

THROMBOTeK PSe

Clotting Assay for Quantitation of Protein S Activity



ThromboTek PSe is a complete kit for the quantitative determination of protein S activity in human plasma by clotting assay.

SUMMARY

Protein S is a vitamin K-dependent plasma protein which serves as a cofactor for the anticoagulant activity of activated Protein C in the degradation of factors Va and VIIIa (1). Deficiencies of protein S are associated with an increased risk of thrombosis (2).

PRINCIPLE

The ThromboTek PSe method is similar to a standard factor assay. Dilutions of normal plasma are mixed with protein S depleted plasma and activated Protein C. After a two minute incubation clotting of the plasma mixture is initiated by addition of an activator reagent that contains rabbit thromboplastin. Under these conditions, the prolongation of clotting time is directly proportional to the concentration of protein S in the patient plasma.

REAGENTS

FOR IN VITRO DIAGNOSTIC USE ONLY.

WARNING: POTENTIAL BIOHAZARD

The plasma used to prepare the Protein S Deficient Plasma and the human Activated Protein C has been tested and found negative for Hepatitis B antigen (HBsAg) and antibodies to HIV and HCV by FDA licensed tests. However, these reagents should be handled with the same precautions as those observed when handling potentially infectious patient plasmas.

(1) Protein S Deficient Plasma

Ingredients: Each vial contains 1 mL of lyophilized human plasma which has been depleted of protein S by immunoadsorption.

Preparation for use: Reconstitute the vial with 1 mL of supplied Hydration Solution. Mix gently; do not shake. Allow to stand room temperature for 20 minutes before use.

(2) Activator S Reagent

Ingredients: Each vial contains 2 mL of lyophilized reagent containing rabbit brain thromboplastin, calcium, buffer, and stabilizers.

Preparation for use: Reconstitute each vial with 2 mL of supplied Hydration Solution. Mix gently; do not shake. Allow to stand at room temperature for 20 minutes before use.

(3) Activated Protein C (aPC)

Ingredients: Each vial contains 1 mL of lyophilized, human activated Protein C with buffer and stabilizers.

Preparation for use: Reconstitute each vial with 1 mL of supplied Hydration Solution. Mix gently; do not shake. Allow to stand at room temperature for 20 minutes before use.

(4) Hydration Solution

Ingredients: Each bottle contains 30 mL of deionized water with an added preservative.

Preparation for use: None.

(5) Imidazole Buffered Saline (IBS)

Ingredients: Each bottle contains 10 mL of buffer containing 15 mM imidazole, 125 mM sodium chloride, and 0.1 % sodium azide as a preservative.

Preparation for use: None.

WARNING: Sodium azide can form highly explosive metal azides if exposed to lead or copper in plumbing. Any azide containing buffer should be discarded into a sink with large volumes of water to minimize such a risk.

STORAGE AND STABILITY

Store the kit components at 2-8°C. Expiry dating is printed on each vial label. After reconstitution the kit is stable (i.e., any shift in recovery is less than twice the total imprecision of the kit) when stored capped for 24 hours at 2-8°C or for 8 hours at room temperature (23°C-25°C).

TEST PROCEDURE

The ThromboTek PSe assay may be performed by acceptable manual methods or by using optical or electromechanical coagulation analyzers. Adaptation protocols for analyzers are available from r² Diagnostics on request.

Specimen Collection and Preparation

Plasma is obtained from whole blood anti-coagulated with 1 part 3.2% sodium citrate to 9 parts whole blood. Process the collected whole blood and

handle the plasma according to the CLSI guideline H21-A5 (or superseding edition) (6).

