D-Dimer and High Molecular Weight Fibrin Degradation Products

Fibrinogen is a blood protein from which fibrin clots are formed upon blood coagulation or thrombotic process. Fibrinogen consists of two identical subunits that contain three polypeptide chains: α, β, and γ. During blood coagulation fibrinogen is first converted into fibrin by thrombin, and these fibrin monomers then polymerize to form fibrin clots. In fibrinolysis, the fibrin clots are digested by plasmin, and fibrin degradation products (FDP) of different molecular weights are released into the bloodstream (Fig. 1). D-dimer (MW 180 kDa) is the final product of fibrin degradation. It consists of the remnants of all three chains (α, β and γ chains) of fibrinogen cross linked by disulfide bonds. The dimeric structure of D-dimer is held by two covalent, intermolecular isopeptide bonds between the γ-chains.

D-dimer in diagnostics

D-dimer levels in healthy individuals are less than 0.5 μg/ml. Elevated levels of D-dimer have been found in the blood of patients with pulmonary embolism (PE), deep vein thrombosis (DVT) and atherosclerosis. The elevated level of D-dimer in blood is believed to be a reliable marker of pathological coagulation that underlies the pathogenesis of most cardiovascular diseases (1, 2). It is widely used to exclude the diagnosis of deep vein thrombosis (3).

Despite the long history of using the D-dimer test in clinical practice, there are a lot of problems concerning the quantitative determination of D-dimer in plasma samples. A patient's plasma contains a wide spectrum of FDP of different sizes along with D-dimer itself. All of these products possess the "D-dimer antigen epitope". Therefore, antibodies specific to D-dimer also recognize FDP. However, there is a great variance between the results obtained by different assays. This can be explained by differences in antibody specificities; some antibodies and antibody pairs recognize D-dimer better than FDP and vice versa. So far all standardization and harmonization attempts have not resulted in satisfying results and this is a continuous cause of problems in daily practice (4).

For an accurate determination of all FDP and D-dimer, and for using D-dimer as a standard, MAbs should detect FDP and D-dimer with equal specificity. In addition, assays for D-dimer must not detect fibrinogen whose concentration in plasma is 1000 times higher than that of D-dimer.

CLINICAL UTILITY

Marker of pathological blood coagulation

Rule out the presence of thrombus causing DVT or PE

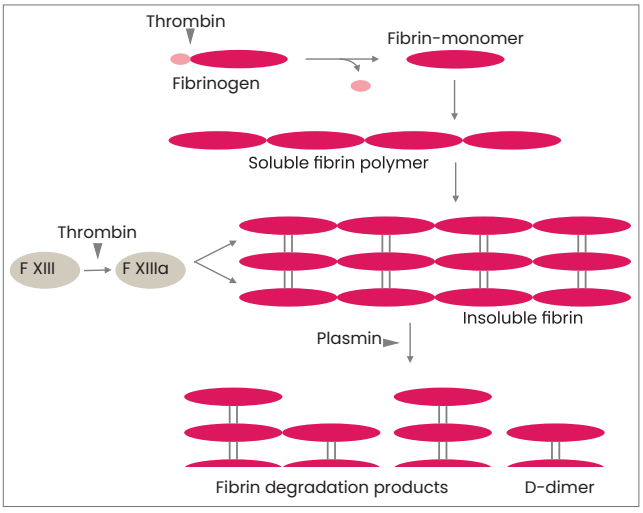

Figure 1.

A scheme of fibrin formation and fibrinolysis.

Assay development and pair recommendations

For development of D-dimer assays, we provide several monoclonal antibodies specific for D-dimer and FDP. In addition to antibodies, we offer D-dimer antigen that is produced from clotted fibrinogen by means of plasmin digestion.

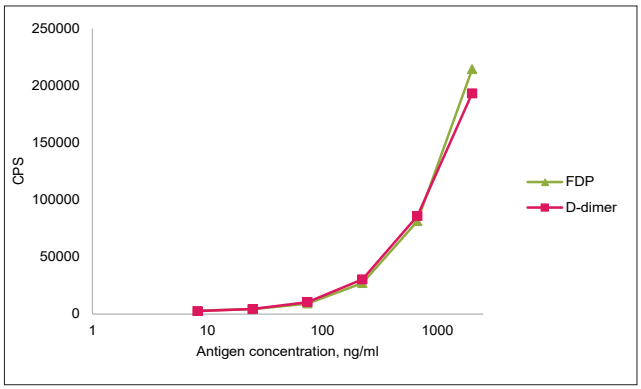

Figure 2.

