Introduction of Products

Overview of *Innovance*® D-DIMER of New D-Dimer Reagent

Nobuo ARAI[*1] and Ann COLLINS[*2]

[*1] Scientific Research Division, Sysmex Corporation, 1-3-2 Murotani, Nishi-ku, Kobe 651-2241, Japan.
[*2] IVD Alliance Group, Sysmex Corporation, 1-5-1 Wakinohama-Kaigandori, Chuo-ku, Kobe 651-0073, Japan.

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INTRODUCTION

D-Dimer is a specific marker for cross-linked fibrin degradation products, and its presence in human plasma is an indicator of fibrinolytic activity. Elevated D-Dimer levels are observed in all diseases and conditions associated with increased coagulation activation, e.g. thromboembolic disease, disseminated intravascular coagulopathy (DIC), acute aortic dissection, myocardial infarction, malignant diseases, obstetrical complications, third trimester of pregnancy, surgery or polytrauma[1-6].

D-Dimer measurement is therefore widely used in the diagnostic work-up of thromboembolic disease, and its determination is used for various diagnostic purposes. The major application of D-Dimer testing is in the exclusion of thromboembolic events, such as for outpatients suspected of having deep vein thrombosis (DVT) or pulmonary embolism (PE) by following a non-invasive diagnostic algorithm[1, 2].

Recently, "*Innovance*® D-DIMER" has been launched by Dade Behring as a high quality, automated, particle-enhanced D-Dimer assay with immunoturbidimetric application on CA analyzers.

Here, we will describe the concept and performance of this new D-Dimer reagent according to Dade Behring's information and evaluation data.

OVERVIEW OF KITS

Innovance® D-DIMER is a particle-enhanced, immunoturbidimetric assay designed for use on coagulation analyzers for the quantitative determination of cross-linked fibrin degradation products (D-Dimers) in human plasma. It is recommended to use this in conjunction with a clinical pre-test probability assessment model for excluding suspected DVT and PE in outpatients.

Innovance® D-DIMER is available in two different pack sizes: a 150 tests kit for medium to small throughput laboratories and a 300 tests kit for medium to high throughput laboratories. These kits consist of four reagents and one calibrator with new product labeling according to color coding and symbology (***Fig. 1*** and ***Table 1***). Two levels of control are separately available. The color on the vial label is the same as the cap and this modification will enable easier and safer product handling of this D-Dimer assay.

COMPONENTS OF KITS

***Innovance*® D-DIMER REAGENT** (green) is a lyophilized latex reagent that contains polystyrene particles coated with monoclonal mouse antibodies (8D3)[7] for determination of D-Dimer in the sample.

Fig. 1 *Color coding and symbology applied to the Innovance® D-DIMER reagents*

Table 1 *Components of the Innovance® D-DIMER Kit*

***Innovance®* D-DIMER Kit (for 150 tests)**	
3× for 4 mL	*Innovance®* D-DIMER Reagent
3× 5 mL	*Innovance®* D-DIMER Buffer
3× 2.6 mL	*Innovance®* D-DIMER Supplement
3× 5 mL	*Innovance®* D-DIMER Diluent
3×	Empty vials for each kit component except calibrator
2× for 1 mL	*Innovance®* D-DIMER Calibrator
***Innovance®* D-DIMER Kit (for 300 tests)**	
6× for 4 mL	*Innovance®* D-DIMER Reagent
6× 5 mL	*Innovance®* D-DIMER Buffer
6× 2.6 mL	*Innovance®* D-DIMER Supplement
6× 5 mL	*Innovance®* D-DIMER Diluent
2× for 1 mL	*Innovance®* D-DIMER Calibrator
***Innovance®* D-DIMER Controls**	
5× for 1 mL	*Innovance®* D-DIMER Control 1 (for the normal range)
5× for 1 mL	*Innovance®* D-DIMER Control 2 (for the pathological range)

***Innovance®* D-DIMER BUFFER** (orange) is a buffered saline solution with detergent and polymeric carbohydrates for signal enhancement.

***Innovance®* D-DIMER SUPPLEMENT** (yellow) is a buffered saline solution that contains a blocking reagent for the inhibition of nonspecific reactions against heterophilic antibodies such as rheumatoid factors and human anti-mouse antibodies (HAMA)

***Innovance®* D-DIMER DILUENT** (white) is a buffered saline solution with detergent for the dilution of sample, calibrator and controls.

