t-PA

Product Monograph 1995

CHROMOGENIX

t-PA

t-PA, Product Monograph 1995

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t-PA

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Tissue-Type Plasminogen Activator (t-PA)

t-PA is a proteolytic enzyme found in blood and tissues. The major physiological function of t-PA is to generate plasmin that can dissolve blood clots in the vasculature. Recent studies suggest that reduced t-PA activity and elevated t-PA and PAI-1 antigen levels may be a risk marker for cardiovascular disease. This monograph provides a brief review of t-PA and presents two modern assay kits for the determination of t-PA levels in plasma.

Introduction

Blood coagulation is an enzymatic event initiated by substances from injured tissues and culminating in the formation clot-forming fibrin monomers. After a few days, the fibrin clot is degraded by the fibrinolytic enzyme system (Figure 1).[1-5]

The central enzyme component in this system is the glycoprotein plasminogen present in plasma and most extravascular fluids. Plasminogen is a zymogen of a serine protease which, following partial cleavage by a plasminogen activator, is converted into its active form plasmin. Plasmin is involved in a variety of biological processes, including cell migration, growth, inflammation and tumour invasion, although its primary function is assumed to be lysis of fibrin in the vasculature. Two plasminogen activators have been found in the human body, the tissue-type plasminogen activator (t-PA) and the urinary-type plasminogen activator (u-PA). t-PA is the principle activator of plasminogen in blood, whereas u-PA has its major function in tissue-related proteolysis and is believed to only be secondary to t-PA in the removal of intravascular fibrin.

Fibrinolysis is initiated and propagated mainly by the fibrin surface, which offers binding sites for an optimal contact between a number of the components of the fibrinolytic system, most notably plasminogen and t-PA. This stimulatory effect ensures a high concentration of plasminogen and t-PA at the fibrin deposits and localizes the plasmin activity. Inhibitory regulation is provided by the plasminogen activator inhibitor-1 (PAI-1) and the plasmin inhibitor. PAI-1 is the most efficient inhibitor of t-PA in plasma and the majority of circulating t-PA is bound to this inhibitor.

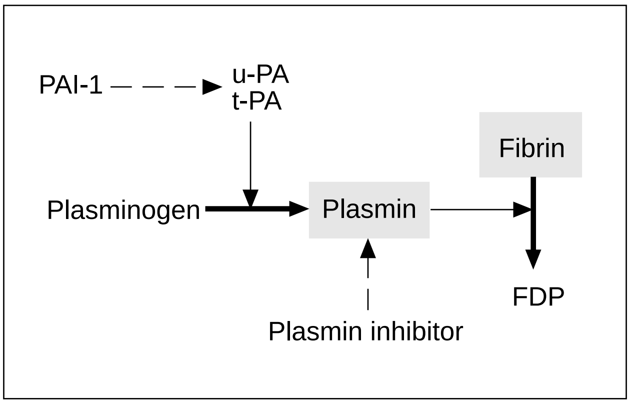


Figure 1a. *The fibrinolytic system.*

Normally, there exists a carefully regulated balance between the formation of fibrin and its subsequent removal. However, in certain pathological conditions and in hereditary deficiencies, this balance is disturbed. Patients with an inherited increase of fibrinolytic activity usually have severe bleedings. In contrast, a decreased fibrinolytic activity may be associated with thrombotic disease.

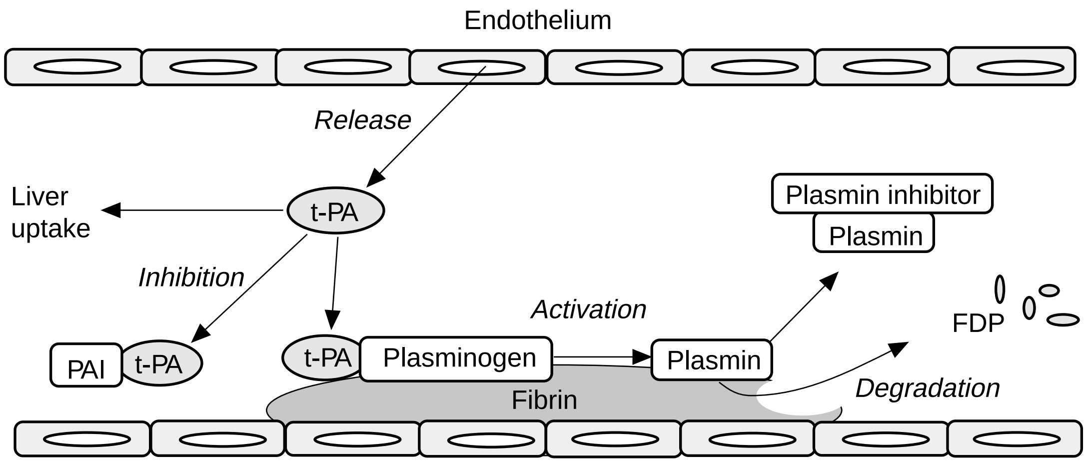


Figure 1b. The fibrinolytic system in vivo *(modified from Wiman et al 1990)***.**
Plasminogen is the proenzyme of plasmin, whose primary target is the degradation of fibrin in the vasculature. The activation of plasminogen to plasmin in blood is catalyzed by t-PA secreted from endothelial cells. Fibrin provides binding sites for both plasminogen and t-PA, thereby optimizing contact between them. This mechanism ensures a high concentration of plasminogen and t-PA at the site of fibrin formation and localizes the action of plasmin. Further regulation of the system is provided by PAI-1 and plasmin inhibitor. Free t-PA, as well as complexed t-PA/PAI-1, is cleared from the circulation by receptors in the liver. Abbreviations: t-PA; tissue-type plasminogen activator, PAI-1; plasminogen activator inhibitor 1, FDP; fibrin degradation products.

