



I. INTENDED USE

Pacific Hemostasis® D-dimer is a latex agglutination assay for the semi-quantitative determination of fibrin D-dimer in human plasma or serum.

II. SUMMARY

D-dimer containing moieties are formed by plasmin degradation of Factor XIIIa cross-linked fibrin. Elevated levels of D-dimer are found in clinical conditions such as deep venous thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC).^{1,3} D-dimer levels rise during pregnancy and high levels are associated with complications.² D-dimer utilizes a monoclonal antibody specific for fibrin D-dimer but not for fibrinogen degradation products.⁴ D-dimer can therefore be determined in plasma samples. Serum samples suited for FDP analysis can also be used.

III. REAGENTS

D-DIMER LATEX, Reagent

Suspension of latex beads coated with mouse monoclonal antibody MA-8D³ directed against D-dimer in HEPES buffer, pH 8.2. Contains 0.02% sodium azide as preservative.

D-DIMER POSITIVE CONTROL, Plasma

Lyophilized human plasma enriched with fibrin D-dimer.

D-DIMER NEGATIVE CONTROL, Plasma

Lyophilized human plasma.

SALINE SOLUTION, pH 7.3

Buffered saline, pH 7.3. Contains 0.02% sodium azide as preservative.

PRECAUTIONS:

D-dimer reagents are for "in vitro Diagnostic Use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws.

D-dimer Positive and Negative Controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HBsAg and for antibodies against HCV, HIV-1 and HIV-2 by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled observing the same safety precautions employed when handling any potentially infectious material.

D-dimer Latex Reagent and Saline Solution contain sodium azide. Sodium azide under acidic conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be discarded by diluting and flushing with large volumes of water. These precautions are recommended to avoid deposits in metal plumbing in which explosive conditions may develop. Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

IV. PREPARATION:

Reconstitute Positive Control Plasma with 0.2 mL Saline Solution. Restopper vial and allow to stand for 10 minutes. Use gentle agitation until dissolution is complete. DO NOT SHAKE.

Reconstitute Negative Control Plasma with 0.2 mL Saline Solution. Restopper vial and allow to stand for 10 minutes. Use gentle agitation until dissolution is complete. DO NOT SHAKE.

Immediately before use, D-dimer Latex suspension should be agitated by repeatedly inverting the vial to disperse sedimented latex particles.

The rubber stoppers in the control vials may be discarded after vial opening.

Buffer Solution is provided ready for use.

V. STORAGE AND STABILITY:

Store reagents in the refrigerator (2–8 °C). Reagent labels bear expiration dates.

Reconstituted Positive and Negative Control Plasma are stable for 1 month at 2–8 °C or 1 month at –20 °C (do not refreeze more than once).

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection and storage be carried out in accordance with NCCLS guideline H21-A3.⁵ No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Venous blood is collected in either 3.8% or 3.2% sodium citrate at a ratio of nine parts blood to one part anticoagulant (1:10 ratio). The ratio is critical. 3.2% sodium citrate is preferred as recommended by NCCLS and WHO. If using commercial vacuum tubes, a full draw must be assured. Trauma or stasis during drawing should be avoided. Blood should not be collected through a heparin lock or other heparinized line. The presence of a clot in a specimen is cause for rejection. Mix tube well by gentle inversion against the stopper or Parafilm® and centrifuge at 1500 x g for 15 minutes. Unless samples are to be processed immediately, transfer the plasma into a plastic tube and store refrigerated (2–8 °C).

Heparinized or EDTA plasma or serum can also be used. Serum must be collected in the presence of fibrinolytic inhibitors (FDP tubes). When EDTA samples are stored for more than 4 hours at room temperature prior to testing, some samples will display nonspecific agglutination.

Samples may be stored at room temperature (18–25 °C) for 4 hours, at 2–8 °C for 8 hours and frozen at –20 °C for 1 month.