***Innovance®* D-DIMER CALIBRATOR** (red) has system specific analytical values and is derived from human plasma containing a D-Dimer preparation of approx. 4.4 mg/L FEU. This calibrator is specific for the kit lot of *Innovance®* D-DIMER to ensure high lot to lot consistency.

***Innovance®* D-DIMER Control 1 and 2** (blue and lilac) have system specific assigned values. Control 1 is a true normal control derived from human plasma containing D-Dimer of approx. 0.3 mg/L FEU. Control 2 is a pathological control derived from human plasma containing a D-Dimer concentration of approx.3.0 mg/L FEU.

Empty labeled vials are pre-labeled and bar-coded. They provide the opportunity to aliquot reagent volume very easily to maintain stability after opening if only a few D-Dimer tests are measured per day (available only with 150 tests kit).

STABILITY AFTER PREPARATION

The stability of the kit after preparation is shown in ***Table 2***. Reagents, calibrator and controls must be stored at +2 to +8°C prior to first opening or reconstitution. Unopened, they expire at the date indicated on the vial and/ or box label.

APPLICATIONS

Applications on CA analyzers (CA-7000, CA-1500 and CA-560) are designed to give comparable performance and the same number of tests per *Innovance*® D-DIMER kit. The results of this kit are provided in mg/L FEU (Fibrinogen Equivalent Units). FEU expresses the concentration of fibrin degradation products in terms of the gravimetrically determined mass of fibrinogen from which they were derived. Approximately 1.7µg/mL FEU have an immuno-reactivity similar to 1µg/mL of purified D-Dimer[8]. The system specific measuring schemes are shown in ***Table 3***.
The *Innovance*® D-DIMER total measuring range is defined by the concentration of the calibrator used and is approximately 0.19 to 4.40 mg/L FEU. The measuring range can be extended to approximately 35.20 mg/L FEU by automatic redilution of samples above 4.40 mg/L FEU. Samples with concentrations above 35.20 mg/L FEU can be further diluted manually with *Innovance*® D-DIMER DILUENT.

PRODUCT PERFORMANCE CHARACTERISTICS

Precision (CV%)

Repeatability is tested by measuring 8 replicates of the same samples per day for 5 days. The within device/laboratory variance is tested by measuring 2 replicates of the same samples per run, 2 runs per day for 20 days. High precision was observed on each CA analyzer in the above-mentioned two methods (***Table 4***). In addition to the controls, 3 samples with different levels of D-Dimer concentration were measured. For samples or controls within the normal range, a within device/laboratory variance of 8.4 % CV or lower was observed. For samples or controls within the pathological range, a within device/laboratory variance of 5.9% CV or lower was observed.

Interference Studies

Innovance® D-DIMER was designed for minimal interference from heterophilic antibodies, rheumatoid factors, triglycerides and other common potentially interfering substances. The interference to heterophilic antibodies was studied using native samples with endogenous levels of rheumatoid factors or HAMA. The results were confirmed by mixing studies. Furthermore, 38 pharmaceuticals, common in the clinical environment have been tested for interference with *Innovance*® D-DIMER. For this category of samples, the test concentrations were beyond therapeutic range or even at toxic levels, depend-

Table 2 *Stability after reconstitution or first opening (closed vial)*

	Reagent	Buffer	Supplement	Diluent	Calibrator	Controls
+2 to +8˚C	4 weeks	4 weeks	4 weeks	4 weeks	N/A	5 days
+15 to +25˚C	N/A	N/A	N/A	N/A	4 hours	6 hours
≤ -18˚C	8 weeks	8 weeks	8 weeks	8 weeks	N/A	2 weeks

Table 3 *The system specific measuring schemes*

	CA-7000	CA-1500	CA-560
Wavelength [nm]	800	800	575
Calibration range [mg/L FEU]	0.19 - 4.40	0.19 - 4.40	0.19 - 4.40
Calibration curve	6 points	6 points	6 points
Measuring time [min]	3	3	3
Measuring range [mg/L FEU]	0.19 - 35.20	0.19 - 35.20	0.19 - 35.20
Re-dilution [mg/L FEU]	≥ 4.40	≥ 4.40	≥ 4.40
	Automatic 1:8	Automatic 1:8	Manual request automatic 1:8
Measuring range for re-dilution [mg/L FEU]	3.65 - 35.20	3.65 - 35.20	3.65 - 35.20

ing on the substance. Guideline CLSI EP7-A2 was followed. In the study, the interfering substances did not interfere with the *Innovance*® D-DIMER assay up to the concentration tested (***Table 5***). However, due to the heterogeneity of heterophilic antibodies, such interference cannot be entirely excluded.