Compound	**Size [kDa]**	**Amino acids**	**Plasma concentration**	**Half-life in circulation**	**Location of synthesis**	**Function**
Glu-plasminogen	92	791	200 µg/ml	2.2 days	Liver	Proenzyme of plasmin
sc.u-PA	54	411	8 ng/ml	5-10 min	Kidney, lung	Plg. activator
sc.t-PA	68	527	5 ng/ml	3-4 min	Endothelium	Plg. activator
Plasmin inhibitor	70	452	70 µg/ml	2.6 days	Liver	Plasmin inhibitor
PAI-1	52	379	20 ng/ml	2-3 hours	Endothelium	Plg. activator inhibitor
PAI-2	60	393	*250 ng/ml	24 hours	Placenta	Plg. activator inhibitor

Table 1. *The major components of the fibrinolytic system. * 30th week of pregnancy*

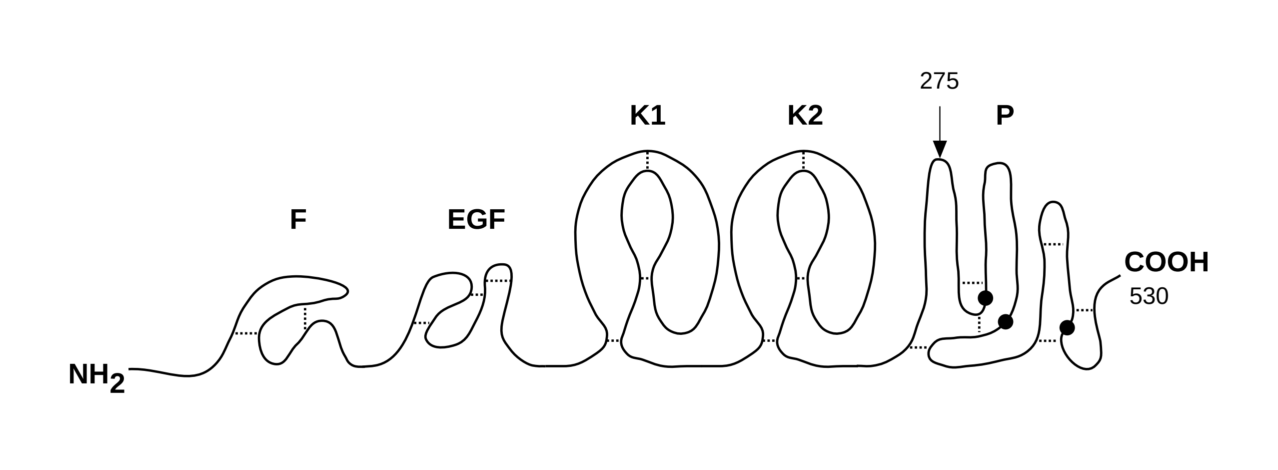


Figure 2. Domain structure of the human t-PA molecule.
Abbreviations: F; finger domain K1-2; kringle domains, EGF; epidermal growth factor domain, P; serine protease domain. The catalytic triad made up of His^{322}, Asp^{371} and Ser^{478}, is illustrated by black dots. The cleavage site for converting the molecule into the two-chain form is shown by an arrow. Disulfide bridges are marked as thin lines.

t-PA biochemistry

t-PA structure and function

Human t-PA is a 68 kDa serine protease composed of 530 or 527 amino acids and containing between seven and 13% carbohydrate. The molecule (Figure 2), is made up of five distinct domain structures with autonomous functions.[6]

The finger domain and the two kringle domains are involved in the binding of t-PA to fibrin, whereas the epidermal growth factor domain is implicated in the rapid hepatic clearance of the molecule. The serine protease domain contains the active site region made up of serine, histidine and aspartic acid, which are situated relatively far apart from each other in the primary structure, but are in close proximity in the folded protein. This region cleaves the Arg^{561}-Val^{562} bond in plasminogen and thus activates it to plasmin.

Synthesis and secretion

t-PA is synthesized mainly in vascular endothelial cells and is secreted into the plasma continuously and also through the acute release of t-PA.[7] The latter situation occurs upon stimulation of certain endothelial cell receptors.[8] Different regions of the vascular system secrete different amounts of t-PA. Upper extremities secrete about four times more t-PA than that of the lower extremities.[9]

There are two forms of t-PA, single-chain t-PA (sct-PA) and two-chain t-PA (tct-PA). The single-chain molecule is the native form of t-PA secreted from endothelial cells, whereas the two-chain form is the result of the proteolytic activity of plasmin. Both forms are catalytically active and have similar enzymatic properties in the presence of fibrin.

Fibrin affinity

t-PA is a relatively poor activator of plasminogen in the absence of fibrin due to the low affinity of t-PA for its substrate. Although, t-PA has a high affinity for fibrin and binding increases its activating capacity up to 1,000-fold. This dramatic increase is attributed to specific binding sites on the fibrin surface that concentrate and correctly orientate t-PA with its substrate, as well as promoting efficient clot lysis.[10]

Concentration/activity

The baseline concentration of t-PA in plasma taken in the morning from a healthy person at rest, is approximately 5 µg/l when measured using immunological methods,[11-13] and about 1 µg/l (corresponding to about 0,5 IU/ml) if measured functionally.[14-16]

This discrepancy between antigen and activity levels can be attributed to the fact that most of t-PA in plasma occurs in non-functional complexes, mainly with its principle inhibitor PAI-1.

An acute increase in t-PA levels is observed in response to stimuli, such as exercise, mental stress, venous occlusion and various drugs.

Half-life and removal

t-PA is removed from the circulation by a rapid clearance mechanism in the liver.[17] The normal half-life is about 4 minutes for free t-PA, although the half-life may decrease considerably if PAI-1 levels are elevated, as is the case in many patients with a thrombotic tendency. When an isolated rat liver perfusion system was studied it was shown that the t-PA/PAI-1 complex is cleared twice as rapidly as that of free t-PA.[18]

Plasminogen activator inhibitors

A number of inhibitors have been identified in plasma and other body fluids with the capacity to inhibit t-PA, including PAI-1, PAI-2 (placenta plasminogen activator inhibitor), PAI-3 (protein C inhibitor), protease nexin, α_2-macroglobulin, trypsin inhibitor and CI-inhibitor.

PAI-1

Plasminogen activator 1 (PAI-1) is the most efficient inhibitor of t-PA in plasma. It is a typical serine protease inhibitor (serpin) that acts as a pseudosubstrate for its target protease, with which it forms an equimolar and inactive complex (i.e active site binding). [19-20]

PAI-1 is unique in the serpin family, in that the molecule exists in two forms. One form is active, but spontaneously loses its activity and has a half-life of 0,5 hour. The second form is a non-active decay product of the active molecule. The reason why PAI-1 undergoes this change is unclear.

PAI-1 is synthesized by several cell types including endothelial cells and hepatocytes and is present in platelets, placenta and serum. Antigen determinations show that platelets contain up to 90% of the total amount of PAI-1 in blood. Platelet PAI-1 is available for release when the platelets are activated, but is much less active than plasma PAI-1 in relation to the amount present. However, it has been calculated that platelet derived PAI-1 accounts for some 50-80% of the total PAI-1 activity, by virtue of their mass.

The normal concentration and activity range of PAI-1 in plasma is reported to be 5-40 µg/l and 0-40 IU/ml, respectively. This large variability is partly due to the marked diurnal variation in PAI-1, with lower values in the afternoon than in the morning.

