REF PF1051-KUS IVD For in vitro diagnostic use

PATHFAST® D-Dimer

<REAGENT FOR PATHFAST®>

60 Tests

Intended Use

PATHFAST® D-Dimer is a product for in-vitro diagnostic use with the in vitro diagnostic system PATHFAST® for the quantitative measurement of cross-linked fibrin degradation products (D-Dimer) in human heparinized or citrated (3.2%) whole blood and plasma. PATHFAST® D-Dimer is for use in clinical laboratory or POC settings.

Summary

The activation of the fibrinolytic system results in the conversion of plasminogen into plasmin. D-Dimer containing fibrin degradation product (XDP) fragments are released when cross-linked fibrin is degradated by plasmin. D-Dimer is a marker of the degradation of fibrin clots and an indirect marker of clot formation. The presence of elevated D-Dimer is reported in several clinical conditions including deep vein thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC).[1-6]

The PATHFAST® D-Dimer is an assay for measurement of D-Dimer in the format of a chemiluminescent enzyme immunoassay (CLEIA). All required components for performing the testing are packed in one reagent cartridge. By loading PATHFAST® D-Dimer into the in vitro diagnostic system PATHFAST®, D-Dimer can be accurately quantified within 17 min.

Package composition

Reagent cartridge 6 cartridges x 10 trays

The reagent cartridge consists of 16 wells. All wells with exclusion of sample well (#1) and counting well (#10) are covered with an aluminum seal having a bar code. All reagents for the test are filled in each well of the reagent cartridge.

Wells	Form	Ingredient	Quantity	Source
# 2	Liquid	Alkaline phosphatase conjugated anti D-Dimer MoAb*	50µL	Calf intestine Mouse
		MES buffer		
		Na azide	< 0.1%	
# 7	Liquid	Anti D-Dimer MoAb* coated magnetic particles	50µL	Mouse
		MOPS buffer		
#13	Liquid	Chemiluminescent substrate CDP-Star®	100µL	
# 11	Liquid	Sample dilution buffer	50µL	
		Tris buffer		
		Na azide	< 0.1%	
# 3, 4, 5	Liquid	Washing buffer	400µL	
		MES buffer		
		Na azide	< 0.1%	

*MoAb = monoclonal antibody

#1, 6, 8, 9, 10, 12, 14, 15, 16 are empty wells.

CDP-Star® is a registered trademark of Applied Biosystems, LLC.

Calibrators

1.	Calibrator 1 (CAL-1)	2.0 mL x 1 bottle	(liquid)
		Na azide (0.05%)	
2.	Calibrator 2 (CAL-2)	For 1.0 mL x 2 vials	(lyophilized)
3.	Calibrator diluent	1.0 mL x 2 bottles	(liquid)
		Na azide (0.05%)	

Note: Cal-2 contains human serum obtained from donors who were confirmed negative for Anti-HIV-1/2, HbsAg and Anti-HCV. Cal-2 also contains D-Dimer from human plasma. Blood samples from the donors were tested by FDA cleared methods and found negative for Anti-HIV-1/2, HbsAg and Anti-HCV. No test offers complete assurance of absence of transmissible pathological agents. Treat as if capable of transmitting infection.

MC ENTRY CARD 1 sheet
Instruction for use 1 sheet

Materials Needed But Not Provided

PATHFAST® Analyzer and consumables
D-Dimer Quality Control Materials

Assay Principle

The PATHFAST® D-Dimer procedure is based on CLEIA and MAGTRATION®. In this procedure, alkaline phosphatase labeled anti D-Dimer monoclonal antibody and anti D-Dimer monoclonal antibody coated magnetic particles are mixed with sample. D-Dimer contained in the specimen binds to the D-Dimer antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound enzyme labeled antibody, a chemiluminescent substrate is added to the immunocomplex. After a short incubation, the luminescence generated by enzyme reaction is detected. The intensity of the measured luminescence is in relationship with the D-Dimer concentration in the specimen which is calculated by means of a standard curve.

*MAGTRATION® is technology of B/F separation where magnetic particles are washed in pipette tip and is a registered trademark of Precision System Science.