Antibody pair DD189-DD255 detects FDP and D-dimer with equal specificity. *The plate wells were coated with 100 µl of MAb DD189 (10 µg/ml in PBS) and incubated for 1 hour at room temperature. After three washes with TBS containing 0.05% Tween 20, 50 µl of Eu^{3+}-labeled MAb DD255 (4 µg/ml in Delfia assay buffer) and 25 µl of D-dimer or FDP dilutions were added and incubated at shaking for 1 hour at room temperature. After washing, 300 µl of Lanfia solution was added to every well, and after 3 minutes of intensive shaking the fluorescent signals were measured in a Victor 1420 Victor™ Multilabel Counter (Wallac, Finland).*

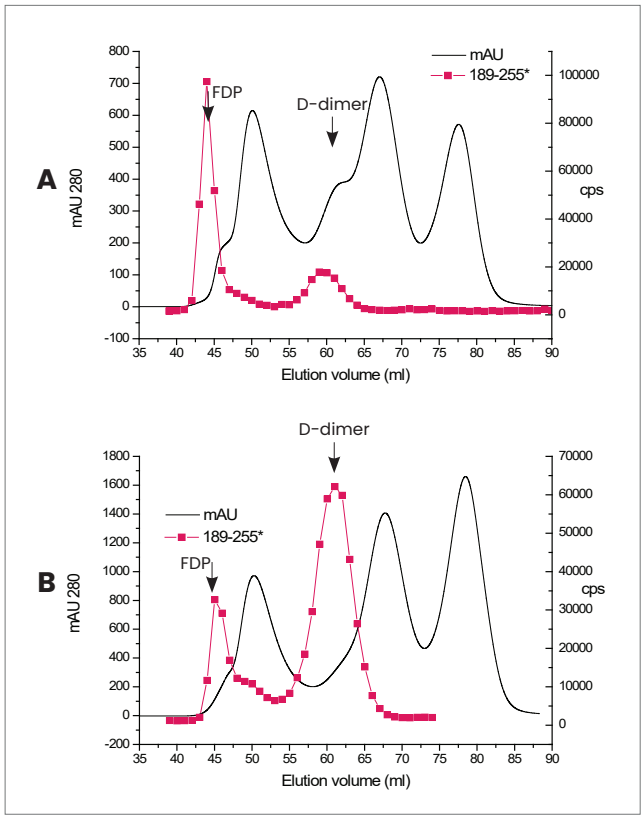

Figure 3.

HPLC gel filtration of plasma samples from patients with thrombosis (A) and after a surgical operation (B). *200-500 µl of plasma was applied to the Superdex 200 column 16/60 at a flow rate of 1 ml/min. 1 ml fractions were analyzed by the DD189-DD255 pair in a sandwich assay as described in Figure 3.*

MONOCLONAL ANTIBODIES SPECIFIC TO D-DIMER AND FDP

FDP and D-dimer, the most degraded form of FDP, appear in human blood as a result of proteolytic degradation of fibrin clots. The ratio of these products is not constant but varies from patient to patient (see Fig. 3). To decrease bias inquantitation of these degradation products, we have developed an assay which recognizes both FDP and D-dimer with equal specificity. This concept could potentially be one step forward in the attempt to achieve D-dimer assay standardization.

A quantitative sandwich immunoassay that is equally specific for D-dimer and FDP

Hytest offers new MAbs (DD189cc and DD255cc) that recognize D-dimer and high molecular weight fibrin degradation products with equal specificity in a sandwich FIA up to 1 µg/ml antigen concentration (Fig. 2). To be analyzed in a sandwich immunoassay, plasma can be diluted ten-fold with 20 mM Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl.

Both MAbs stained D-dimer in Western blotting under reducing and non-reducing conditions (Fig. 6 A and B).

The development and characterization of these MAbs is described in the article by Kogan et al. (5).

