NESPI-FRDD-001EN(V.0.1)

NanoEntek

CE IVD

FREND™ D-Dimer

Fibrinogen degradation product

Intended use

The FREND™ D-Dimer is designed for *in vitro* quantitative measurement of Fibrinogen degradation product (FDP, D-dimer) in human plasma (Sodium citrate) by fluorescence immunoassay. The FREND™ D-Dimer is indicated for use in conjunction with a clinical pretest probability assessment model to exclude deep vein thrombosis (DVT) and pulmonary embolism (PE) disease in outpatients suspected of DVT or PE.

Principle of the assay

The FREND™ D-Dimer test cartridge is a one-time-use rapid "Sandwich" immunoassay utilizing fluorescent nanoparticle in microfluidic flow to capture and quantify total D-dimer in sodium citrate plasma specimens. The drop of 35 µL patient sample is added to the FREND™ D-Dimer cartridge, interacting with D-dimer antibodies conjugated fluorescent nanoparticles. The mixture moves via Capillary action to the detection region, where fluorescent nanoparticle complexes are grabbed. The fluorescence intensities from the complexes are measured and D-dimer concentration is calculated by the FREND™ System.

Materials provided

Q'ty	Contents	Catalogue number
25	Cartridge	FRDD 025
30	Disposable pipette tip	
01	Code chip	
01	Package insert	

Materials required but not provided

- The FREND™ System
- Micro-pipette capable of delivering 35 µL
- Personal protective equipment and biohazard waste equipment

Warnings and Precautions

- The FREND™ D-Dimer cartridges are intended for *in vitro* diagnostic use only.
- FREND D-Dimer cartridges are only to be used on the NanoEntek FREND™ System.
- FREND D-Dimer cartridges are disposable devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for 15-30 minutes prior to use.
- Cartridges should not be frozen.
- The humidity in the laboratory must be 10-80% range when running tests.
- Avoid high humidity, direct sunlight or heat when storing cartridges.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Testing of contaminated samples may cause erroneous results.
- Using specimens containing clotted fibrin could result in erroneous results.
- Overloading or underloading the cartridge with the sample may result in inaccurate results. Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package insert and User manual.
- Keep the cartridge sealed in the pouch until ready for use.
- Use the cartridge immediately after opening the pouch.
- Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the laboratory may be derived from human materials, use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples
- Wash hands thoroughly after handling cartridges or samples.
- Do not ingest the silica gel in the cartridge pouch.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twelve months from the date of manufacture.
The expiration date is clearly indicated on the product box and the cartridges.

Material	Catalogue number
Refrigerator temperature storage (2~8 ℃) FREND™ D-Dimer cartridges	FRDD 025
Room temperature storage (18~25 ℃) Pipette tips	None

Specimen collection and handling

- Human plasma (Sodium citrate) samples are suitable for use with FREND™ D-Dimer cartridges.
- Follow instructions detailed in this package insert as well as the specimen collection tube manufacturer's instructions for specimen collection and preparation (centrifugation time and speed).
- For Sodium citrate plasma, a venous blood sample is collected aseptically with the designated additive. After allowing the specimen to sufficiently mix with anticoagulant at room temperature, the sample tube can be centrifuged for 10 minutes at 3,000 rpm.
- Sample may be stored at 2~8°C for up to 6 hours prior to analysis. If the analysis is scheduled to be done at some later time, the sample should be stored frozen at -20°C or below for future use. Repeated freeze-thaw cycles should be avoided.
- Prior to assay, slowly bring frozen samples to room temperature (64~77°F or 18~25°C) and mix gently but thoroughly before test.
- For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter.
- When pipetting into the FREND™ D-Dimer cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedures

Calibration

There is no need for calibration to be performed by the end user. All calibration statistics and information were stored in the FREND™ D-Dimer Code chip included in the FREND™ D-Dimer Cartridge box. The FREND™ D-Dimer Code chip is specific for each lot of FREND™ D-Dimer cartridges.
Always run external quality control samples to verify that the D-Dimer results obtained on the FREND™ System meet acceptable criteria of the laboratory for each lot of FREND™ D-Dimer cartridge.