PAI-2

PAI-2 is a 47 kDa serpin with a higher affinity for u-PA than for t-PA. While hardly detectable in nonpregnant plasma, levels up to 100 µg/l are observed in the third trimester of pregnancy. It does not inhibit sct-PA and is 100-fold less effective as an inhibitor of tct-PA than PAI-1. The more efficient inhibition by PAI-2 of u-PA indicates that its primary role may be in regulating extracellular u-PA.

t-PA facts

Name:	Tissue-type plasminogen activator
Synonyms:	t-PA, vascular activator, extrinsic plasminogen activator
History:	Discovered by Astrup et al 1947
Synthesis:	Endothelial cells, mesothelial cells, megacaryocyte
Molecular weight:	68.000 Dalton
Carbohydrate:	7-13%
Gene:	36.6 kb, 14 exons, located at chromosome 8, p12-q11.2
Amino acids:	527 or 530
Concentration:	5 µg/l (antigen) 1 µg/l, 0.5 IU/ml, (activity)
Half-life:	4-5 min
Type:	Serine protease
Function:	Activates plasminogen into plasmin.
Importance:	Decreased t-PA activity and elevated t-PA and PAI-1 antigen may be a risk marker for cardiovascular disease.

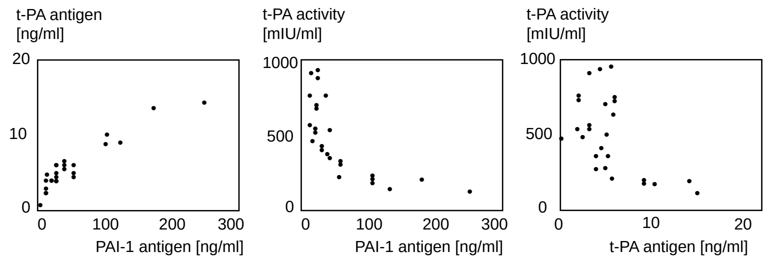


Figure 3. *Relationships between t-PA antigen, PAI-1 antigen and t-PA activity (BIA) in plasma from 25 healthy subjects.[21]*

The t-PA/PAI-1 system and disease

Regulation of t-PA

The interaction between t-PA and PAI-1 and their coordinated apperance in plasma is highly complex. The total amount of t-PA and PAI-1 antigen in plasma usually show a strong positive correlation, suggesting that synthesis and/or clearance of t-PA and PAI-1 are biologically linked. However, an increased t-PA production due to increased PAI-1 levels does not automatically result in increased amounts of active t-PA. Instead, t-PA activity appears to show a negative correlation with both PAI-1 and t-PA antigen (Figure 3).[21] This latter correlation may seem confusing, since the increase in the total amount of t-PA results in a decrease of t-PA activity.

Deep vein thrombosis

Several studies have observed a fibrinolytic deficiency in patients with idiopathic or recurrent deep venous thrombosis (DVT).[22-23] About one third of these patients exhibited impaired fibrinolytic activity, either due to a poor release of t-PA after venous occlusion, or due to increased PAI-1 levels. In two prospective studies carried out on postoperative DVT in patients subjected to hip replacement, preoperative values of increased PAI-1 appeared to be predictive of postoperative venous occlusion.[24,25]

Studies regarding a reduced t-PA activity and elevated PAI-1 levels as a risk factor for thromboembolic disease remain to be further evaluated. Baseline levels of PAI-1 and t-PA antigen, do not appear to predict the occurence of DVT or pulmonary embolism among healthy individuals.[26]

Cardiovascular disease

t-PA and PAI-1 have in several independent studies been identified as risk markers for cardiovascular disease. Elevations of t-PA antigen have been linked to persons at risk in several studies involving cardiovascular events in patients with angina pectoris and coronary artery stenosis,[27] myocardial infarction (MI),[28] and stroke.[29] Furthermore, a strong support for the link between PAI-1 elevation and risk of having a MI was obtained from a study of men who had survived a first MI before the age of 45 years.[30] Reduced t-PA activity has been reported as predictive for MI,[31] for MI in patients with angina pectoris,[32] and in ischaemic disease in younger men.[33]

The results from these studies put together suggest that a state of elevated t-PA and PAI-1 antigen and reduced activity (Table 2; situation I) is the condition associated with cardiovascular disease.[21]

Situation I	Situation II
high t-PA antigen	low t-PA antigen
high PAI-1 antigen	low PAI-1 antigen
low t-PA activity	high t-PA activity*

** At least in normal range; subject to a lower limit for total t-PA*

Table 2*. The possible relationship between t-PA and PAI-1.[21]*

Malignant disease

The production of t-PA and u-PA by leukemic cells can be used to predict the prognosis and response to chemotherapy in patients with acute myeloid leukemia. Patients whose cells produce only t-PA have a lower chance of survival and fail to respond to chemotherapy. In contrast, patients with u-PA producing cancer cells have a higher chance of survival and a better response to chemotherapy.[34]

Thrombolytic therapy

Cardiovascular diseases, such as acute myocardial infarction, stroke, and venous thromboembolism, are probably the major cause of death and disability in an adult population. The immediate underlying etiology in these conditions is often a thrombotic obstruction of critically situated blood vessels, causing a loss of blood flow to vital organs.

One approach to the treatment of thrombosis consists of the intravenous infusion of plasminogen activators as clot-dissolving drugs.[35] Reduction of infarct size and mortality has been obtained with streptokinase (SK), anisoylated plasminogen streptokinase activator complex (APSAC), and recombinant t-PA (rt-PA).

rt-PA is probably the most effective thrombolytic agent as fibrin affinity of t-PA not only localizes the catalytic activity to the clot, but it also increases its catalytic efficiency. rt-PA in combination with the adjunctive infusion of the anticoagulant drug heparin recanalizes approximately 75% of occluded coronary arteries within 90 minutes and reduces mortality by about 25%. The use of thrombolytic agents for clinical use may occasionally require close monitoring of the components of the plasminogen activation system. Excessive thrombolytic activity is likely to cause bleeding, particulary cerebral hemorrhage, as a side-effect.