The ratio of D-dimer and FDP varies between patients

We analyzed plasma from patients with two different disorders using gel filtration. The results show that the ratio of D-dimer and FDP is not constant (Fig. 3). This finding further supports the idea that an immunoassay should equally recognize D-dimer and FDP to allow for a more accurate determination of all products resulting from fibrin degradation.

Table 1.

Recommended pairs to be used in a sandwich immunoassay for D-dimer detection in human plasma. *Note, these recommendations and observations are based on results obtained using our in-house DELFIA® immunoassay platform.*

Pair (capture-detection)	Remarks
DD189cc – DD255cc	Equal specificity for D-dimer and high MW fibrin degradation products
DD2 – DD41cc	Slightly more specific for high MW fibrin degradation products
DD2 – DD4*	Approximately equal specificity for D-dimer and high MW fibrin degradation products.

* Due to the cross-reactivity of DD4 with fibrinogen, we strongly recommend to use it as the detection antibody. In a sandwich immunoassay, plasma must be diluted at least two-fold with 10 mM Tris-HCl, pH 7.5, 1 M NaCl, 0.1 % Tween 20 in order to avoid nonspecific binding. Each step in the assay should be followed by an incubation and wash: coating with the capture MAb, addition of the sample and addition of the (conjugated) detection MAb.

Antibody recommendations for quantitative sandwich immunoassays

The recommended pairs are listed in Table 1. They are specific to cross-linked material (D-dimer and high molecular weight fibrin degradation products) in samples and do not detect fibrinogen (Fig. 4).

Our pair recommendations for chemiluminescence (CLIA) and Lateral flow (LF) platforms are described in Table 2. The new recommended pairs show good correlation with commercially available D-dimer assay (Fig. 5).

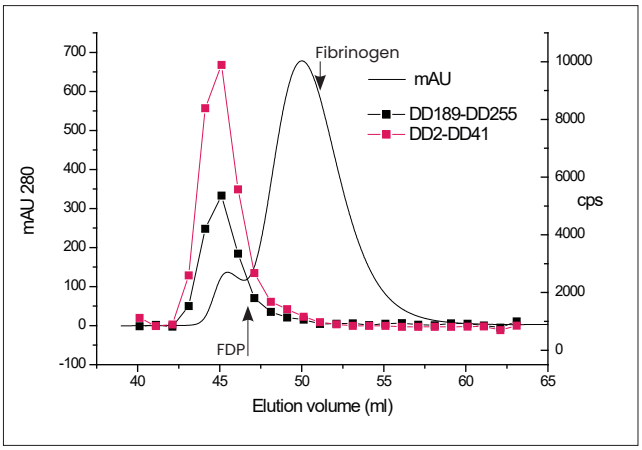

Figure 4.

Immunoassays with DD2-DD41 and DD189-DD255 show no cross-reactivity with fibrinogen. *5 mg of fibrinogen (Calbiochem) was applied to the Superdex 200 column 16/60 using TBS, pH 7.5 at a flow rate of 1 ml/min. 1 ml fractions were analyzed by DD2-DD41 and DD189-DD255 pairs in a sandwich assay as described in Figure 3. The results demonstrate that the D-dimer assays do not detect fibrinogen, however, some high molecular weight fibrin degradation products are present in the preparation.*

Table 2.

New pair recommendations for chemiluminescence and lateral flow platforms.

Capture	Detection	Platform
DD189cc	DD255cc	CLIA
DD255cc	DD41cc	CLIA, LF
DD3cc	DD46cc	CLIA, LF

Anti-D-dimer MAbs can be used in Western blotting

Anti-D-dimer antibodies can be used in Western blotting to detect D-dimer. All MAbs stained non-reduced D-dimer and some of them stained reduced D-dimer as well (Fig. 6 A and B, respectively).

