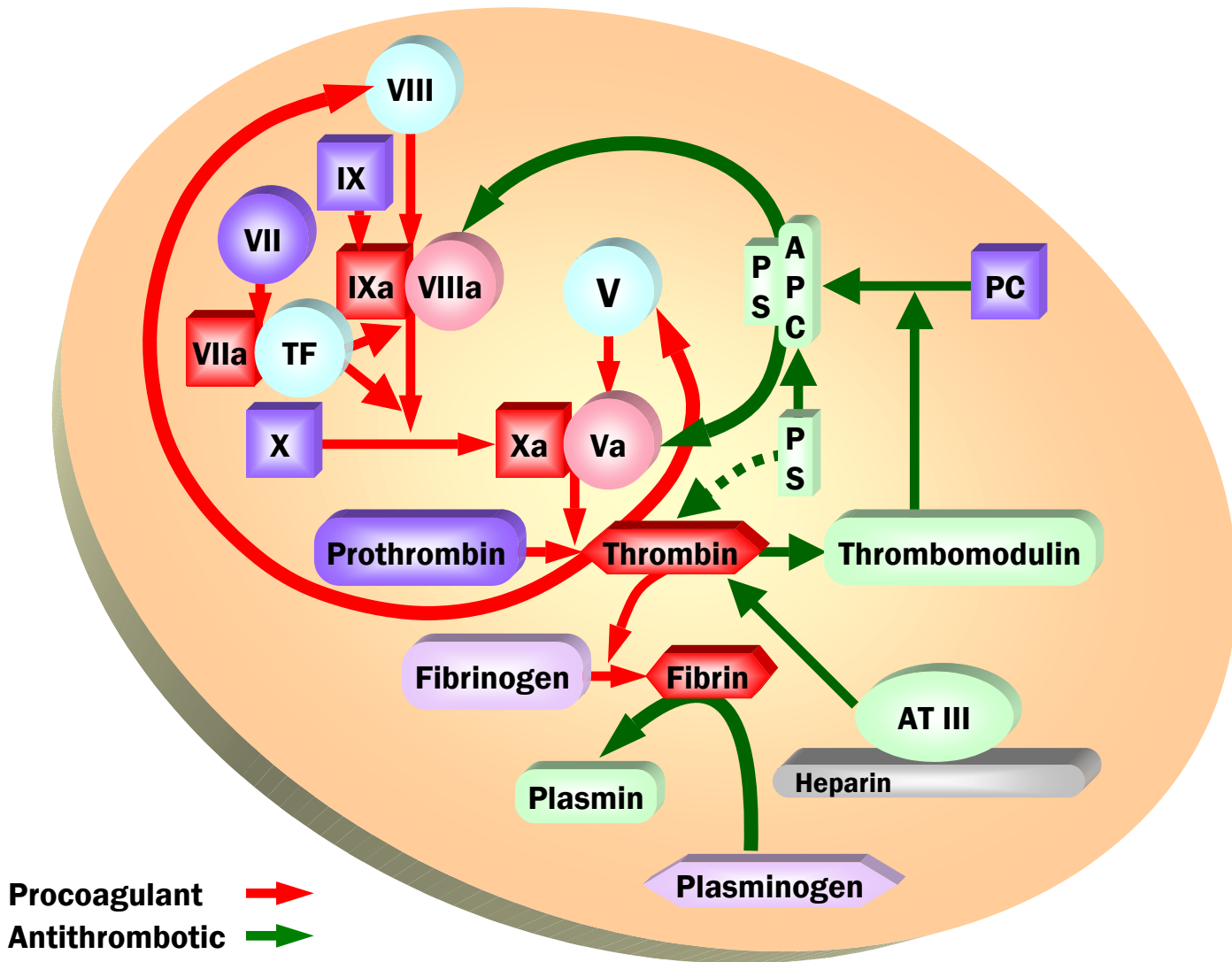


LABORATORY ASSAYS FOR ANTICOAGULANT/ANTITHROMBOTIC PROTEINS



FUNCTIONAL AND IMMUNOASSAYS FOR MEASURING

PROTEIN C - PROTEIN S - ANTITHROMBIN

Form AH15
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Assay principle

Protein C is a vitamin K dependent human Protein, which inhibits and regulates coagulation through specific cleavages of Factors Va and VIIIa, suppressing their procoagulant cofactor activity.