Reference Range

Plasma specimens obtained from apparently healthy donors (n=150) were tested using the *Innovance*® D-DIMER assay on the CA-7000, CA-1500 and CA-560 System with the following results:
90^{th} percentile 0.55 mg/L FEU
The mean and median of the tested normal population were well below the cutoff (0.5 mg/L FEU). Increases in D-Dimer concentration observed with thromboembolic events can be variable due to localization, extension and age of the thrombus. Therefore, a thromboembolic event cannot be excluded with certainty solely on the basis of an increased D-Dimer concentration being within the reference range of ostensibly healthy persons[9].

Method Comparison

A study was performed to compare the *Innovance*® D-DIMER assay with a commercially available assay, VIDAS® D-Dimer Exclusion™ (bioMérieux) for the measurement of D-Dimer. The results from the Passing-Bablok regression analysis are summarized in ***Table 6***. Excellent correlation was observed against this commercially available assay on each CA analyzer (***Fig.2-4***). Furthermore, the CA analyzers were compared with each other (***Table 7***). In a multicenter study, the system to system correlation coefficient r was determined to be 0.977 or higher (***Fig.5*** and ***6***). In this multicenter study, > 98.2% same patients were tested positive, and >95.7% same patients were tested negative.

Diagnostic Sensitivity and Specificity

A cutoff of 0.50 mg/L FEU for the exclusion of DVT and PE, when using *Innovance*® D-DIMER, was clinically derived and validated. A result of ≥ 0.50 mg/L FEU is considered positive, and a result of < 0.50 mg/L FEU is considered negative for DVT or PE. Performance of *Innovance*® D-DIMER for the exclusion of DVT and PE was evaluated in a multi-center management study. Samples were collected prospectively from outpatients with suspected DVT and/or PE. Patients were subjected to follow-up for 3 months. The excellent diagnostic efficiency of DVT and PE exclusion in combination with a clinical pre-test probability assessment was demonstrated by an NPV of 99.5% or higher with a cutoff at 0.50 mg/L FEU (***Table 8*** and ***Fig. 7-9***). In this study, *Innovance*® D-DIMER was compared with two (2) commercially available assays, Stratus® CS D-Dimer (Siemens) and VIDAS® D-Dimer Exclusion™. Both assays revealed comparable performance. A total of two (2) samples tested false negative with *Innovance*® D-DIMER. These samples were from patients assessed with a low pre-test probability score according to Wells[10]. These patients had distal DVT and both samples also tested false negative with both comparison assays.

Table 4 *Precision results*

	CA-7000		CA-1500		CA-560	
	repeatability	within device/lab	repeatability	within device/lab	repeatability	within device/lab
Innovance® D-DIMER CONTROL 1	1.9 %	2.3 %	2.2 %	2.5 %	5.3 %	6.7 %
Innovance® D-DIMER CONTROL 2	1.4 %	2.8 %	3.6 %	4.9 %	3.9 %	5.2 %

Table 5 *Potential interference*

	CA-7000	CA-1500	CA-560
Rheumatoid factor [IU/mL]	1330	1330	1330
Triglyceride [mg/dL]	400	600	400
Hemoglobin [mg/dL]	200	100	200
Bilirubin [mg/dL]	60	15	12

Table 6 *Correlation with a commercially available assay*

	Commercially available assay		
System	CA-7000	CA-1500	CA-560
N	1427	1412	1410
Concentration range of plasma samples investigated	0.17 mg/L FEU to 35.2 mg/L FEU		
Regression equation	y = 1.392x - 0.204 [mg/L FEU]	y = 1.384x - 0.213 [mg/L FEU]	y = 1.361x - 0.188 [mg/L FEU]
Coefficient of Correlation	r = 0.956	r = 0.956	r = 0.961

