Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

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, intermolecularisopeptide bonds between the γ-chains.

CLINICAL UTILITY

- **Marker of pathological blood coagulation**
- **Rule out the presence of thrombus causing DVT or PE**

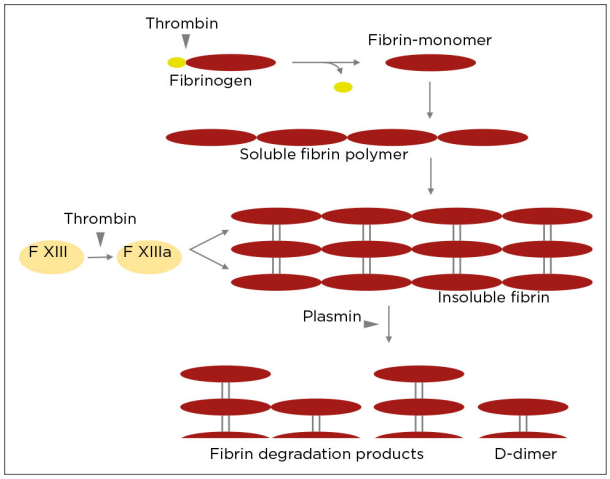

FIGURE 1. A scheme of brin formation and brinolysis.

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 atherosclero-sis. 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 speci city. In addition, assays for D-dimer must not detect fibrinogen whose concentration in plasma is 1000 times higher than that of D-dimer.

Assay development and pair recommendations

For development of D-dimer assays, we provide several monoclonal antibodies specfic for D-dimer and FDP. The recommended capture-detection pairs for sandwich

immunoassays are shown in Table 1. In addition to antibodies, we offer D-dimer that is produced from clotted fibrinogen by means of plasmin digestion.

Human D-dimer

We offer a highly purified D-dimer prepared from human plasma.

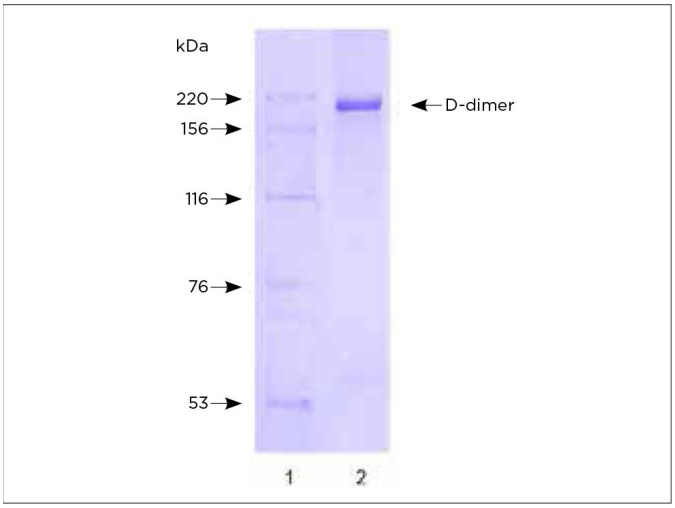

FIGURE 2. SDS-PAGE of purified D-dimer under non-reducing conditions.
The gel was stained using Coomassie Brilliant Blue R-250.
Lane 1: MW standard
Lane 2: D-dimer (3 µg)

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. 4). To decrease bias in quantitation 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

Advanced ImmunoChemical offers new MAbs (DD189 and DD255) 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. 3). To be analyzed in a sandwich immunoassay, plasma can be diluted ten-fold with 20 mM Tris-HCl bu er, 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).

