COAMATIC® Protein S-Free - 82 4003 63

ENGLISH - Insert revision 05/2003

CE

Intended use

Automated latex ligand immunoassay for the quantitative determination of free Protein S (PS) in human citrated plasma on automated instruments.

Background and summary

Protein S is a vitamin K-dependent cofactor for the anticoagulant and the profibrinolytic effects of activated Protein C.[1] Two forms of Protein S are present in plasma: free Protein S (40%), and Protein S linked to the complement C4b-binding protein (C4BP) (60%). Only free Protein S has functional cofactor activity.[2]

Protein S deficiency may be hereditary[3] or acquired. Acquired deficiency may be observed during pregnancy, oral anticoagulant therapy, oral contraceptive use, in liver disease, in newborn infants as well as in other clinical conditions.[4,5,6] Deficiency of Protein S has been associated with a high risk of developing venous thromboembolism especially in young people.[7,8]

Measurament principle

The COAMATIC® Protein S-Free assay determines the presence of free Protein S by measuring the increase of turbidity produced by the agglutination of two latex reagents. Purified C4BP adsorbed onto the first latex reagent reacts with a high affinity for free Protein S of patient plasma in the presence of Ca^{2+} ions.[9,10] The free Protein S adsorbed on the C4BP latex triggers the agglutination reaction with the second latex reagent, which is sensitized with a monoclonal antibody directed against human Protein S. The degree of agglutination will be directly proportional to the free Protein S concentration in the test sample.

Reagents

The COAMATIC® Protein S-Free kit consists of:

C4BP Buffer: 3 vials of Borax buffer containing bovine serum albumin, stabilizers and preservative.

C4BP Latex: 3 vials of a lyophilized suspension of polystyrene latex particles coated with purified human C4BP containing bovine serum albumin, stabilizers and preservative.

Anti PS MAb Latex: 3 vials of a suspension of polystyrene latex particles coated with a monoclonal antibody directed against human Protein S containing bovine serum albumin, stabilizers and preservative.

CAUTION:

The material in this product was tested by FDA approved test methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), Anti-HCV and HIV 1/2 antibodies. Handle as if potentially infectious.[11]

PRECAUTIONS AND WARNINGS:
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.

Reagent required but not provided

1. Calibration plasma	Art No 82 3534 63
2. Abnormal control plasma Level 1/2	Art No 82 3559 63
3. Saline (0.9% NaCl)	

Reagent preparation

C4BP Buffer: The reagent is ready for use.

C4BP Latex: Dissolve the contents of each vial of C4BP latex by pouring the entire contents of one vial of C4BP buffer into the latex reagent vial. Replace the stopper and swirl gently for a minimum of 20 seconds to completely dissolve the lyophilized latex.
Make sure of the complete reconstitution of the product. It must appear as a homogenous and slightly milky suspension. Keep the reagent at 15-25°C for 30 minutes and invert to mix before use.
Do not shake.
Anti PS MAb Latex: Invert to mix before to use. Do not shake and AVOID FOAM FORMATION.

Storage condition and stability

Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

C4BP Latex - Stability after reconstitution: 1 month at 2-8°C in the original vial. Do not freeze.

Anti PS MAb Latex - Opened reagent is stable 1 month at 2-8°C. Do not freeze.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

Specimen collection and preparation

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to NCCLS Document H21-A3 for further instructions on specimen collection, handling and storage.[12]

Frozen plasma samples should be rapidly thawed at 37°C while gently mixing before testing. After thawing the assay must be performed within 2 hours.

Quality control

Normal and abnormal controls are recommended for a complete quality control program.[13] Abnormal control plasma Level 1/2 Art No 82 3559 63 are designed for this program. The assigned values of these Controls are traceable to the International Standard. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance to good laboratory practice. Refer to the instrument's Operator's Manual for additional information. Refer to Westgard *et al* for identification and resolution of out-of-control situtations.[14]

Results

Free Protein S results are reported in % normality. The assay results should be used with other information, including the clinical context, in forming a diagnosis.

Limitations/interfering substances

Free Protein S results are not affected by heparin (UF heparin or LMW heparin) up to 1.5 IU/mL, bilirubin up to 18 mg/dL, hemoglobin up to 200 mg/dL, lipids up to 1280 mg/dL, platelets up to 10^{11}/L and rheumatoid factor up to 350 IU/mL.
Hemolyzed and turbid samples should not be assayed.

COAMATIC® Protein S-Free assay is not affected by Factor V Leiden mutation (APC-R).

Reference values

A normal range study was performed using the COAMATIC® Protein S-Free kit on ACL Futura.

Sex	N	ACL Futura % fFree PS
Male	130	64.4 - 128.8
*Female	102	53.2 - 109.1

* Note: Age and hormonal status may affect the normal range for females.[15]

Ranges were calculated as recommended by the International Federation of Clinical Chemistry (IFCC).[16] These results were obtained using a specific lot of reagent. Due to many variables, which may affect results, each laboratory should establish its own free Protein S normal range.

Calibration

A standard curve is obtained by analyzing different dilutions of Calibration Plasma Art. No 82 3534 63 in saline, which should be traceable to the International Standard.

Procedures

All conditions included in this package insert are referred to ACL Futura. Detailed instrument application setting including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix.

Calculation

The changes in absorbance for the standards are plotted against free Protein S %. The samples results are then calculated from the linear equation obtained from the standard curve. This procedure is automatically handled by the instruments.

Performance characteristics

Precision:

Within run precision was assessed over multiple runs.

ACL Futura	CV% (Within run)	CV% (Between run)
Mean (%FPS)		
97.7 (n=60)	1.9	0.8
57.0 (n=60)	2.7	1.1
22.4 (n=60)	2.9	1.0

Correlation:

System	slope	intercept	r	Comparative method
ACL Futura	0.918	2.32	0.981	Free Protein S EIA
ACL 9000	0.933	5.09	0.972	Free Protein S EIA

The precision and correlation results were obtained using specific lots of reagents and controls.

Linearity:

System	
ACL Futura	12 - 135 (% Free Protein S)

Sample results above 135% should be manually diluted 1:2 with saline (200 µL of sample + 200 µL of saline) and reassayed.
The printed results must be multiplied by 2 to correct for the dilution.

Detection Limity:

System	
ACL Futura:	6.4 % Free Protein S

Determinations/Kit

On ACL Futura 75 tests (approx.)

Instrumentation Laboratory Company - Lexington, MA 02421-3125 (USA)
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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