Circadian variations

An intriguing feature of the fibrinolytic system is the circadian variation in t-PA and PAI-1 that has been observed (Figure 4). Free t-PA levels are lowest in the morning, increase during the day and reach their peak activity level in the late afternoon. t-PA and PAI-1 antigen are highest in the early morning and decrease during the day.[36]

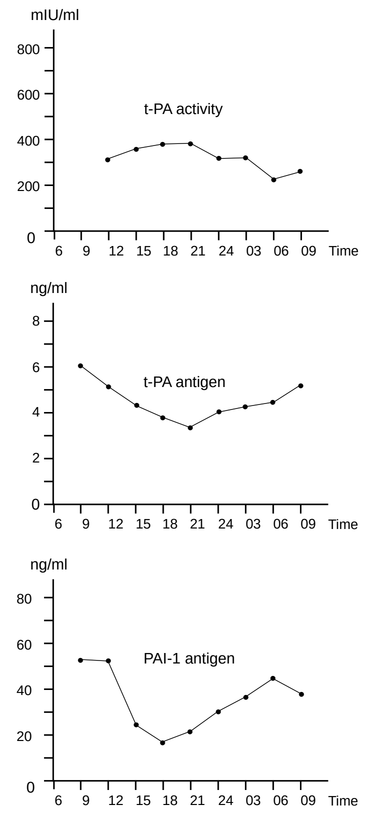


Figure 4. *Plasma levels of t-PA and PAI-1 in healthy, active young subjects over a 24-hour period using blood sampling in Stabilyte® media and BIA t-PA analysis in water. (modified from C. Kluft 1993).*

It has been suggested that the high incidence of myocardial infarction[37] and cerebral thrombosis[38] in the morning hours, may be connected to the circadian rhythm of fibrinolytic activity.

From mortality statistics in Greenland it is known that Eskimos have a low prevalence of myocardial infarction. This has been related to their diet, although it may also be due to the observation that Eskimos have a rapid increase in t-PA activity in the morning and a more rapid decrease in PAI-1 activity and antigen compared to Caucasians.[39]

Because of the diurnal variation in fibrinolytic activity, sampling should always take place at the same time during the day (usually between 8 a.m-9 a.m).

Physiological aspects

Different stimuli, drugs, and environmental factors have been reported to modulate the fibrinolytic activity in the experimental animal as well as in humans. Examples of these are listed below in alphabetical order.

Physiologic stimuli
Acetylcholine
Acidosis
Adrenaline
Basic fibroblast growth factor
Bradykinin
Calcium
cAMP
Circadian rhythm
Endothelin
Exercise
Histamine
Hyperoxia
Insulin
Interleukin 1
Oxygen radicals
Platelet-activating factor
Prostacyklin, prostaglandins
Substance P
Temperature
Thrombin
Tumor necrosis factor
Vasopressin
Vasoactive agents
Vasoact. intestinal polypeptide
Venous occlusion
Vitamin D

Environment, food
Age
Alcohol
Coffee
Diet
Electroshock
Garlic
Mental stress
Onions
Sex
Smoking

Age

Age has little influence on t-PA or PAI-1 activity, although t-PA antigen levels are correlated to increasing age.[40]

Alcohol

Most reports on alcohol and fibrinolysis show an increase in plasma PAI-1 levels following alcohol consumption that causes an acute decrease in t-PA activity.[41,42] In a recent study of moderate alcohol consumption in a group of healthy men, it was observed that t-PA activity falls sharply after alcohol intake for the first 5 hours, although it then rises and becomes significantly higher after 13 hours.[43] Moderate alcohol consumption may therefore be associated with a lower risk of coronary heart disease 'the day after'.

Anabolic steroids

Stanozolol produces profound change in the coagulation and fibrinolytic systems after prolonged oral administration. A significant decrease in t-PA and PAI-1 activity has been reported.[44]

DDAVP

1-deamino-8-d-arginine vasopressin or DDAVP induces a powerful release of t-PA, factor VIII and von Willebrand factor when injected into humans.[45] Peak t-PA concentrations are seen after 40 to 60 minutes of administration. Patients who are poor responders in the venous occlusion test may show efficient t-PA release after DDAVP.[46]

Exercise

A rise in the fibrinolytic activity after exercise has been reported by many authors and attributed mainly to the acute release of t-PA from the vascular endothelium.[47] The increase in t-PA activity is related to both the intensity and the duration of exercise and may reach 30 times the normal after a Marathon race.[48] When comparing physically active and inactive men, it was found that t-PA activity increases more in active men and that they have a lower PAI-1 activity.[49]

Food

A diet rich in high-complex carbohydrates and low in fat has been reported to lower both t-PA and PAI-1 antigen. The net effect was an enhancement of fibrinolytic potential, due to the greater fall in PAI-1.[50] When comparing fish with lean meat, it has been observed that a fish diet leads to higher t-PA and PAI-1 antigen levels.[51]

Basal t-PA activity may increase following a 24 hour fast. This is probably a secondary reaction due to decreased PAI-1 activity.[15]

Heparin

Heparin can form 1:1 complexes with t-PA. The complex has higher catalytic activity for plasminogen activation than t-PA alone, but heparin appears to inhibit the potentiating effect of fibrin on t-PA-induced plasminogen activation.[52]

Prolonged administration of unfractionated heparin and LMW heparin, induces a rise of circulating t-PA antigen levels.[52]

Drugs
Anabolic steroids
Antidiabetics
Aspirin
Batroxobin
Benzodiazepines
Bezafibrate
ß-Blockers
ß-Pyridylcarbinol & nicotinic acid
Carbachol
DDAVP
Defibrotide
Disulfiram

Drugs
Erythropoietin
Flufenamate
Furosemide
Fucoidan
Gemfibrozil
Heparin & LMW heparin
Molsidomine & metabolite SIN-1
Nitroprusside
Oral anticoagulation
Oral contraceptives
Phytosterol
Polyions
Retinoic acid

Insect venom

The release of t-PA from the endothelium may be involved in the pathogenesis of anaphylactic shock induced by insect venom. Levels have been found to increase about 10-fold following a controlled insect-sting challenge in patients with a previous history of insect-sting induced anaphylactic reaction.[53]

Mental stress

Mental stress releases t-PA [54] in a similar manner to that of adrenaline,[55] with increases in heart rate and systolic and diastolic blood pressures.

Stress-induced release must be avoided during blood sample collection. The subject must rest both mentally and physically for 20-30 minutes prior to venepuncture.

Oral contraceptives

Oral contraceptives may produce a significant increase in t-PA activity, not due to decreases in PAI-1 or plasminogen concentration.[56]

Pregnancy

Pregnancy induces marked changes in the coagulation mechanism and fibrinolytic system, changes that aim to secure hemostasis during pregnancy and delivery. The increase of t-PA antigen and the decrease of PAI-1 antigen and activity after the 38th week contributes to sustain a fibrinolytic potential capable of degrading large fibrin deposists.[57]

Smoking

Chronic smoking induces higher baseline levels of t-PA and PAI-1 antigen and lower t-PA activity. In addition, the release of t-PA after a venous occlusion is impaired in chronic smokers.[58]

Venous occlusion

The venous occlusion test is often used to test patients for their capacity to release t-PA from the occluded venous segment. A test takes 5-20 minutes and involves a blood pressure cuff on the upper arm, inflated midway between the systolic and diastolic blood pressure. The t-PA activity rises 3-12 fold and antigen levels increase 2-6 times.