VI. PROCEDURE

MATERIALS PROVIDED:

D-Dimer Assay Kit (80 det.): Latex Reagent 1.7 mL (1), Positive Control (2), Negative Control (2), Saline Solution (2), Disposable Test Cards (16), Mixing Sticks (50)

D-Dimer Assay Kit (20 det.): Latex Reagent 0.5 mL (1), Positive Control (1), Negative Control (1), Saline Solution (1), Disposable Test Cards (4), Mixing Sticks (13)

D-Dimer Latex Reagent: 1.7 mL Latex (1)

MATERIALS/EQUIPMENT REQUIRED BUT NOT PROVIDED:

Pipettes, 20 µL, 50 µL and 200 µL

Test tubes

QUALITATIVE METHOD:

Allow reagents to warm to room temperature for a minimum of 10 minutes before use.

Agitate the D-dimer Latex by repeatedly inverting the vial for 5 seconds immediately before use.

Place 20 µL of a sample and Positive and Negative Control Plasmas in circles on a test card.

Place 20 µL of D-dimer Latex in a nearby area of each circle. Quickly mix the samples and Latex using clean mixing sticks for each sample. Start the timer.

Rock the test card gently back and forth and read agglutination between 180 and 200 seconds.

NOTES:

The “+” agglutination response is clearly non-homogenous. Aggregated particles in the mixture appear suspended in a milky or clear solution. Positive (+) or negative (–) agglutination are compared to results obtained using the Positive and Negative controls. The Positive Control is merely qualitative and should not be further diluted. Non-agglutinated latex means the sample is normal and no further testing is required.

A small number of samples, when mixed with the latex, may exhibit white flakes which should not be confused with agglutination.

SEMI-QUANTITATIVE METHOD:

(Performed only on samples tested positive)

Serially dilute the 50 µl of sample 1:2, 1:4 and 1:8 with 50 µl Buffer Solution using small test tubes.

Tube #	Plasma Dilution	Plasma	Buffer
1	1:2	50 µl	50 µl
2	1:4	50 µl of 1:2 dilution	50 µl
3	1:8	50 µl of 1:4 dilution	50 µl

Mark the positions of sample dilutions on the test card and mix with latex suspension as described previously. Refer to “Results” section to determine D-dimer concentration.

VII. QUALITY CONTROL:

The positive and negative control provided in the D-dimer kit should be used for quality control of the kit. It is recommended that both positive and negative controls be tested each time the kit is used. If either positive or negative control fails to elicit the appropriate response, patient results obtained on that occasion should not be used.

The failure to meet Quality Control Specifications should be investigated and resolved. The assay should then be repeated. If the problems cannot be resolved, contact Fisher Diagnostics Technical Services.

VIII. RESULTS

Agglutination occurs within 180 to 200 seconds for samples containing more than 0.25 µg/mL D-dimer. By testing serially diluted samples, semi-quantitative results can be obtained.

(µg/mL)	Undiluted	Dilution		
		1:2	1:4	1:8
<0.25	–	–	–	–
0.25-0.5	+	–	–	–
0.5-1.0	+	+	–	–
1.0-2.0	+	+	+	–
>2.0	+	+	+	+

Agglutination may be more pronounced and appears more rapidly at higher D-dimer concentrations. If you wish to express the results in fibrinogen equivalent units (FEU) multiply the D-dimer levels in the table above by 2, e.g. <0.25 µg/mL becomes <0.50 µg/mL.

As discussed in several reports,⁶ some commercial latex tests do not have the claimed sensitivity when compared to commercial ELISA.

IX. INTERPRETATION OF RESULTS

Agglutination occurs within 180 to 200 seconds for samples containing more than 0.25 µg/mL D-dimer tested with the D-dimer test. The mean level of D-dimer in a healthy population is between 0.008 and 0.135 µg/mL, therefore undiluted plas-

ma or serum from normal, healthy individuals should not agglutinate. If no agglutination is observed, a thrombotic condition is unlikely. The negative predictive value of the D-dimer for thrombosis is high.⁷ The circulatory half-life of D-dimer is about 12 hours. Elevated D-dimer levels can therefore persist for some time after the active process has ceased.

In clinical studies on normal subjects, patients with phlebographically confirmed DVT, patients with DIC and patients with pre-eclampsia (Pre-EC) the following results were obtained:

Patient Group	Total Number	Number of Patients with Titer†				
		Neg	1:1	1:2	1:4	>1:8
Normal	101	100	1	–	–	–
DVT	48	3	*10*	*7*	*14*	*14*
DIC	29	0	3	3	4	19
Pre-EC	6	2	1	3	–	–

† Titers indicate the highest dilution at which the samples show agglutination.