Precautions

Reagent cartridge

1. Do not use reagents beyond its labeled expiration date.
2. Do not reuse a reagent cartridge. It is designed for single use only.
3. Do not peel off the aluminum seal.
4. Handle the reagent cartridge by holding the edge of it and do not touch the aluminum seal and the black well with your fingers.
5. When the reagent cartridge is dropped and damaged, do not use it.
6. Store the reagent cartridges in an upright position at all times.
7. Avoid contamination of saliva in the black well.
8. Avoid contamination and exposure to direct sunlight.
9. During storage or shipment, there may be some reagents adhered to the aluminum foil. If such a condition is observed, gently tap the cartridge on table before use.
10. Azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.
11. Dispose in accordance with applicable national and local institution's regulations. Follow general precautions, and handle all components as if capable of transmitting infectious agents.
12. Do not use any reagent cartridge which was left at room temperature over 8 hours.

Storage Instructions

Store at +2 to +8 °C. Do not open the cartridge tray until immediately prior to use.

Expiration

The expiration date is listed on reagent cartridge, cartridge tray and package.

Sample Collection

1. Use whole blood or plasma collected with qualified collection tube containing heparin-Na, heparin-Li or citrate-Na (3.2%).
2. Whole blood samples must be analyzed within 4 hours of collection when stored at +2 to +8 °C.
3. It should be ensured that fibrin clots and other insoluble materials are not present in plasma sample, otherwise such material has to be removed by centrifugation or filtration.
4. Plasma samples are stable for one week at +2 to +8 °C and for 2 weeks at –20 °C or lower. Plasma samples should not be repeatedly frozen and thawed more than 3 times before testing.

Preparation and procedure

Reagent preparation

1. Reagent cartridge
   Ready to use.
2. Cal-1
   Ready to use.
   After opening calibrators are stable for the expire date which is printed on the bottle label, if they are stored at +2-+8°C.
3. Cal-2
   Reconstitute each vial of CAL-2 with one bottle of Calibrator diluent. Stand for 15 minutes at room temperature after the reconstitution. Mix gently and ensure that the calibrator is completely dissolved. Reconstituted calibrators are stable for 3 days at +2 to +8 °C or 1 month at –20 °C or lower. Do not exceed five freeze/thawing cycles.

   NOTE: Use the same lot of CAL-1, CAL-2, Calibrator diluent and PATHFAST® reagent cartridge. Never mix different lots of CAL–1, CAL-2, Calibrator diluent and PATHFAST® reagent cartridge.

Installation of master calibration curve

1. Installation of the master calibration curve is necessary when a new reagent lot is used.
2. Install the master calibration curve by reading the bar code on MC ENTRY CARD, which is enclosed in each package, with the hand-held bar code reader of the PATHFAST®. Refer to the PATHFAST® operator's manual for more information.

User calibration

1. The PATHFAST® D-Dimer result is given in µg/mL FEU (fibrinogen equivalent unit). The unit µg/mL FEU is equivalent to mg/L FEU and µg/mL FEU corresponds to ng/mL FEU x 1,000.
2. User calibration is necessary when a new reagent lot is used after installation of the master calibration curve on MC ENTRY CARD.
3. User calibration is also necessary every 4 weeks after the first user calibration (MC ENTRY CARD is not required).
4. The calibrators, CAL-1 and CAL-2, must be tested both **in duplicate**.

Therefore 4 reagent cartridges, two for CAL-1 and two for CAL-2 are necessary for user calibration.

5. Set the reagent cartridges in the cartridge rack, and then dispense approximately 100 µL of CAL-1 and CAL-2 into the appropriate sample wells and load the cartridge rack onto the PATHFAST®.
6. Carry out user calibration in accordance with the PATHFAST® operator's manual.

Quality Control Assay (QC Assay)

1. Refer to the PATHFAST® operator's manual. A QC Assay is performed after every calibration to check the calibration curves and store data from QC samples for quality control. A QC Assay is indispensable for assuring validity of sample results. After each calibration, with each new shipment of previously calibrated test kit, or whenever the institution wishes to verify the performance of the system, analyze two levels of quality control material with known levels of D-Dimer.
2. Good laboratory practices recommend the use of appropriate quality controls. It is recommended that federal, state and local guidelines for quality control be followed. If controls do not perform as expected, do not use the test results. Repeat the controls and the test or call your authorized PATHFAST® distributor for technical service.

Sample assay

1. Use heparinized or citrated whole blood or plasma sample (heparin-Na or heparin-Li, citrate-Na (3.2%)).
2. Set the reagent cartridge in the cartridge rack, then dispense approximately 100 µL of sample into the appropriate sample well of a cartridge and load the cartridge rack onto the PATHFAST®.
3. Carry out sample assay in accordance with the PATHFAST® operator's manual.