Materials Provided in Kit

80 Determinations

- 4 vials of Protein S Deficient Plasma
- 4 vials of Activator S
- 4 vials of Activated Protein C
- 1 bottle of IBS
- 1 bottle of Hydration Solution

Reagents and equipment required but not provided

- Coagulation analyzer or 37°C water bath and timer.
- Variable volume pipettes (50 – 1000 µL)
- Graph paper or computer spreadsheet program

Additional supplies available from R² Diagnostics:

- PlasmaRef ARN (Assayed Reference Normal Plasma)
- PlasmaRef ARL-2 (Abnormal Reference Control Plasma)

Step-by-Step Procedure

A. Disposables

All test tubes and pipette tips should be plastic

B. Assay Calibration

Pooled normal plasma (PNP) (at least 10 normal donors), which has been collected in the same manner as plasmas to be tested should be used for preparation of protein S calibration standards. This PNP will be assumed to have 100% protein S activity. Alternatively, a commercially available assayed reference plasma (PlasmaRef ARN) in which protein S activity has been determined may also be used. Assayed reference plasmas are also available from recognized international standards organizations.

Prepare plasma protein S calibration standards just before testing as follows:

Standard	Sample	IBS Buffer
100%	100% PNP or reference plasma	--
50%	500 µL 100% standard	500 µL IBS
25%	500 µL 50% standard	500 µL IBS
12.5%	500 µL 25% standard	500 µL IBS
6.25%	500 µL 12.5% standard	500 µL IBS

Use immediately after preparing.

C. Testing of samples

1. Reconstitute reagents as described above.
2. Prepare plasma and standard dilutions as described above.
3. Transfer appropriate volumes of ThromboTek PSe Activator S reagent to a 37°C water bath or to a reagent reservoir in the instrument. Prime reagent delivery tubing if necessary.
4. To an instrument cuvette or test tube:
 - Add 50 µL of a 1:10 dilution in IBS of patient sample or standard.
 - Add 50 µL of aPC.
 - Add 50 µL of Protein S depleted plasma.
 - Incubate for 2 minutes at 37°C.
 - Initiate the reaction by adding 100 µL of Activator S reagent, and note the time to clot formation.
 - Obtain duplicate determinations for each sample or reference plasma.
5. Using linear graph paper, plot the % Protein S activity of the calibration standards on the x-axis versus the mean clotting time of the standards on the y-axis. Draw the line of best fit between the resulting points. Commonly available spreadsheet software programs are often used as an alternative to graph paper and manual calculation. Determine the % Protein S of patient samples by interpolating from the standard curve.
6. If the assigned Protein S level of the reference plasma used to construct the standard curve is not 100%, then the patient result must be corrected to account for the true reference value. Alternatively, the true assigned value can be used in the calibration curve.

QUALITY CONTROL

Quality control of coagulation tests involves multiple components, including reagents, pipettes, distilled water, buffers and instruments. Each laboratory should establish a Quality Control program that includes both normal and abnormal control plasmas. All assays should include controls, and if any of

the controls are outside the established reference ranges, then the assay should be considered invalid and no patient results should be reported. A normal reference control plasma such as [PlasmaRef ARN](#) and an abnormal reference control plasma such as [PlasmaRef ARL-2](#) can be used to verify instrument and reagent performance.

INTERPRETATION OF RESULTS

A decrease in protein S activity does not necessarily indicate a decrease in plasma concentration. Protein S (total) is present in plasma as free protein and as protein bound to C4b binding protein (C4bBP). Only the free protein S acts as a cofactor for activated protein C. When the protein S activity is decreased, it is important to establish the plasma levels of both free protein S and C4bBP bound protein S.

Congenital deficiency of protein S has been identified as a risk factor for thrombosis.(4) Various attempts to characterize the deficiency based on the relationships between total and free protein S activity and antigen values have been suggested but there is no agreement on how to express this (5).

Acquired protein S deficiency can also be seen in liver disease, DIC and with oral anticoagulant therapy. Congenital protein S deficiency has been reported to segregate with activated protein C resistance (APC-R) in about 25% of cases

LIMITATIONS OF PROCEDURE

The presence of heparin or lupus-type anticoagulants may interfere with the assay results, prolonging the clotting time and hence giving an artificially high protein S value. In patients with marked inflammatory responses an apparent protein S deficiency can occur because of high levels of the C4bP, which may be found in this condition. It is important to repeat testing at a different time or test a family member before classifying a patient as protein S deficient.