Code chip installation

Please refer to the FREND™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions are as follows:

(1) Insert the FREND™ System power cord into an appropriate outlet.

(2) Insert the Code chip into the Code chip slot at the rear of the system following the arrows.

(3) Press the 'Setup' button on the 'Main' screen.

(4) Press the 'Code chip' button on the 'Setup' screen.

(5) The information embedded on the FREND™ D-Dimer Code chip is automatically saved on the FREND™ System.

(6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.

(7) Press the 'Item' button on the 'Setup' screen.

(8) Check the FREND™ D-Dimer cartridge lot number and the installation date of the Code chip.

(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

Quality control

•FREND™ System QC cartridge

The FREND™ QC Cartridge contains multiple controls that check the optics of the system. By testing the QC Cartridge, (1) laser power (2) alignment, and (3) mechanical integrity components of the system are confirmed.

For each testing day of the patient, perform QC Cartridge testing. Refer to the quality control procedures section in the User manual of the FREND™ System. In brief, perform QC Cartridge testing for the following conditions:

- Upon initial setup of the system
- Each day of patient testing
- When the system has been transported or moved
- Whenever there is uncertainty about the performance of the system
- Whenever required by your laboratory's quality control requirements

•Internal procedural controls

The FREND™ D-Dimer test cartridge contains a built-in control feature. Fluorescence signal in the reference zone of each cartridge shows: (1) that enough sample volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this reference zone signal is missing or lower than the threshold, the FREND™ System considers it an incorrect or failed test and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

•External quality control testing

Commercially available controls from a variety of manufacturers are available that contain D-Dimer as a measure analyte. It is recommended that a minimum of two (2) levels of controls runs at least once per month or once for each new lot, whichever comes earlier. However, controls should be run according to the local requirements for each laboratory. Each laboratory should establish its own criteria based on the following parameters.

- Each new lot
- Each new shipment (even if from the same lot previously received)
- Each new operator (an individual who has not run the tests for a least two weeks)
- Monthly, as a continued check on storage conditions
- Whenever problems (storage, operator, or other) are identified,
- Or other times as required by your laboratory's standard QC procedures.

Individual laboratory policy will dictate exactly which control materials and lot numbers should be run, the frequency with which controls are to be tested, criteria for acceptance if the results and required corrective action to be taken if results do not meet laboratory criteria. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument of software malfunction. Do no assay patient samples on the FREND™ System using the FREND™ D-Dimer if quality control results do not fall within the acceptable ranges. Each laboratory operates under a different set of regulations. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to whom the laboratory is responsible.

Specimen processing

•Preparation

Prepare sufficient FREND™ D-Dimer cartridges from the refrigerator to test the number of patient specimens. Allow the sample tubes and the sealed pouches containing the cartridges to come to room temperature for 15-30 minutes prior to the start of the testing procedure.
If using refrigerated patient samples, remove those from the refrigerator and allow them to come to room temperature (64~77°F or 18~25°C) prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing. Testing should not begin on these frozen samples until they have reached room temperature.
There are no other reagents or sample preparations necessary.

•Assay procedure

(1) Prepare the FREND™ D-Dimer cartridges and specimen at room temperature.

(2) Record the Patient ID on the cartridge in the designated area.

(3) Drop the specimen (35 µL) into the sample inlet on the cartridge using a calibrated micro-pipette with a fresh pipette tip.

(4) Press the 'Test' button on the 'Main' screen of the FREND™ System.

(5) The screen of FREND™ System moves to the Patient ID screen automatically.

(6) Type the Patient ID and press the 'Enter' button to begin the test.

(7) Insert the cartridge into the cartridge slot using the cartridge arrow as a guide.

⚠ ***Caution:*** *Check the direction of the cartridge before insertion and assure the insertion is complete. It is recommended to insert the cartridge after the sample loading after 30 seconds elapsed in less than 5 minutes to obtain the optimal result of test.*

(8) When the reaction in the cartridge is completed, the FREND™ System will automatically begin the reading process.