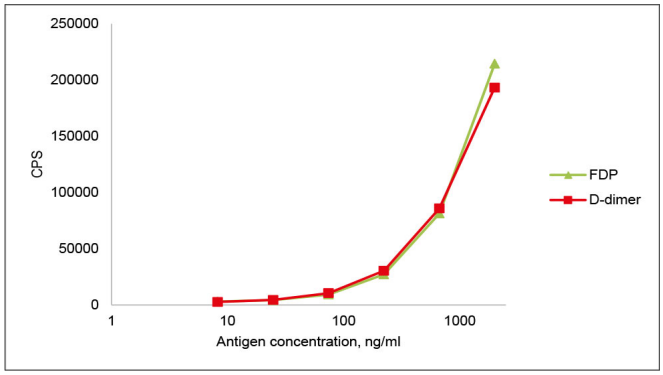

FIGURE 3. Antibody pair DD189-DD255 detects FDP and D-dimer with equal specificity.

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).

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. 4). 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.

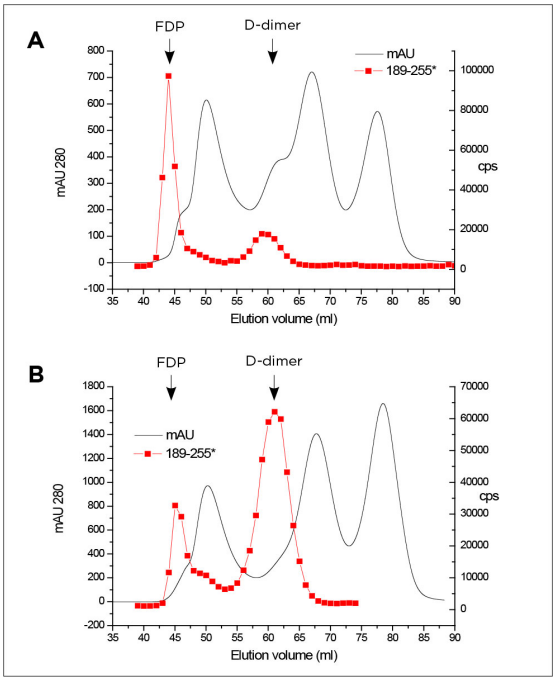

FIGURE 4. HPLC gel ltration of plasma samples from patients with thrombosis (A) and after a surgical operation (B).

200-500 µl of plasma was applied to the Superdex 200 column 16/60 at a ow rate of 1 ml/min. 1 ml fractions were analyzed by the DD189-DD255 pair in a sandwich assay as described in Figure 3.

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. 5).

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
DD189 – DD255	Equal specificity for D-dimer and high MW fibrin degradation products
DD2 – DD41	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.*

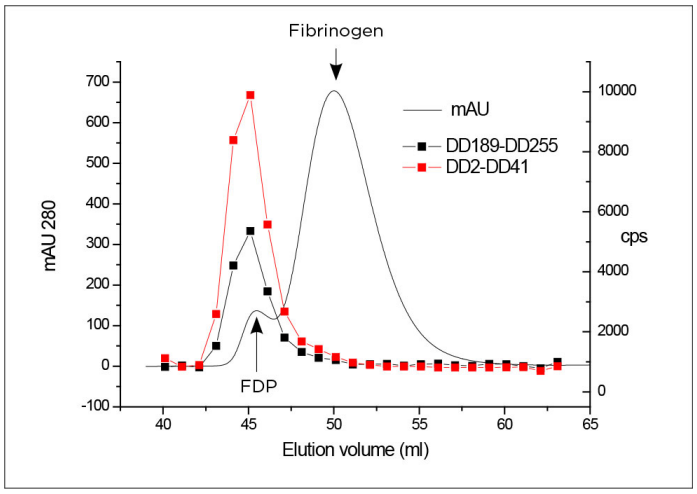

FIGURE 5. Immunoassays with DD2-DD41 and DD189-DD255 show no cross-reactivity with fibrinogen. *5 mg of fibrinogen (Calbiochem) was applied to the Superdex 200 column 16/60 using TBS, pH 7.5 at a flow rate of 1 ml/min. 1 ml fractions were analyzed by DD2-DD41 and DD189-DD255 pairs in a sandwich assay 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.*

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).

