COATEST® APC™ Resistance V - 82 3120 63

**Intended use**

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

**Background and summary**

The APC resistance phenotype 5 is in more than 90% of the cases due to a mutation in the factor V gene, resulting in a replacement of Arg506 (R) with Gin (Q) in the factor V protein 6 . 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 plasma from untreated and patients on oral anticoagulant therapy, the selectivity 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 6 .

**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 CaCl₂, in the absence and presence of APC, and the time for clot formation is recorded.

**Rawents**

1. V-DEF Plasma
   - 4 vials
   - Stabilized, lyophilized human plasma, with a low level of factor V activity, containing the heparin antagonist Protamine 6 . Reconstitute with 4.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

2. CaCl₂
   - 1 vial
   - 8 mL of 5% chromatographically pure CaCl₂ in Tris buffer containing 0.5% bovine serum albumin.

3. 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.

4. APC/CaCl₂
   - 4 vials
   - Human activated protein C colyophilized with CaCl₂. Reconstitute with 2.0 mL of preservative. Mix thoroughly on a Vortex mixer before use.

5. Control Plasma Level 1
   - 1 vial
   - Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

6. Control Plasma Level 2
   - 2 vials
   - Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

7. V-DEF Plasma
   - 40 mL of 1:4 plasma dilution
   - V-DEF Plasma. Pre-diluted plasma should be analyzed within 45 minutes.

8. Specimen collection
   - The patient should be at rest for 10 min. before sampling. Collect blood (9 volumes) in a standardized way ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate.

9. Measurement principle
   - Stabilized, lyophilized human plasma, with a low level of factor V activity, containing the heparin antagonist Protamine. Reconstitute with 4.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

10. Controls
   - Control Plasma Level 1
      - 1 vial
      - Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

11. Controls
   - Control Plasma Level 2
      - 2 vials
      - Lyophilized human plasma. Reconstitute with 1.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

12. Controls
   - V-DEF Plasma
      - 4 vials
      - Stabilized, lyophilized human plasma, with a low level of factor V activity, containing the heparin antagonist Protamine. Reconstitute with 4.0 mL of NCCLS type II water 6 . Allow to stand for 30 minutes at 20-25°C. Swirl gently before use.

13. Controls
   - Specimen collection
      - The patient should be at rest for 10 min. before sampling. Collect blood (9 volumes) in a standardized way ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate.

14. Materials required but not provided:
   - Deionized water, filtered through 0.22 µm or NCCLS type II water 6
   - Calibrated pipettes
   - Automated or semi-automated coagulation instruments which employ mechanical or optical detection method, as used by the operator manual from the instrument manufacturer for exact procedures.

**Storage conditions and stability**

The sealed vials 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 and constitution 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. CaCl₂
   - Open vial in the original vial is stable for 1 week at 15-25°C or 1 month at 2-8°C.

3. APTT reagent
   - Open vial in the original vial is stable for 1 week at 15-25°C or 1 month at 2-8°C.

4. APC/CaCl₂
   - Stability after reconstitution is 6 hours at 37°C, 8 hours at 15-25°C, 5 days at 2-8°C or 20°C 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.

7. Control Plasma Level 1 and Level 2 were used for validation of the assay series. Level 1 shows a normal response to APC whereas Level 2 shows a response consistent with the presence of the factor V Q 506 mutation. Ranges of expected APC-V ratios are provided with each assay series. 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 of Coatest Plasma Level 1 and 2).

**Quality controls**

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 available for the APC-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 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 for blood sampling 1 . Alternatively, freeze plasma within 30 min. 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 salting down mixture and not be freeze dried. Treat specimens as potentially infectious. For more information see NCCLS document H21-A3 15 .

**Procedure**

1. All reagents must be brought to room temperature before use. Frozen plasma samples should be handled in a standardized way ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate.
2. Pre-warm a sufficient venter of V-DEF Plasma and APC/CaCl₂ at 37.0°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, to 37°C must be used.
5. Add one volume of CaCl₂, and simultaneously begin timing of clot formation. Record the time for clot formation.
6. Perform a second analysis on the plasma exchanging CaCl₂ with APC/CaCl₂ and record the time for clot formation.

Result calculation

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 5%. In case a satisfactory performance has already 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 more.
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 reported APC-V ratios are only valid for 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 as V:Q 506 and H:Q 2024 15 ).

**Performance characteristics**

**Precision**

APC-V ratios were calculated from 22 single replicate analyses of Control Plasma Level 1 on an AACL instrument using 11 different reagent combinations on 11 different occasions. Basal APTT times for untreated individuals may differ moderately as compared to the original CoATEST APC Resistance assay.

**Reference values**

The APC-V ratios obtained from analysis of plasma from 61 healthy individuals on AACL and ST-4 and from 390 healthy individuals on Thrombolyzer were in the range 2.3-5.6. No difference is found between sexes.
Bibliography / Literatur / Bibliografia / Bibliographie / Bibliografia / Bibliografía / Bibliografia / Litteraturförteckning / Bibliografía


US Patent 5,443,960; EP 0 608 235; EP 0 690 991; Australia 666 484; 690 535; Japan 2562000; Canada 2,119,761; New Zealand 261 190.

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

| IVD | LOT | Control | Biological risks | Manufacturer |
| In vitro diagnostic medical device | batch code | Control | Biological Risks | Manufacturer |
| In-vitro Diagnostikum | Chargen-Bezeichnung | Kontrollen | Biologisches Risiko | Hergestellt von |
| De uso diagnóstico en vitro | Identifizierung número de lote | Controlle | Riesgo biológico | Fabricado por |
| Dispositif médical de diagnostic in vitro | Désignation du lot | Controllo | Risque biologique | Producent |
| Per uso diagnostic in vitro | Número del loto | Controle | Rischio biologico | Producente |
| Dispositivo médico para utilização em diagnóstico in vitro | Número de lote | Controllo | Risco biológico | Tillerkare |
| ’in vitro’ diagnóstico udstyr | Batch nr. | Kontrollen | Milje oplysninger | Fabrikant |
| In vitro diagnóstico medicinal producto | Tillverkningskod | | Biologiska risker | |
| Γράφημα για διαγνωστική χρήση | Α, Παρθένος | | Τιλερκαρε | |
| Συμβολικά για διαγνωστική χρήση | | | Κατασκευαστής | |
| In vitro | | | | |