Note:

1. When a whole blood sample is used, the whole blood contained in a blood collection tube should be mixed gently just before dispensing (do not use vortex mixer). After dispensing the whole blood sample and loading the cartridge rack on the PATHFAST®, the assay must be started immediately.
2. When samples are left for more than 5 minutes after dispensing into a sample well, a lower result may be obtained analyzing whole blood because of blood sedimentation and a higher result may be obtained analyzing plasma because of possible evaporation.
3. Whole blood or plasma samples above 5 µg/mL FEU should not be diluted, and result should be reported as > 5 µg/mL FEU.

Expected Value

Note: Assay cut off of 0.686 µg/mL FEU was established by calculating the 95th percentile of sodium citrated (3.2%) plasma from 113 apparently healthy individuals. Each laboratory should validate the cutoff based on their reference patient population.

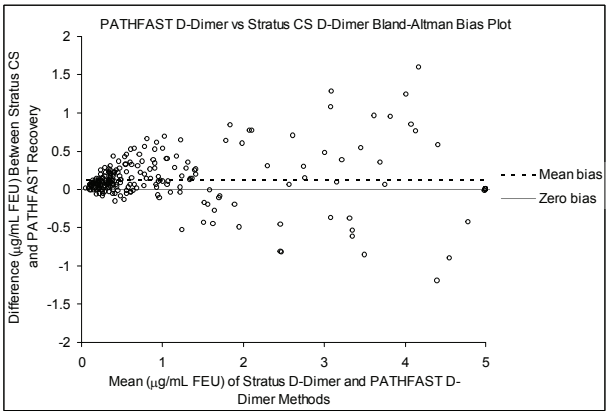
1. Expected values were determined in accordance to the Clinical and Laboratory Standards Institute CLSI (formerly NCCLS) Approved Guideline – Second Edition, NCCLS – document C28-A2 (ISBN 1-56238-406-6) – NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087-1898. The expected values/reference values may vary from laboratory to laboratory and from country to country depending on various factors. It is therefore recommended that each institution establish its own expected values for D-Dimer as performed on the PATHFAST® Analyzer
2. The expected value for each sample matrix was calculated non-parametrically and represent the 90th percentile of the population tested.
   Using the PATHFAST® D-Dimer assay, the expected value for of 128 apparently healthy individuals is:
   Less than 0.683 µg/mL FEU (lithium heparin plasma)
   Plasma samples ranged from 0.031 to 1.07 µg/mL FEU with a mean of 0.336 µg/mL FEU. Samples consisted of 97 males and 31 females with age 19 to 57 years.
   Using the PATHFAST® D-Dimer assay, the expected value for 113 apparently healthy individuals are:
   Less than 0.666 µg/mL FEU (lithium heparin whole blood)
   Whole blood samples ranged from 0.080 to 1.32 µg/mL FEU with a mean of 0.348 µg/mL FEU. Samples consisted of 54 males and 59 females with age 22 to 60 years.
   Using the PATHFAST® D-Dimer assay, the expected value for 113 apparently healthy individuals are:
   Less than 0.474 µg/mL FEU (3.2% sodium citrate plasma)
   Plasma samples ranged from 0.064 to 1.07 µg/mL FEU with a mean of 0.272 µg/mL FEU. Samples consisted of 54 males and 59 females with age 22 to 60 years.
   Using the PATHFAST® D-Dimer assay, the expected value for 113 apparently healthy individuals are:
   Less than 0.593 µg/mL FEU (3.2% sodium citrate whole blood)
   Whole blood samples ranged from 0.064 to 1.20 µg/mL FEU with a mean of 0.309 µg/mL FEU. Samples consisted of 54 males and 59 females with age 22 to 60 years.

Specific performance data of the assay

Representative performance data on the PATHFAST® is given below.

1. Assay range: 0.005 – 5.00 µg/mL FEU
2. Correlation between lithium heparin plasma and other sample matrices
   y=0.955x + 0.073; r =0.990, n= 56
   (y：lithium heparin whole blood; x: lithium heparin plasma)
   y=1.022x + 0.001; r=0.992, n =56
   (y： sodium heparin plasma; x: lithium heparin plasma)
   y=1.022x + 0.030; r =0.988, n=56
   (y：sodium heparin whole blood; x: lithium heparin plasma)
   y=0.942x - 0.015; r =0.991, n=56
   (y：sodium citrate (3.2%) plasma; x: lithium heparin plasma)
   y=1.029x + 0.041; r =0.984, n=56
   (y：sodium citrate (3.2%) whole blood; x: lithium heparin plasma)
3. Methods comparison (plasma samples):
   y = 1.01x + 0.069, r = 0.982, n = 235
   (y：this method; x：Dade Behring Stratus® CS D-Dimer)
   Stratus® is a registered trademark of Dade Behring Inc.