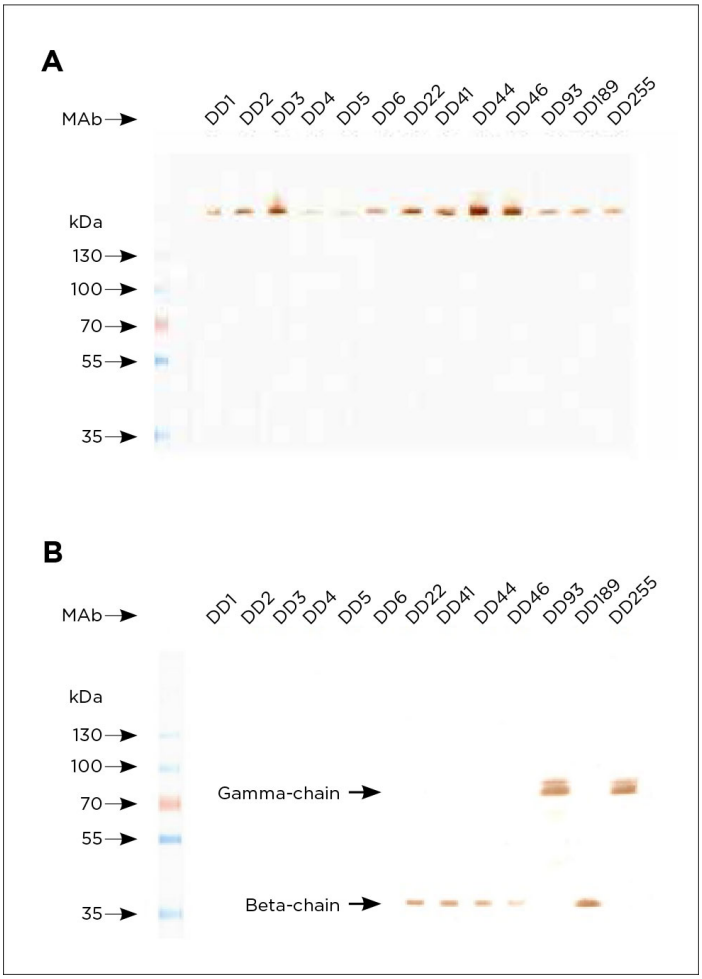

FIGURE 6. Western blot of D-dimer. *D-dimer was run in SDS-PAGE under non-reducing (A) or reducing (B) conditions using a 7.5–12.5% separating gel and transferred onto a nitrocellulose membrane. The membrane was blocked by 7% milk in PBST for 30 minutes and the protein bands were stained by different 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 visualized by DAB/ hydrogen peroxide in 50 mM Tris-HCl bu er, pH 7.5.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
D-dimer	2-DD1	DD1	IgG2a	EIA, WB, N/cr with fibrinogen
		DD2	IgG2b	EIA, WB, N/cr with fibrinogen
		DD3	IgG2b	EIA, WB, N/cr with fibrinogen
		DD4 *	IgG2b	EIA, WB, N/cr with fibrinogen (use as detection MAb)
		DD5 *	IgG2b	EIA, WB, N/cr with fibrinogen (use as detection MAb)
		DD22	IgG2a	EIA, WB, N/cr with fibrinogen
		DD41	IgG2a	EIA, WB, N/cr with fibrinogen
		DD44	IgG2b	EIA, WB, N/cr with fibrinogen
		DD46	IgG2a	EIA, WB, N/cr with fibrinogen
		DD93	IgG1	EIA, WB, N/cr with fibrinogen
		DD189 *	IgG1	EIA, WB, N/cr with fibrinogen
		DD225 *	IgG1	EIA, WB, N/cr with fibrinogen

Ordering Information:

ANTIGEN

Product	Cat #	Purity	Source
D-dimer	8-DD1	>90%	Human Plasma

References

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 brin 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.

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