COATEST® APC™ Resistance - 82 2643 63

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Intended use

To measure the anticoagulant response in human plasma to activated protein C (APC) in individuals with suspected or verified thromboembolic disease.

Background and summary

APC resistance is a disorder in the protein C anticoagulant pathway, which constitutes an important risk factor for thromboembolic disease. The APC resistance phenotype is characterized by an abnormally low anticoagulant response in human plasma on addition of human APC [1-5]. More than 90% of the cases are due to a mutation in the factor V gene. The mutation renders one of the APC cleavage sites in factor Va less susceptible to cleavage due to an amino acid substitution (Arg ^{506} ->Gln) [6,7].
For remaining cases, other inherited or acquired conditions contribute to the APC resistance phenotype [8-10]. Coatest APC Resistance provides an APTT-based assay for the determination of the response towards human APC [11,12]. The prolongation of the basal APT clotting time after addition of APC is shorter in plasma from subjects with the APC resistance phenotype than from subjects with a normal response to APC.

Measurement principle

Plasma is 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 human APC and the time for clot formation is recorded.

REAGENTS

1. **$CaCl_2$** 1 vial
   8 mL of calcium chloride, 0.025 mol/L, in Tris buffer containing 0.5% bovine serum albumin.
2. **APTT reagent** 1 vial
   16 mL of purified phospholipids with colloidal silica as contact activator. Contains a preservative. Mix thoroughly on a Vortex mixer before use.
3. **APC/$CaCl_2$** 4 vials
   Human activated protein C colyophilized with $CaCl_2$. Reconstitute with 2.0 mL of NCCLS type II water [13]. Please note that a different reconstitution volume may be chosen (see Calibration). Allow to stand at 20-25°C for 30 min.
   Swirl gently before use. Do not shake.

Reagents 2 and 3 are not interchangeable between lots.

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 [13].
— 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. **$CaCl_2$**
   Opened reagent in the original vial is stable for 1 week at 15-25°C or 1 month at 2-8°C.
2. **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!
3. **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.

*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 2 should be used for validation of the assay series. 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. Level 1 shows a normal response and Level 2 an abnormal response to APC. Ranges of expected APC 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).

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 7 hours at 2000 x g for 20 min. at room temperature. Separate the plasma carefully from the blood cells within 30 min. Utilize only the middle portion of the plasma in order to avoid contamination of platelets. Analyse the plasma within 8 hours from blood sampling [12]. 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 information 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 losses of activity of labile coagulation factors and no presence of cryoprecipitate.
2. Pre-warm (37±0.5°C) a sufficient volume of $CaCl_2$ and APC/$CaCl_2$.
3. Add one volume (50-100 µL) 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.
4. Add one volume of $CaCl_2$ and simultaneously begin timing of clot formation. Record the time for clot formation.
5. Perform a second analysis on the plasma, exchanging $CaCl_2$ with APC/$CaCl_2$ and record the time for clot formation.

NOTE: When using automated instruments, it is preferable to extend the time for detection of clot formation to at least 150 seconds.

Results

Calculate the APC ratio for the samples and controls:

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

A low response consistent with the APC resistance phenotype is indicated when the APC ratio is below or equal to the cut-off value (see Calibration). In case a low response is obtained, it is recommended that analysis of a second, independently collected sample is performed for confirmation.

NOTE: If fresh samples are used it is important that a normal range is derived from fresh plasma samples from healthy individuals. Likewise if frozen plasma is used, the normal range should be obtained from frozen plasmas. Fresh samples generally provide slightly higher APC ratios.

Performance Characteristics

REPEATABILITY AND PRECISION

The imprecision of the clotting times obtained in the presence and absence of APC has been determined through analyses of three plasma samples with various response to APC. The analyses were performed on 7 different occasions, including 5 replicates of each plasma according to NCCLS reference EP5-T2 [15] .

	$CaCl_2$ (s)	CV% (Within series)	CV% (Between series)
High response plasma	29.9	1.8 %	4.2 %
Control Plasma Level 1	32.0	2.0 %	3.9%
Control Plasma Level 2	32.2	1.1%	0.8%

	APC/$CaCl_2$ (s)	CV% (Within series)	CV% (Between series)
High response plasma	122	2.4%	2.0%
Control Plasma Level 1	116	4.3%	5.0%
Control Plasma Level 2	64.5	1.4%	1.6%

	APC ratio	CV% (Between series)
High response plasma	4.1	5.0%
Control Plasma Level 1	3.6	3.3%
Control Plasma Level 2	2.0	1.4%

In general the APC ratio shows a lower imprecision at low APC ratio values. The above study was performed on an ACL 300 instrument. Duplicate or triplicate analysis of 17-20 plasma samples on 9 different types of instruments resulted in within series CV = 2.0% (range 0.3-4.2%) in the absence of APC and 3.9% (range 0.8-11.3%) in the presence of APC.

Calibration

The actual APC ratio corresponding to a borderline response to APC may vary slightly due to the instrumentation used as well as the instrument condition. It is recommended that each user establishes the performance of his own instrument as well as arrives at an estimation of the APC resistance cut-off value by using the following procedure:

1. Perform five independent determinations of the APC 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 ratio is below 7%.
2. Determine the APC ratios for 50-100 plasma samples from healthy individuals in the age range 20-65 years, including about equal numbers of men and non-pregnant women. It is recommended that the selected healthy individuals reflect the regional community. Include Control Plasma Level 1 and Control Plasma Level 2. It should be recognized that the APC ratio distribution is not Gaussian.
3. Verify that the APC ratios for the Control Plasmas are within their specified ranges.
4. Calculate the median APC ratio. If this value is 2.6 or lower, it is recommended that the APC/$CaCl_2$ vial is reconstituted in 1.5 mL water instead of 2.0 mL, in order to obtain a higher resolution.
5. Calculate the APC resistance cut-off value as 0.80 times the median APC ratio when below 3 and as 0.75 times the median APC ratio when 3 or higher.
6. The APC ratio for the Control Plasma Level 1 should be within the normal range. The APC ratio for the Control Plasma Level 2 should be below the cut-off value.

Interpretation of results

If an individual is found to carry the APC resistance phenotype from analysis of two independently collected blood samples, a follow up analysis is recommended with a test for the presence of a factor V mutation.

Limitation/interfering factors

The APT time should be within the normal range. Plasma samples showing prolonged APT times due to e.g. deficiencies in intrinsic coagulation factors or the presence of phospholipid-protein antibodies will not allow a reliable conclusion to be made from the effect of addition of APC. Individuals to be investigated should not be on heparin or vitamin K antagonist therapy. Treatment should in the latter case be discontinued for at least one week until the baseline prothrombin time has been re-established. As with any APTT-based assay, care should be taken to avoid contact activation of samples since this may lead to activation of FVIII and FV. For similar reasons it is important to avoid platelet contamination of plasma samples (see under "Specimen collection").

Reference values

The APC ratios obtained from analysis of plasmas from 100 healthy individuals (51 men, 49 women, age range 20-58 years) on five different coagulation instruments were in the range 2-5.

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US Patent 5,443,960; EP 0 608 235; Australia 666 484; Japan 2562000; Canada 2,119,761.

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

IVD	LOT				CONTROL				
In vitro diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Control	Biological risks	Manufacturer	Authorised representative	EC REP
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	

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