PATHFAST® D-Dimer vs. Stratus CS D-Dimer Bland-Altman Bias Plot



4. Standardization
   The calibrators for the PATHFAST® D-Dimer consist of high molecular weight fraction of human cross-linked fibrin degradation products obtained by plasmin action.
5. Imprecision
   Precision was determined using the present method, 4 plasma-based control materials as follows: Four samples were assayed in duplicate for 20 non-consecutive days. The within-run and total standard deviations were calculated according to the Clinical and Laboratory Standards Institute CLSI (formerly NCCLS) EP-5A [ISBN 1-56238-368-X]. The following results were obtained.

		Interassay imprecision		Total imprecision	
Sample	Mean (µg/mL FEU)	S.D. (µg/mL FEU)	C.V. (%)	S.D. (µg/mL FEU)	C.V. (%)
Control-LL	0.024	0.001	3.8	0.002	6.1
Control-L	0.249	0.007	2.8	0.015	6.0
Control-M	0.654	0.020	3.0	0.029	4.5
Control-H	2.45	0.120	4.9	0.174	7.1

6. Limit of Quantitation: 0.003 µg/mL FEU
   The limit of quantitation is the lowest analyte concentration that can be reproducibly measured with a between-run coefficient of variation of 10 %.
7. Hook Effect
   High molecular weight samples from above 5 to 800 µg/mL FEU and low molecular weight samples from above 5 to 160 µg/mL FEU were tested and results above 5 µg/mL FEU were observed.

Possible interferences

The following factors were found to have an effect less than 10 % on the assay at the concentrations indicated in parentheses.

Free bilirubin	(60 mg/dL)
Conjugated bilirubin	(60 mg/dL)
Triglyceride, lipemia in sample	(1000 mg/dL)
Hemoglobin (hemolysis)	(Hb 500 mg/dL)
Rheumatoid Factor	(500 IU/mL)

High concentration of fragment E, such as found in patients receiving thrombolytic therapy, can lead to measurement of lower values[7].

Limitations of procedure

1. The instrument reporting system contains error codes to warn the operator of specific malfunctions. Any report slip containing such error codes should be kept for follow-up. See the PATHFAST® operator's manual.
2. Patient samples may contain heterophilic antibodies that could react in immunoassays to give a falsely elevated or depressed result. This assay has been designed to minimize interference from heterophilic antibodies. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with cautions.
3. D-Dimer results for specimens collected in 3.2% sodium citrate may be slightly lower than results from specimens collected in lithium

heparin.

References

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2) Bick R.L. Baker WF. Diagnostic efficacy of the D-Dimer assay in disseminated intravascular coagulation (DIC). Thromb Res 1992; 65: 785 – 790.
3) Bounameaux H, de Moerloose P, Perrier A, Reber G. Plasma measurement of D-Dimer as diagnostic aid in suspected venous thromboembolism: an overview. Thromb. Haemost. 1994; 71: 1 – 6.
4) Bounameaux H, de Moerloose P, Perrier A, Miron MJ. D-Dimer testing in suspected venous thromboembolism: an update. QJ Med 1997; 90: 437 – 442.
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6) Perrier A, Desmarais S, Miron MJ et al. Non-invasive diagnosis of venous thromboembolism in outpatients. Lancet 1999; 353: 190 – 195.
7) Olexa A, Budzynski A. Primary soluble plasmic degradation product of human cross-linked fibrin. Isolation and stoichiometry of the (DD)E complex. Biochem 1979; 18: 991 – 995.

Symbols

CE : EU Conformity

IVD : For in vitro Diagnostic Use

Lot : Batch Code

REF : Product Number

: Manufactured by

EC REP : Authorized Representative

Σ : Contains sufficient for

: Temperature Limitation

: Use by

: Caution, refer to accompanying documents

: Consult IFU

CAL 1 : Calibrator 1

CAL 2 : Calibrator 2

DILUENT : Diluent

CARTRIDGE : Reagent Cartridge

MC ENTRY CARD : Entry Card for Master Standard Curve data

*PATHFAST : US Registered Trademark No.3074207

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