Comparison of preocclusion with the postocclusion blood sample gives an estimate of the fibrinolytic capacity of the individual.[59]

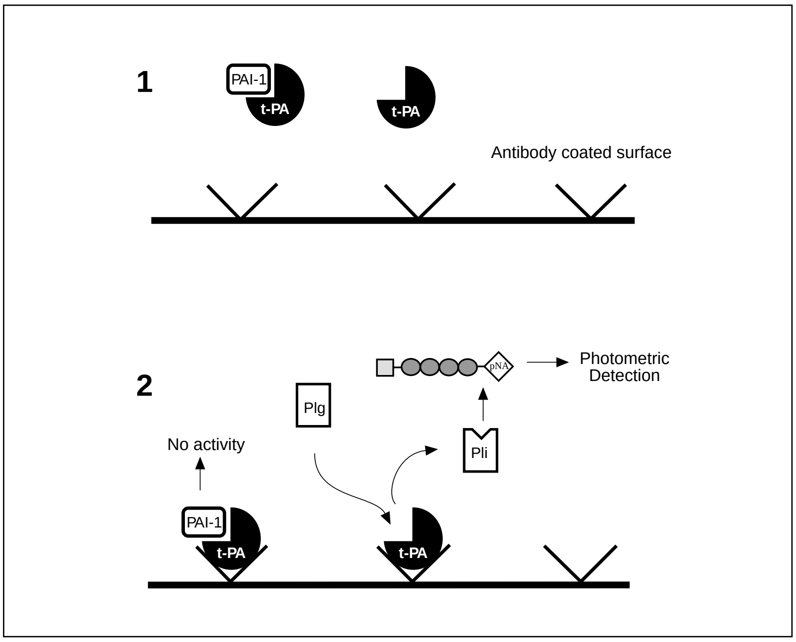


Figure 5. Bioimmunoassay for t-PA.
[1] Microplate wells are coated with a monoclonal anti-t-PA antibody that binds t-PA without interfering with its active site. [2] A mixture of Glu-plasminogen (Plg) and a chromogenic plasmin substrate is added. t-PA activates plasminogen to plasmin (Pli), which causes the chromogenic substrate to release pNA (yellow colour). The amount of pNA is proportional to the amount of active t-PA originally present in the sample.

t-PA assay methods

Introduction

A large variety of methods for measuring t-PA levels in human plasma have been described. These can be divided into specific t-PA assays, and non-specific global tests. Two common methods today are the global, euglobulin clot lysis time[60] and the fibrin plate assay.[61] Specific t-PA assays include immunological methods which measure t-PA antigen (i.e. both free t-PA and t-PA/PAI-1),[11-13] and functional methods. The latter are either chromogenic assays using chromogenic plasmin substrates and stimulators,[14,62-63] or bioimmunoassays that use a combination of monoclonal antibodies and chromogenic plasmin substrates.[64-65]

Blood collection and handling

In recent years there have been considerable improvements in the methodology of blood collection routines and sample handling.[66]

As large number of variables influence fibrinolytic activity it is important to standardize as many aspects as possible of blood collection. Samples should be taken in the morning (8 am- 9 am) from a subject who has been fasting, although a light breakfast without fat and tea or coffee can be chosen. Smoking must be avoided for at least one hour and alcohol for at least 24 hours prior to taking the sample. It is also important that the subject is resting mentally and physically prior to venepuncture.

In vivo, the concentration of active t-PA is regulated by endothelial secretion, hepatic clearance, and PAI-1, which results in a relatively stable steady-state level of active t-PA. However, when a blood sample is taken the secretion and clearance mechanisms are removed. PAI-1 will therefore continue to react with free t-PA unless preventive measures are taken. The collection of plasma at low pH (about pH 6) using Stabilyte® tubes is an effective way of "freezing" the in vitro complex formation between t-PA and PAI-1.[15,67]

This requirement to prevent t-PA complexing in vitro is not important for immunological assays of total t-PA antigen (free plus complexed), but is essential for assays measuring t-PA activity. The latter type of test includes chromogenic substrate assays and bioimmunoassays.[16,68]

Bioimmunoassays

t-PA activity assays utilizing the technique of immobilized antibodies for adsorbing t-PA from plasma is referred to as a BIA or bioimmunoassay (Figure 5).

In the first step, t-PA complexes to an immobilized monoclonal anti-t-PA antibody (without blocking the active site) and unbound proteins are washed away. This results in the complete removal of interfering factors.

In the second step, human Glu-plasminogen and a chromogenic plasmin substrate are added. Plasminogen is converted to plasmin by the active t-PA, and the plasmin generated cleaves the chromogenic substrate, releasing pNA. The amount of pNA released is proportional to the amount of t-PA originally present in the sample and is measured photometrically. The sensitivity of the bioimmunoassay makes venous occlusion tests optional and the active t-PA can be measured directly using undiluted plasma.

Euglobulin clot lysis time (ECLT)

ECLT is a global test that measures the fibrinolytic potential in plasma, and is used for estimating the t-PA activity. The basic idea is to separate the inhibitors that interfere with the assay from the components with fibrinolytic activity.

Citrated plasma at 4° C is diluted and acidified with acetic acid. The precipitated euglobulin fraction is then separated by centrifugation, resuspended in a buffer and clotted by adding thrombin. The time taken for the clot to lyse is measured visually or using an automated clot lysis recorder. Fibrinolytic activity is inversely proportional to the lysis time.

Normal values are approximately 2 to 8 hours and 20 minutes to 1 hour following venous occlusion. The major disadvantage with clot lysis time is that it is not only affected by activator content but also by the levels of inhibitors and the fibrinogen and plasminogen content of the sample.

Fibrin plate assay

The fibrin plate assay measures the fibrinolytic activity by calculating the amount of lysis produced by a standard volume of euglobulin fraction applied to a standardized plasminogen-rich fibrin plate (Figure 6). The test is more accurate than clot lysis time, although as it is time-consuming and labour-intensive it is not suitable for acute situations.