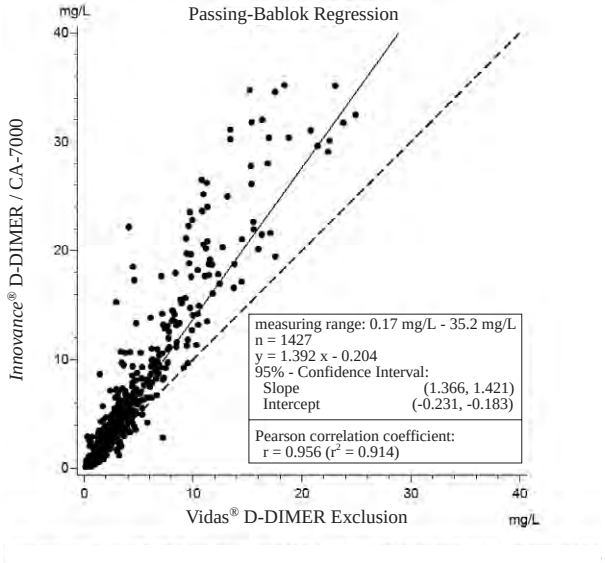

Fig. 2 *Method comparison of Innovance® D-DIMER/ CA-7000 vs. VIDAS® D-Dimer Exclusion™*

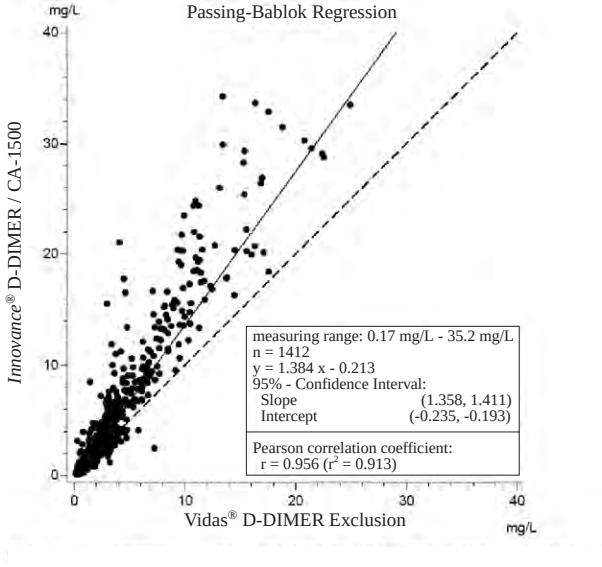

Fig. 3 *Method comparison of Innovance® D-DIMER/ CA-1500 vs. VIDAS® D-Dimer Exclusion™*

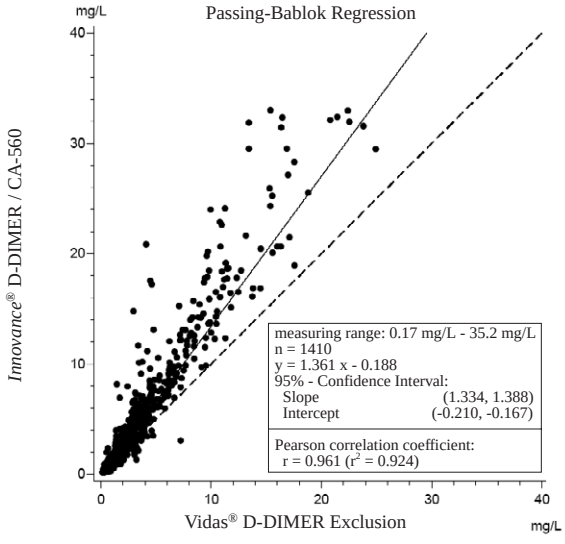

Fig. 4 *Method comparison of Innovance® D-DIMER/ CA-560 vs. VIDAS® D-Dimer Exclusion™*

Table 7 *Agreement and kappa coefficients for each CA analyzer*

System	Comparison system	Kappa coefficient*	Same patient tested positive	Same patient tested negative
CA-7000	CA-560	0.963	99.2%	96.7%
CA-1500		0.965	99.8%	95.7%
CA-7000	CA-1500	0.959	98.2%	98.4%