Using the BIOPHEN Protein C assay, Protein C is measured following a specific activation with Protac®, an enzyme extracted from snake venom (Agkistrodom C Contortrix).

Activated Protein C (APC) then specifically cleaves the specific substrate SaPC-21, releasing para-nitroaniline (pNA), which colour is measured at 405nm. There is a direct relationship between colour development and Protein C activity in the tested plasma.

References

Chromogenic assays for Protein C:

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|------------------|----------------|-----------|
| • BIOPHEN PC 2.5 | (Ref. A221202) | 75 tests |
| • BIOPHEN PC 5 | (Ref. A221205) | 200 tests |

BIOPHEN Antithrombin



Assay principle

Antithrombin is the major physiological coagulation inhibitor. It inhibits coagulation serine esterases, especially Thrombin, Factor Xa and Factor IXa, regulates coagulation pathway and prevents from thrombosis. When complexed to heparin, Antithrombin becomes a potent and fast acting inhibitor of coagulation serine esterases.

BIOPHEN Antithrombin assay is a kinetics method based on the inhibition of Factor Xa, which is at a constant concentration and in excess, by Antithrombin in presence of heparin. The remaining Factor Xa is then measured by its amygdolitic activity on a Factor Xa specific chromogenic substrate, which releases pNA.

The amount of pNA generated is inversely proportional to the Antithrombin concentration present in the tested plasma.

The assay being insensitive to heparin, Antithrombin can be assayed on plasmas from heparin treated patients.

References

Chromogenic assays for Antithrombin using an anti-Xa method:

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|----------------------------|----------------|-----------|
| • BIOPHEN Antithrombin 2.5 | (Ref. A221102) | 50 tests |
| • BIOPHEN Antithrombin 5 | (Ref. A221105) | 200 tests |

ZYMUTEST Free and Total Protein S



Assay principle

One-step immunoassay for Free or Total Protein S.

First, the immunoconjugate, which is a monoclonal antibody specific for Free or Total Protein S, coupled to horse radish peroxidase (HRP), is introduced into the microwells coated with another monoclonal antibody specific for Free or Total Protein S. Then, the diluted tested plasma or biological fluid is immediately introduced, and the immunological reaction starts. When present, the Free or Total Protein S binds onto the monoclonal antibody coated solid phase through one epitope, and fixes the second monoclonal antibody coupled to HRP by another epitope.

Only Free or Total Protein S is bound. Following a washing step, the peroxidase substrate, 3,3',5,5'-Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H2O2), is introduced and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human Free or Total Protein S in the tested sample. The assay for Free Protein S does not measure Protein S bound to C4b-BP.

References

One-step, two site ELISAs for Free or Total Protein S:

- | | | |
|----------------------------|----------------|----------|
| • ZYMUTEST Free Protein S | (Ref. ARK015A) | 96 tests |
| • ZYMUTEST Total Protein S | (Ref. ARK021A) | 96 tests |

Calibrators and Controls



Assay principle

BIOPHEN Normal and Abnormal Control Plasma and BIOPHEN Plasma Calibrator contain 12 vials of normal citrated human plasma proposed for the calibration and the quality control of some coagulation factors.

The various parameters measured, using assays from HYPHEN BioMed or from other manufacturers and according to the package inserts, are Factor VII+X, Protein C, Antithrombin III and Lupus Anticoagulant.

The BIOPHEN Normal and Abnormal Control Plasma are tested for the absence of Lupus Anticoagulant and can be used as a negative control for this investigation.

References

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|-----------------------------------|----------------|---------|
| • BIOPHEN Plasma Calibrator | (Ref. A222101) | 12x1 mL |
| • BIOPHEN Normal Control Plasma | (Ref. A223201) | 12x1 mL |
| • BIOPHEN Abnormal Control Plasma | (Ref. A223301) | 12x1 mL |

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