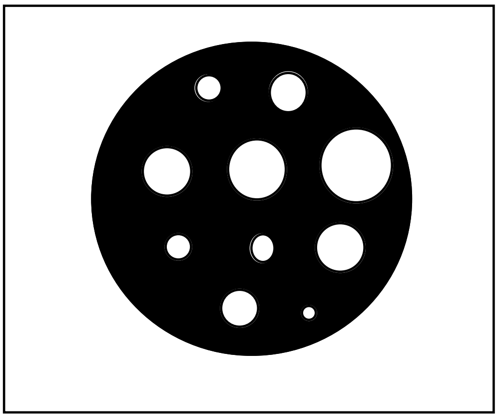


***Figure 6:** Principle of a fibrin plate test. Euglobulin fractions are placed on the fibrin plate and incubated overnight. The white circles represent digested fibrin and the grey area undigested fibrin. 1-5 are standard solutions. 6-10 are samples with different fibrinolytic activity. The white lysed area is proportional to the fibrinolytic activity.*

Immunological methods

Commercially available t-PA antigen kits are generally the enzyme-linked immunosorbent assay (ELISA). In contrast to the functional activity assays in which only free t-PA is measured, immunological methods also include the non-functional t-PA bound to inhibitors. The two most common t-PA/inhibitor complexes are t-PA/PAI-1 and t-PA/plasmin inhibtor.

Chromogenic substrate assays

Numerous chromogenic substrate assays are available for the indirect determination of t-PA by a plasmin-mediated reaction. The majority of these assays employ different types of fibrin-like stimulators (e.g. soluble fibrin, fragmented fibrin(ogen), poly-L-lysine) to enhance the activation of plasminogen by t-PA (Figure 7).

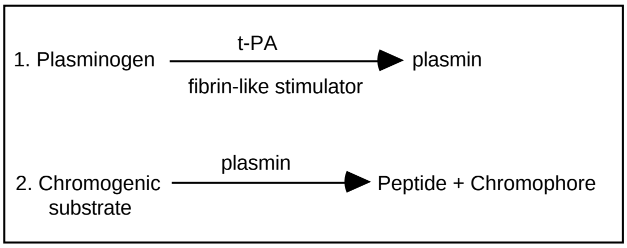


***Figure 7.** Chromogenic substrate assay for t-PA*

Diagnostic kits from Chromogenix

Product range

Chromogenix has a wide range of chromogenic peptide substrates as well as complete assay kits with applications extending from routine analysis to front-line research in both coagulation and fibrinolysis (Table 3).[74] The tests can be performed manually or on automated analytical systems with high specificity, sensitivity and accuracy. Important, non-chromogenic based products include kits for APC resistance (an APTT-test) and several ELISA kits (Table 4).

t-PA kits

COATEST® BIA t-PA is the first convenient, specific and sensitive bioimmunoassay that not only measures the functional level of t-PA, but also discriminates between high and low levels of PAI-1. The test can be used with undiluted plasma and does not require venous occlusion. Blood samples may be taken at low pH in Stabilyte® (acid citrate) tubes, followed by centrifugation.

COALIZA® t-PA is a conventional solid phase enzyme immunoassay (ELISA) for the detection of t-PA antigen in biological fluids. The assay is highly specific for one-chain and two-chain t-PA.

HEMOSTASIS
Prekallikrein
Factor VII
Factor VIII
Factor X
Soluble fibrin
Antithrombin
Heparin/LMW heparin
Protein C
α_2-macroglobulin
α_1-antitrypsin
Plasminogen
t-PA
PAI-1
Plasmin inhibitor

ENDOTOXIN
Endotoxin

Table 3. *Substances that can be determined with diagnostic kits based on synthetic peptide substrates (Examples from Chromogenix product range 1995).*

HEMOSTASIS
APC resistance
Anti-Cardiolipin IgG, IgM
D-dimer
Lipoprotein(a)
t-PA
PAI-1

INFLAMMATION/ SEPSIS
EndoCAb
IL-6
TFNa
Endotoxin

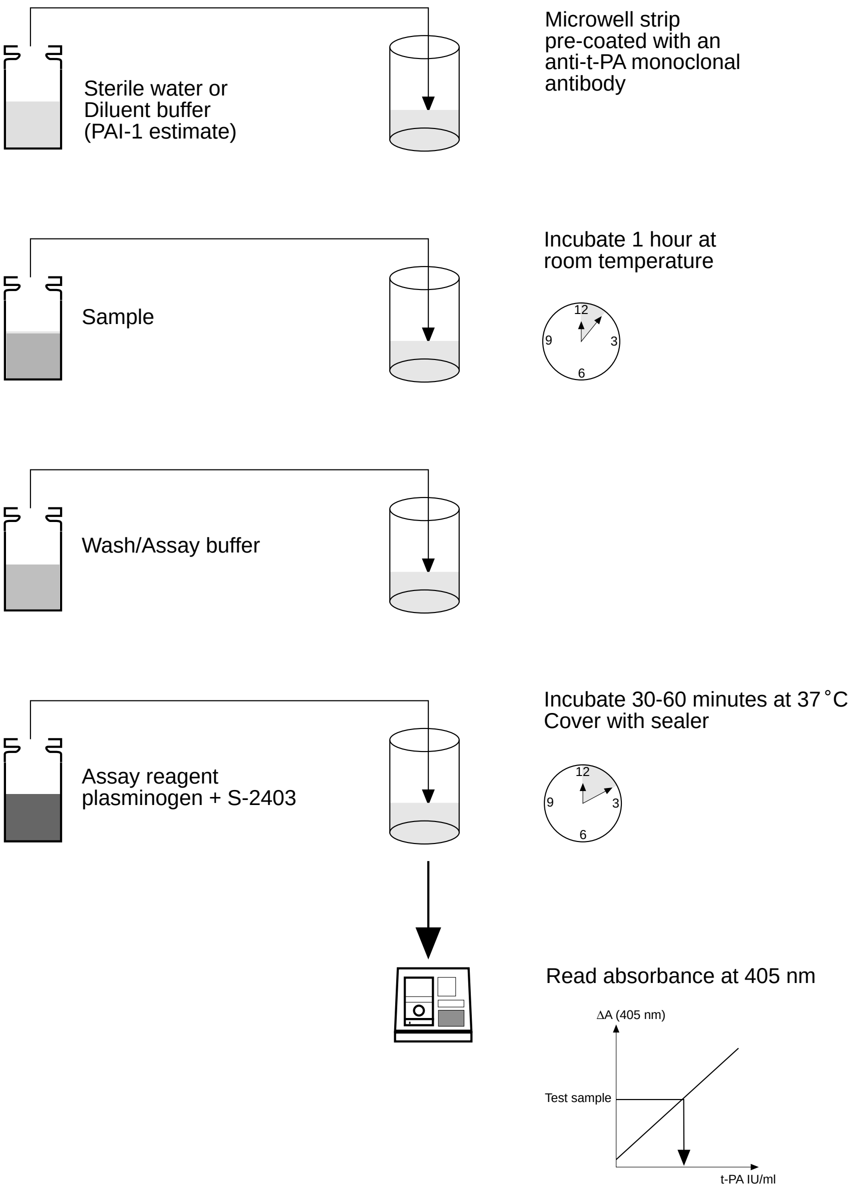
Table 4. *Substances that can be determined with kits from Chromogenix, based on ELISA or clotting techniques .*

t-PA Diagnostic Indications

- **Cardiovascular disease**
- **Deep vein thrombosis**
- **Bleeding diathesis**
- **Monitoring thrombolytic therapy**
- **Malignancy**

Assay Procedure

COATEST® BIA t-PA



COATEST®
BIA t-PA

COATEST® BIA t-PA is a solid-phase bioimmunoassay designed for the determination t-PA activity and for the estimation of PAI-1 activity in plasma.