* Cohen's kappa coefficient is a statistical measure of inter-rater reliability

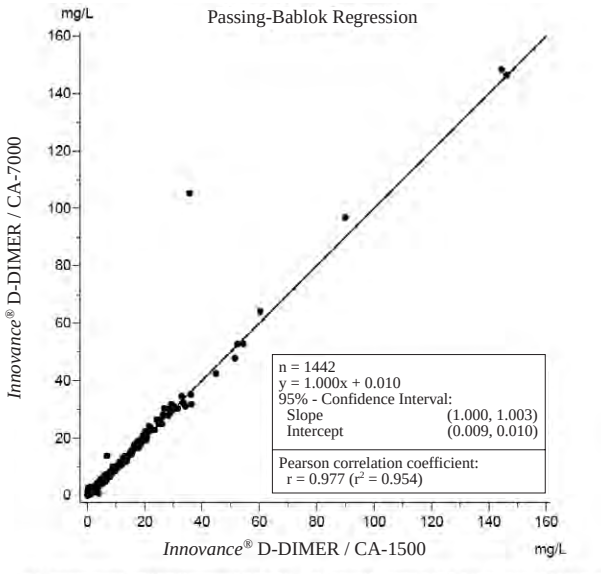

Fig. 5 *Method comparison of CA-7000 vs. CA-1500*

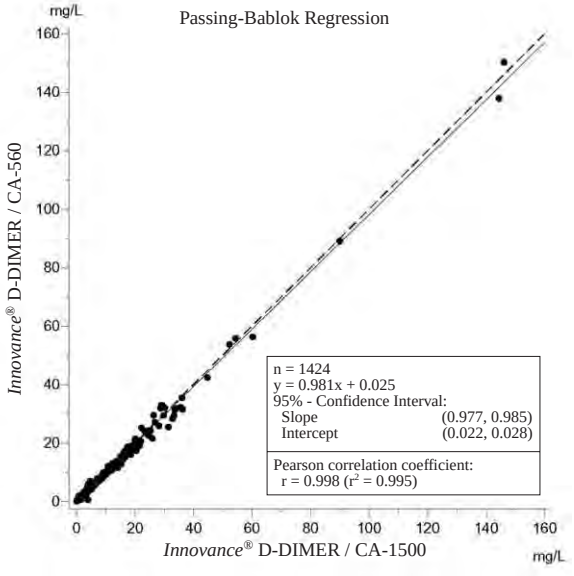

Fig. 6 *Method comparison of CA-560 vs. CA-1500*

On-board Stability

The reagent's stability on board the CA-7000 is 48 hours, on-board the CA-1500 is 24 hours and on-board the CA-560 is 8 hours. It is permissible to allocate the allowed holding time into several portions provided that the controls, which accompany each new test run, lie within the target values of the given acceptance range.

INNOVANCE® D-DIMER AND D-DIMER PLUS

Although the clinical utility has been demonstrated for both D-Dimer PLUS and *Innovance*® D-DIMER, quantitative results generated with *Innovance*® D-DIMER should NOT be compared with those of D-Dimer PLUS or other assays. Both assays use different monoclonal antibodies (MAb), different calibrators, different system settings and were standardized differently; results generated can therefore not be compared and so the quantitative results are quite different for both assays. Technical differences between the two reagents are shown in ***Table 9***.

Because there is no international standard for the D-Dimer assay, each manufacturer may employ a specific monoclonal antibody for their product which may recognize different epitopes of the fibrinogen or D-Dimer molecule as well as may show different degrees of cross-reactivity with similar antigens. For this reasons, different assays show a different sample response.

CONCLUSION

The D-Dimer cutoff of 0.5 mg/L FEU is accepted globally as the "consensus" cutoff for exclusion of DVT and PE. *Innovance*® D-DIMER has been harmonized with the cutoff used for VIDAS® D-Dimer Exclusion™, as this assay is considered as a benchmark for the exclusion of DVT and PE in the marketplace.
Innovance® D-DIMER provides the benefit of a D-Dimer test that offers best in class quality with less labor and expense. This kit is a fast, automated, particle-enhanced D-Dimer assay with excellent clinical performance that can be tested in parallel with other coagulation assays in clinical laboratories of all different sizes.

Table 8 *Diagnostic sensitivity and specificity*

	N	Cut-Off [mg/L FEU]	N false negative	Sensitivity / LCL* [%]	Specificity / LCL* [%]	NPV+ LCL* [%]
CA-7000	1425	0.50	2	99.4 / 98.0	38.3 / 35.9	99.5 / 98.7
CA-1500	1425	0.50	2	99.4 / 98.0	39.3 / 36.9	99.5 / 98.7
CA-560	1425	0.50	2	99.4 / 98.0	37.8 / 35.4	99.5 / 98.6

*: Lower 95% confidence limit
+: Negative Predictive Value
The study design is described in the respective publications[10, 11].