* The agglutination was inhibited by addition of D-dimer specific antibody (0.2 mg/mL) MA-8D3 but not with a non-related antibody PAM-1.

There are indications that some commercial latex tests have a lower sensitivity than claimed. With the D-dimer, 60–70% positive samples are expected for suspected DVT; some other latex tests exhibit a lower frequency.⁷

X. PERFORMANCE CHARACTERISTICS

SPECIFICITY:

The monoclonal antibody used in this kit is specific for D-dimer by virtue of the screening method used in the selection of the hybridoma.³ A hybridoma secreting antibodies which reacted positively with purified D-dimer but not with the whole fibrinogen and fragment D of fibrinogen was selected. No cross-reactivity with fibrinogen or des-AA-fibrinogen was observed when either analyte was substituted for plasma in this assay.

Plasma from 16 patients with rheumatoid arthritis were tested and 14 were found to be non-agglutinating with the D-dimer test. The two agglutinations could be inhibited by addition of the D-dimer specific monoclonal antibody MA-8D³ but not with addition of monoclonal of the same subgroup, IgG1k (PAM-1). This suggests that the D-dimer is insensitive to rheumatoid factor disturbances.

REPRODUCIBILITY:

Three plasma samples were selected to test reproducibility of the assay. Each sample was tested 10 times on each of three different days. In each case where a positive result was obtained the sample was titrated. The results were as follows:

Sample	D-dimer Level	Result
Normal	<0.25 µg/mL	always showed a negative reaction
Intermediate	3 µg/mL	always showed a titer of 1:8
High	>16 µg/mL	always showed a titer of 1:64

ACCURACY:

D-dimer was compared to another commercially available assay. Both products gave a negative reaction when tested on 25 normal specimens known not to contain more than 0.25 µg/mL of fibrin D-dimer. When 30 patient plasma samples were tested with ELISA and D-dimer, all samples with ELISA values above 225 ng/mL showed agglutination with D-dimer.

XI. LIMITATIONS

A negative D-dimer test does not completely rule out thrombosis. A negative predictive value for patients with suspected DVT has been found to be 94% with the D-dimer kit.⁷ Detection of elevated levels of D-dimer should be used with other clinical information in forming a diagnosis. Agglutination with samples containing normal D-dimer levels may occasionally occur due to non-specificity.

XII. REFERENCES

1. Elms, MJ et al: Rapid detection of cross-linked fibrin degradation products in plasma using monoclonal antibody-coated latex particles. *J Clin Path* 85:360, 1986
2. Ballegeer, V et al: Fibrinolytic response to venous occlusion and fibrin fragment D-dimer levels in normal and complicated pregnancy. *Thromb Haemost* 85:1030, 1987
3. Declerck, PV et al: Fibrinolytic response and fibrin fragment D-dimer in patients with deep vein thrombosis. *Thromb Haemost* 58:1024, 1987
4. Holovet P, et al: Binding properties of monoclonal antibodies against human fragment D-dimer of cross-linked fibrin to human plasma clots in an in vivo model in rabbits. *Thromb Haemost* 61(2):307–313, 1989
5. National Committee for Clinical Laboratory Standards: Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays, 1998. NCCLS Document H21-A3
6. Bounameaux H, et al: Measurement of plasma D-dimer for diagnosis of deep venous thrombosis. *Am J Clin Path* 91:82–85, 1989
7. Hansson PO, Eriksson H, Eriksson E, Jegenburg R, Risberg B: Can laboratory testing improve screening strategies for deep vein thrombosis at an emergency unit? *J Intern Med* 235:143–141, 1994

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ORDERING INFORMATION

Cat No.	Description	Contents
100660	D-dimer Assay	80 Test
100662	D-dimer Assay	20 Test
100659	D-dimer Latex Reagent	1.7 mL
100650	FDP Assay	30 Test
101654	FDP Sample Collection Tubes	100
100654	FDP Sample Collection Tubes	30
100651	FDP Latex Reagent	1x5 mL
100665	Glycine Buffered Saline	4x50 mL
100656	FDP Control Set	3x2 mL



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