Measurement principle

A microwell strip is coated with mouse IgG monoclonal antibodies that bind t-PA in plasma without interfering with the molecule's active site. During incubation of a sample, t-PA will be bound to the solid phase. Unbound substances are removed by washing followed by the addition of a mixture of plasminogen and the chromogenic plasmin substrate S-2403. Active t-PA bound to antibodies will activate plasminogen to plasmin, which then cleaves the chromogenic substrate, resulting in the release of pNA. The colour change is proportional to the amount of t-PA present in the sample and can be read photometrically at 405 nm.

PAI-1 estimations

Estimates of PAI-1 levels can be obtained by the incubation of the sample with the microwell strip at pH 6 (preventing PAI-1 interference), or at pH 8 (allowing PAI-1 interference). The ratio of t-PA activities, at neutral pH and low pH, shows the influence of PAI-1.

Article number: 82 24 60

The kit contains

Anti-t-PA coated microwell strips	12 x 8
Diluent buffer	1 vial
Wash/Assay buffer	2 vials
Assay reagent (S-2403, human Glu-plasminogen)	1 vial
t-PA Standard	1 vial
t-PA Control	1 vial

Storage and stability

Microwell strips	2–8 °C, 1 year
Buffers	2–8 °C, 1 month
Assay reagent	2–8 °C, 6 hours
	-20 °C, 6 months
t-PA Standard/Control	2–8 °C, 1 month

Measuring range

Low range: 0,02 - 0,5 IU/ml
Normal range: 0,25 - 2,0 IU/ml

Detection limit

0,015 IU/ml

Repeatability

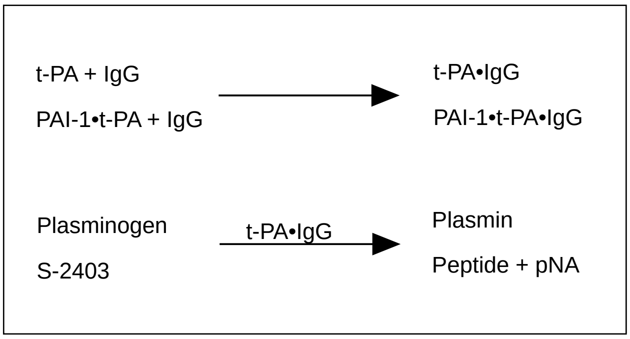
t-PA	CV within series	CV between
0.4 IU/ml	7%	-
0.8 IU/ml	5%	-
0.4–1.4 IU/ml	-	<5%

Specificity and interfering factors

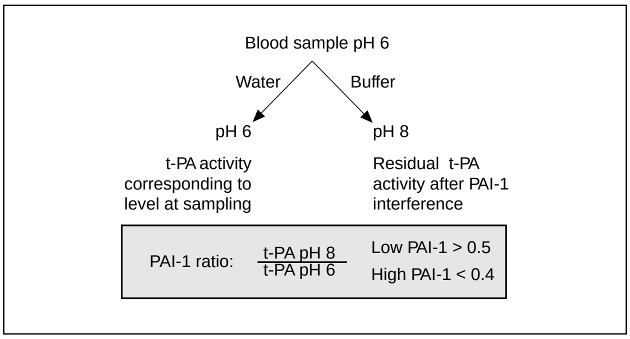
No influence of PAI-1 if analysed in Stabilyte®/aq.
No influence from α_2-macroglobulin, plasmin inhibitor, endogenous levels of scu-PA or u-PA. Aprotinin levels up to 400 KIU/ml have no effect on the assay.[69]

Applications per kit

96 microwell strips



Schematic principle of Coatest® BIA t-PA using a mouse lgG monoclonal antibody against t-PA (See also Figure 5).



Schematic principle of PAI-1 estimates.

COALIZA® t-PA

COALIZA® t-PA is a solid-phase enzyme-linked immunosorbent assay (ELISA) designed for the detection of human t-PA antigen in biological fluids such as plasma, tissue extract and culture supernatant.

Measurement principle

A microwell strip, coated with mouse monoclonal anti-t-PA antibodies, is incubated with a sample and t-PA antigen present is bound to the solid phase. Unbound substances are then removed by washing. An enzyme-labelled anti-t-PA monoclonal antibody (conjugate) is then added. The conjugate forms a sandwich-like complex with the antibody-antigen complex formed in the previous step. Unbound conjugate is then removed by a second washing. Finally, enzyme substrate is added. The action of the conjugated enzyme on the substrate produces a blue colour which turns yellow when the reaction is stopped with acid. The colour change is proportional to the amount of t-PA present in the sample and can be read photometrically at 450 nm.

Article number: 82 15 38

The kit contains

Anti-t-PA coated microwell strips	12 x 8
Conjugate, stock solution	1 vial
Conjugate diluent	1 vial
Sample diluent	2 vials
t-PA standards	6 vials
Substrate buffer	1 vial
Substrate, stock solution	1 vial
Washing buffer, stock solution	2 vials