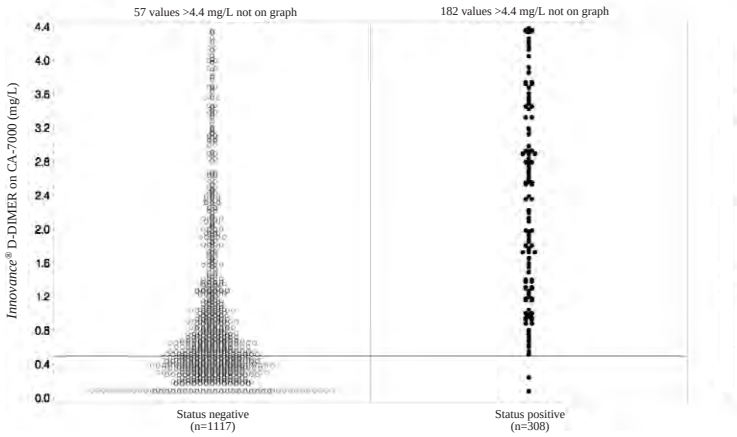

Fig. 7 *Distribution graphs for sensitivity and specificity: CA-7000*

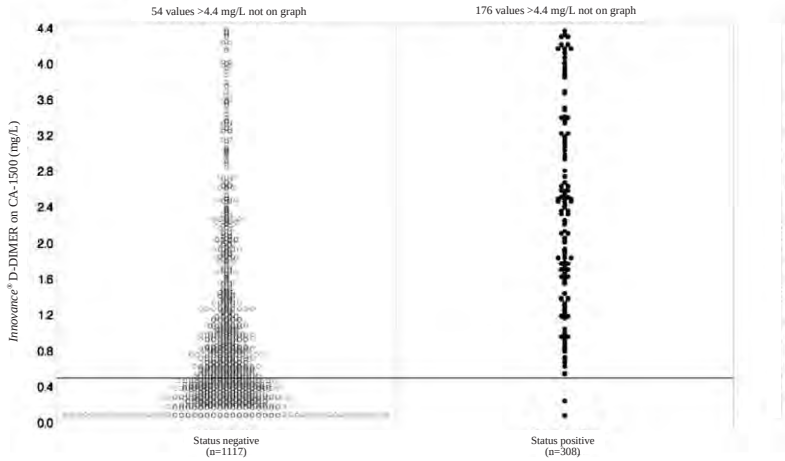

Fig. 8 *Distribution graphs for sensitivity and specificity: CA-1500*

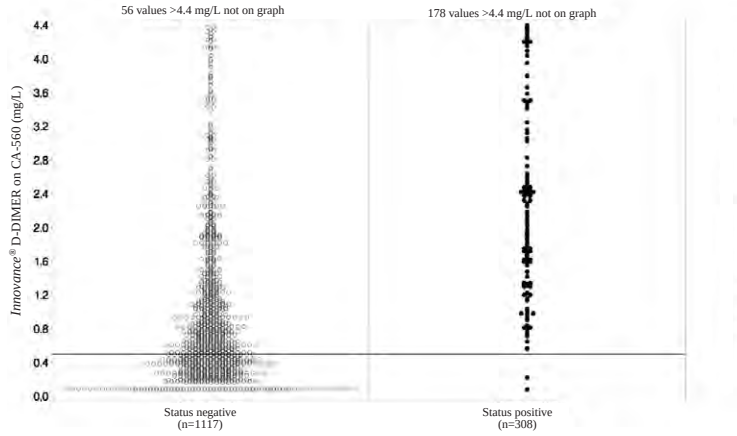

Fig. 9 *Distribution graphs for sensitivity and specificity: CA-560*

Table 9 *Technical difference between Innovance® D-DIMER and D-Dimer PLUS*

	Innovance® D-DIMER	D-Dimer PLUS
Antibody	MAb 8D3	MAb DD5
Antigen material for calibrator	Fibrin degradation products	Purified D-dimer
Calibrator	Lot specific	Open lot
Controls	Normal range and pathological range	Both controls in the pathological range
Cutoff	0.5 mg/L FEU for all applications	130 or 160 µg/L depending on the application
Unit of results	mg/L FEU	µg/L D-Dimer
Wavelength	800 nm (CA-7000, CA-1500) 575 nm (CA-560)	575 nm (CA-7000, CA-1500, CA-560)
Sample volume	15 µL	50 µL
Number of reagents on board	4 reagents	2 reagents
Standardization	Standardized using an in-house reference preparation consisting of a plasma spiked with FDP's (plasmin degraded cross-linked fibrin) and then evaluated in a mulitcenter management study for derivation and validation of the cutoff for the exclusion of DVT and PE.	Dade Behring standardization

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