	Prodotto da	Rappresentanza autorizzata
Dispositivo médico para utilização em diagnóstico *in vitro*	Número de lote	Data límite de utilização	Límite de temperatura	Consultar as instruções de utilização	Controlo	Risco biológico	Fabricado por	Representante autorizado
"in vitro" diagnostisk udstyr	Batch nr.	Anvendelse	Temperatur begrænsninger	Se vejledning for anvendelse	Kontrol	Miljø oplysninger	Producent	Leverandør
In vitro diagnostisk medicinsk produkt	Tillverkningskod	Användning	Temperatur gräns	Ta del av instruktionen före användning	Kontroll	Biologiska risker	Tillverkare	Auktoriserad representant
Προϊόν για διαγνωστική χρήση *In vitro*	Αρ. Παρτίδας	Χρήση έως	Περιορισμοί θερμοκρασίας	Συμβουλευτήτε τις οδηγίες χρήσης	Υλικό ποιοτικού ελέγχου	Βιολογικοί κίνδυνοι	Κατασκευαστής	Εξουσιοδοτημένος αντιπρόσωπος