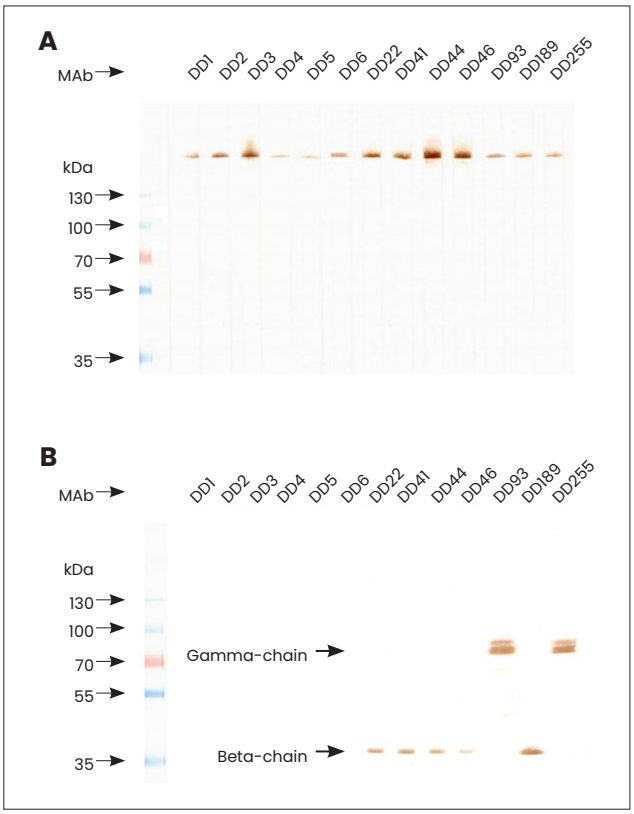

Figure 6.

Western blot of D-dimer. *D-dimer (Cat.# 8D70) was run in SDS-PAGE under non-reducing (A) or reducing (B) conditions using a 7.5–12.5% separating gel and transferred onto a nitrocellulose membrane. The membrane was blocked by 7% milk in PBST for 30 minutes and the protein bands were stained by different anti-D-dimer MAbs (10 µg/ml) for 1 hour using a Mini-Protean® II Multi Screen (Bio-Rad). After washing with PBST, goat anti-mouse Fc-specific IgG labeled with horseradish peroxidase (diluted as recommended by the manufacturer) was added and incubated for 1 hour. After washing with PBST, the immune complexes were visual-ized by DAB/hydrogen peroxide in 50 mM Tris-HCl buffer, pH 7.5.*

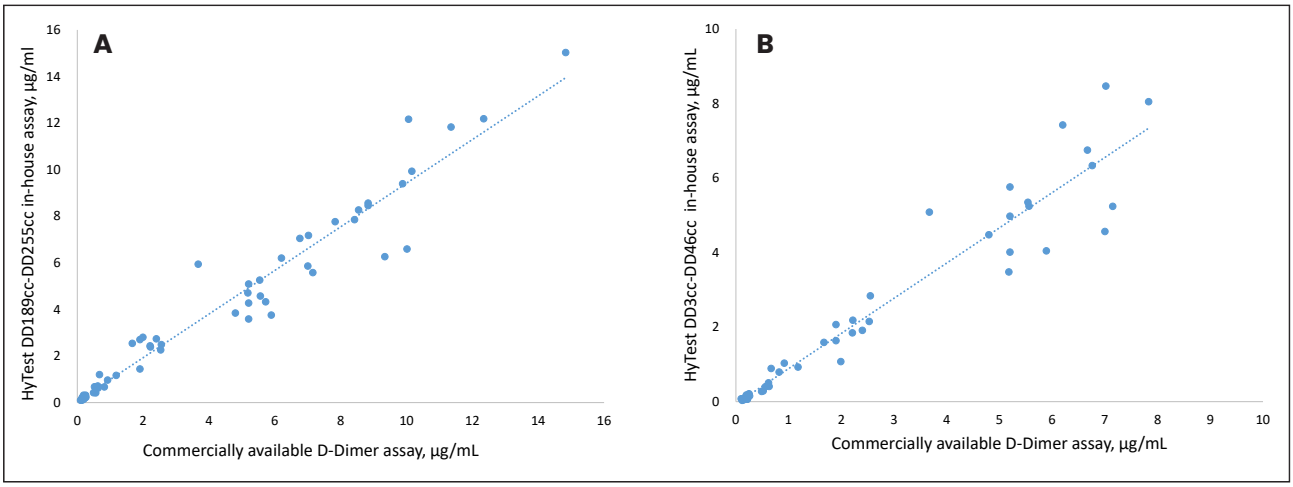

Figure 5. ***Correlation study of Hytest in-house assay and commercially available D-dimer assays.***

A) Hytest in-house assay DD189cc-255cc with clinical sample amount of 65. B) Hytest in-house assay DD3cc-DD46cc with clinical sample amount 65.