PERFORMANCE CHARACTERISTICS

Precision: Precision estimates of three lots of ThromboTek PSe were determined in a two run per day, twenty day exercise on an AMAX 200 analyzer in mechanical mode using a normal plasma and an abnormal plasma as described in the CLSI guideline EP5-A2 (7). The average precision results as %CV were:

Plasma	Repeatability	Total
Normal	4.9%	5.7%
Abnormal	7.8%	9.2%

Linearity: Linearity studies of three lots of ThromboTek PSe were determined on Stago ST4 semi-automated coagulation analyzer. The ThromboTek PSe assay was linear from 10% Protein S to the maximum tested concentration of 156% Protein S.

Analytical Sensitivity: The lower limit of detection for three lots of ThromboTek PSe were determined by replicate measurement of IBS alone as the sample on an AMAX 200 analyzer in mechanical mode, and the % PS activity was calculated from the sum of the mean and 3 standard deviations. The lower limit of detection of the assay was 1% PS.

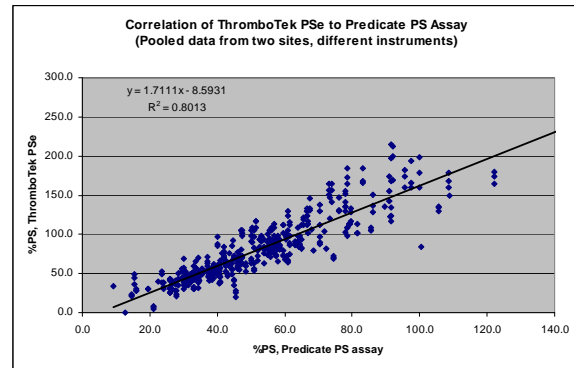
Interferences: Interference studies of three lots of ThromboTek PSe were determined on an AMAX 200 analyzer in mechanical mode. Interferant was spiked into pooled normal plasma and a dilution series prepared. The maximum concentration tolerated in the assay was defined as the highest concentration of interferant wherein any consistent shift relative to the recovered value of the base PNP was less than 10%. The maximum concentrations were:

Interferant class	Added interferant	Maximum concentration tested	Maximum tolerated concentration
Hemolysis	Hemoglobin	500 mg/dL	500 mg/dL
Icterus	Unconjugated bilirubin	20 mg/dL	20 mg/dL
Lipemia	IntraLipid®	2,000 mg triglyceride/dL	2,000 mg triglyceride/dL
Heparin	Heparin	2.0 Unit/mL	1.0 U/mL

Intralipid® is a registered trademark of Fresenius Kabi.

Method Comparison:

A total of one hundred seventy-four patient samples were assayed for Protein S activity with multiple lots of ThromboTek PSe and two lots of another commercially available Protein S activity assay. The data was collected in two sites, one using an AMAX 200 analyzer and the other a STart4 analyzer. As determined by Kruskal-Wallis analysis the data was pooled and then further analyzed by linear regression. The correlation coefficient was 0.895 (95% CI, 0.875-0.912) and the coefficient of determination was 0.801, with a slope and intercept of 1.71 and -8.59 respectively.



Normal Reference Range:

In a representative study one hundred twenty healthy donors were analyzed for Protein S activity with each of three lots of ThromboTek PSe on an AMAX 200 analyzer in mechanical mode. Assay calibration was performed using the SSC/ISTH Secondary Coagulation Standard Lot #3 available from NIBSC. The geometric means and standard deviations were calculated, and the ranges were calculated as the mean +/- 2 standard deviations. The results were:

Donors	Number	Mean % PS	Range %PS
All	120	120%	47% - 193%
Males only	35	135%	62% - 209%
Females only	85	114%	45% - 183%

These values should be considered illustrative only. Each laboratory should establish its own normal reference range.

The performance of any assay should be reviewed for the individual analyzer(s) in use in each laboratory according to the CLSI guideline EP15-A2 (8) or to a similar guideline.

References

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