Storage and stability

Microwell strips	2-8 °C, 8 weeks
Others	2-8 °C, exp. date

Measuring range
1 - 20 ng/ml

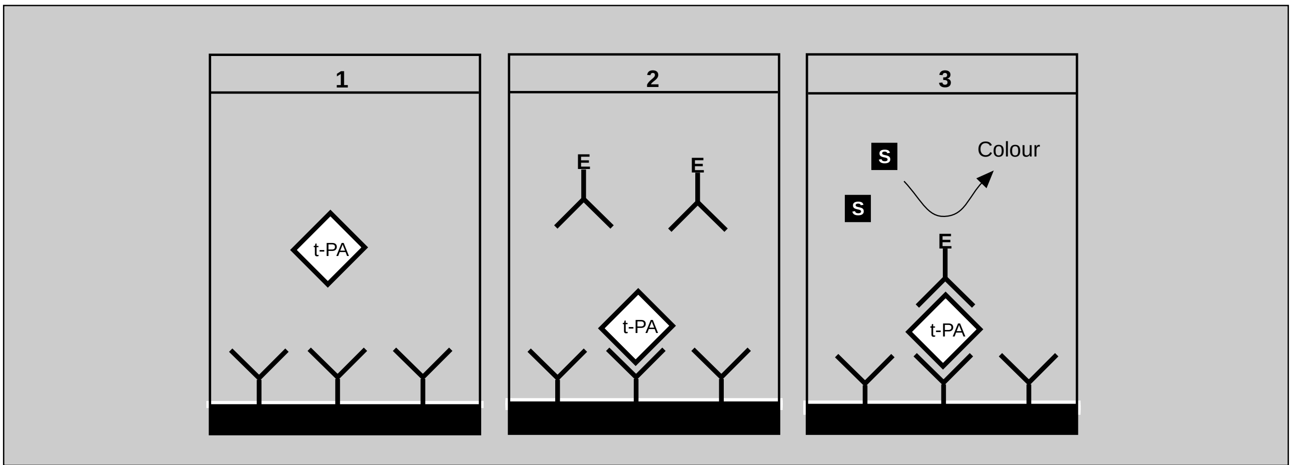
Detection limit
0,5 ng/ml

Repeatability

t-PA	CV within series	CV between
1 ng/ml	-	7%
10 ng/ml	7%	4%
20 ng/ml	7%	-

Specificity and interfering factors
Heparin concentrations below 2 IU/ml will not affect the assay

Applications per kit
Microwell 96 (12 x 8)



Measurement principle COALIZA® t-PA

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Allele. One of an array of possible mutational forms of a gene at a specific locus

Amino acids. Basic building blocks of all proteins

Antibody. A molecule produced by animals in response to antigen.

Antigen. A molecule which induces the formation of antibody.

APC resistance. An hereditary defect caused by a point mutation in the gene coding for factor V and characterized by a poor anticoagulant response to activated protein C.

Anaphylactic shock. A term used to denote the immediate, transient kind of allergic reaction characterized by contraction of smooth muscle and dilation of capillaries.

Autosome. A chromosome other than a sex chromosome.

Cardiovascular disease. Mainly comprising coronary artery disease leading to myocardial infarction, cerebrovascular disease causing stroke, venous thrombosis predisposing to pulmonary embolism, and the post-phlebitic syndrome.

Chromosome. The darkly staining bodies within the cells made up of a large number of genes and a centromere region.

Circadian. Relating to biological variations or rythms with a cycle of 24 hours

Embolism. Obstruction or occlusion of a vessel by a transported clot.

Endothelium. Cells lining blood vessels and lymphatics which control the passage of materials into and out of the bloodstream.

Enzymes. A protein with catalytic power.

Exon. Gene segment encoding protein.

Fibrin. An elastic filamentous protein derived from fibrinogen by the action of thrombin, which releases fibrinopeptides A and B from fibrinogen.

Fibrinogen. Factor I; a globulin of the blood plasma that is converted into the coagulated protein, fibrin, by the action of thrombin in the presence of calcium ions.

Fibrinolysis. The hydrolysis of fibrin by plasmin.

Gene. The unit of inheritance, located at a specific region on the chromosome.

Glycoprotein. One of a group of protein-carbohydrate compunds

Hemostasis. Process which arrests the escape of blood from injured vessels.

Homozygous. Condition of having identical alleles at one or more loci under consideration

Heparin cofactor II. Serpin with heparin cofactor abilities. Specific inhibitor of thrombin.

Heterozygous. Having a dissimilar alleles at one or more loci.

Idiopathic deep vein thrombosis. Is usually defined as the occurence of deep vein thrombosis in patients younger than 45 who do not present any underlying disease (cancer, cardiac failure etc.).

Intron. Gene segment between exons not encoding protein.

Locus. The position on a chromosome at which a particular gene is found.

Platelets. A little disk-shaped blood cell, containing granules in the central part and peripherally, clear protoplasma, but no nucleus. Numbering 200,000 to 300,000/µl.

Proteases, proteinases. Enzymes hydrolyzing native protein, or polypeptides, making internal cleavages; they include pepsin, chymosin, trypsin, papain etc.

Proteins. A class of macromolecules that are built from a repertoire of twenty amino acids.

Proteoglycan. A macromolecular glycoconjugate composed of sulfated glycosaminoglycans covalently linked to a protein core.

Proteolysis. Enzymatic cleavage of protein.

Prothrombin. Factor II, zymogen of thrombin; a glycoprotein formed and stored in the parenchymal cells of the liver. Present in blood at approximately 100 µg/ml.

Receptor. A cell surface molecule which binds specifically to particular proteins or peptides in the fluid phase.

Sepsis. A clinical syndrome of serious bacterial infection.

Serine protease. Proteolytic enzyme with a serine residue at its enzymatically active site.

Thrombocyte. Blood platelet

Thrombocytopenia. A conditon in which there is an abnormally small number of platelets in the circulating blood (usually less than 150,000/µl).

Thromboembolism. Refers to either thrombosis or embolism or a combination of both.

Thrombolytics. Biological and synthetic substances capable of activating the fibrinolytic system in plasma.

Thrombin. Active protease deriving from prothrombin (factor II). Induces conversion of fibrinogen into clot-forming fibrin monomers resulting in the coagulation of blood.

Thrombophilia. A disorder in which there is a tendency to develop thrombosis.

Thrombosis. The formation of a thrombus (blood clot).

Thrombotic. Relating to, caused by, or characterized by thrombosis.

Zymogens. The enzymatically inactive precursors of proteolytic enzymes.

Notes

t-PA

Chromogenix Product Monographs

Antithrombin
COAMATIC® Antithrombin
COAMATIC® AT 400
COAMATIC® LR Antithrombin
COATEST® Antithrombin
COACUTE® Antithrombin R

APC resistance
COATEST® APC Resistance
COATEST® APC Resistance – C
COATEST® APC Resistance – S
COATEST® APC Resistance – SC
COATEST® APC Resistance V
COATEST® APC Resistance V-S
COASET® FV-506

D-dimer
SimpliRED® D-dimer
DIMERTEST® GOLD EIA

Factor VIII
COAMATIC® Factor VIII
COATEST® Factor VIII
COATEST® VIII:C/4

Heparin
COATEST® Heparin
COATEST® LMWHeparin/Heparin
COACUTE® Heparin

Plasminogen
COAMATIC® Plasminogen
COATEST® Plasminogen

Protein C
COAMATIC® Protein C

t-PA
COASET® t-PA
COALIZA® t-PA

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Complete enzyme-immunoassay (ELISA)-based kits for antigen determinations.

COACUTE®
For a small number of tests. All the reagents are freeze-dried in a single test cuvette.

COASET®
A group of products aimed for research applications.

COAMAB®
Monoclonal antibodies for research purpose.

t-PA_mono_v1.1. Printed in Sweden on non-acid paper.

CHROMOGENIX

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