HUMAN D-DIMER

Hytest has been one of the world's leading suppliers of D-dimer antigens for more than 10 years. We offer a highly purified D-dimer prepared from human plasma.

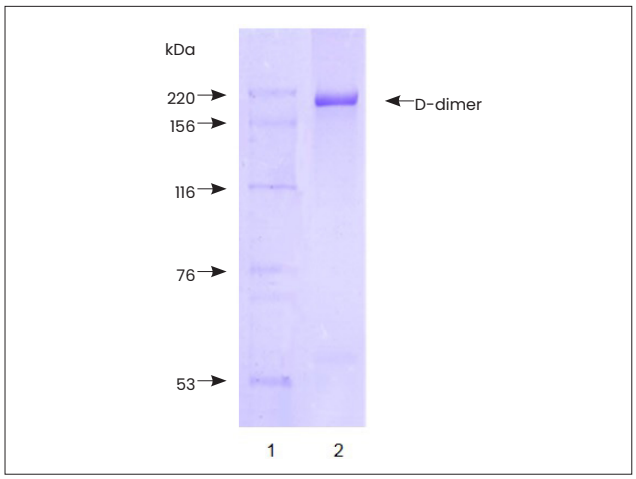

Figure 2.
SDS-PAGE of purified D-dimer under non-reducing conditions. *The gel was stained using Coomassie Brilliant Blue R-250.*
Lane 1: MW standard
Lane 2: D-dimer (3 µg)

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1. **Bounameaux H, et al.** (1994) Plasma measurement of D-dimer as diagnostic aid in suspected venous thrombo-embolism: an overview. Thromb Haemost. 71, 1-6.
2. **Rowbotham BJ, et al**. (1987) Measurement of crosslinked fibrin derivatives – use in the diagnosis of venous thrombosis. Thromb Haemost. 57, 59-61.
3. **Righini M, et al.** (2008) D-Dimer for venous thrombo-embolism diagnosis: 20 years later. J Thromb Haemost. 6, 1059-71.
4. **Reber G. and de Moerloose P.** (2009) Standardization of D-dimer testing. Quality in Laboratory and Thrombosis 99-109.
5. **Kogan, AE et al.** (2016) Monoclonal antibodies with equal specificity to D-dimer and high-molecular-weight fibrin degradation products. Blood Coagul. Fibrinolysis. 27: 542–550.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
D-dimer	4D30	DD1	IgG2a	EIA, WB, N/cr with fibrinogen
		DD2	IgG2b	EIA, WB, N/cr with fibrinogen
		DD3cc	IgG2b	*In vitro*, EIA, WB, N/cr with fibrinogen
		DD4 *	IgG2b	EIA, WB, C/r with fibrinogen (use as detection MAb)
		DD5 *	IgG2b	EIA, WB, C/r with fibrinogen (use as detection MAb)
		DD6cc *	IgG2a	*In vitro*, EIA, WB, C/r with fibrinogen (use as detection MAb)
		DD22	IgG2a	EIA, WB, N/cr with fibrinogen
		DD41cc	IgG2a	*In vitro*, EIA, WB, N/cr with fibrinogen
		DD44cc	IgG2b	*In vitro*, EIA, WB, N/cr with fibrinogen
		DD46cc	IgG2a	*In vitro*, EIA, WB, N/cr with fibrinogen
		DD93	IgG1	EIA, WB, N/cr with fibrinogen
		DD189cc *	IgG1	*In vitro*, EIA, WB, N/cr with fibrinogen
		DD255cc *	IgG1	*In vitro*, EIA, WB, N/cr with fibrinogen

* Note. If used as a capture antibody, the assay may give false positives for patients treated with streptokinase.

ANTIGEN

Product name	Cat. #	Purity	Source
D-dimer	8D70	>90%	Human plasma

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • HYTEST.FI