(9) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠ ***Caution:*** *Do not disconnect power cord or shut off power on the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.*

(10) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.

For more detailed instructions, please refer to the FREND™ System User manual.

Procedural notes

Samples cannot be diluted for D-dimer determinations.
Samples which read "> 10,000 ng/mL (FEU)" should be reported as such.

Calculation of results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been loaded to the sample inlet in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process. blank reading is subtracted after which the net rate is automatically converted to D-dimer concentration in ng/mL (FEU) based upon information stored on the FREND™ D-Dimer Code chip. This result is then output on the screen and to the optional printer. It is also stored in memory on the FREND™ System.

Screen displayed for various concentration scenarios

Displayed result	Description
	D-Dimer concentration Less than 50,00 ng/mL (FEU)

Displayed result	Description
	D-Dimer concentration Not less than 50,00 ng/mL (FEU) and not higher than 10,000,00 ng/mL (FEU)
	D-Dimer concentration Higher than 10,000,00 ng /mL (FEU)

Limitation of the procedure

When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, medical history, therapy, etc.).
The FREND™ System paired with a FREND™ D-Dimer cartridge, is programmed to report 10,000 ng/mL (FEU) as the highest concentration of D-dimer measurable without dilution. The lowest measurable concentration is 50 ng/mL (FEU) – the assay limit of quantitation. If the result is below the lowest reportable range, it should be reported as such < 50 ng/mL (FEU).

Performance characteristics

Precision

A single lot imprecision study was performed at the NanoEntek laboratory as described in the CLSI guideline EP05-A3. Three serum pools were assayed for 20 days, 2 runs per day in duplicate using a single lot of FREND™ D-Dimer cartridge. The results are summarized below:

	Mean (ng/dL)	Repeatability		Between-run		Between-day		Within-laboratory	
		SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
Low	500,00	41,923	8.4	4,861	1.0	6,532	1.3	42,707	8.6
Medium	2,000,00	145,388	7.1	70,590	3.4	18,011	0.9	162,619	7.9
High	4,000,00	313,297	7.9	155,689	3.9	82,391	2.1	359,420	9.1

Linearity

To demonstrate the linearity of the assay, a plasma base pool with an elevated D-Dimer (13,271 ng/mL) was prepared and diluted to a total of 11 levels according to the dilution protocol outlined in CLSI guideline EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. At each dilution level, the samples were tested in duplicate to determine the experimental value of D-Dimer. Linearity was demonstrated from <50,00 ng/mL to >10,000,00 ng/mL. The measuring range for the FREND™ D-Dimer is 50,00~10,000.00 ng/mL.

Method comparison

Method comparison studies were performed in a CLIA-certified laboratory using de-identified fresh and frozen citrate plasma specimens. The reference method was the VIDAS® D-Dimer Exclusion II on the Biomerieux VIDAS System. All samples (194) analyzed in the clinical testing were split and tested by both the VIDAS and the FREND™ D-Dimer test systems. Passing-Bablok regression analysis gave a slope of 0,9512 and a correlation (R) of 0,976, Comparability using CLSI guideline EP09-A3 shows that the two methods compare favorably.

Reference interval

Plasma (citrate) sample from a total of 137 normal, apparently healthy adult individuals were assayed on 3 lots of the FREND™ D-Dimer assay using a single FREND™ System. The reference interval was determined according to CLSI guideline C28-A, was found to be 550 ng/mL (FEU).

Sensitivity

The Limit of Detection (LoD) for the FREND™ D-Dimer was established according to the CLSI guideline EP17-A2 and was determined to be 48,67 ng/mL. The functional sensitivity (LoQ) was determined to 48,67 ng/mL.

Specificity and Interferences

Interference was defined as recovery values outside of 10% of the known specimen mean concentration. Recovery within 90% to 110% of the expected D-Dimer was considered as lack of interference. The interference studies were performed as recommended in the CLSI guideline EP07-A2 using two concentrations of D-Dimer. Results are summarized in the table below

Interferent type	Interferent (Concentration tested)	% Recovery, Low D-dimer	% Recovery, High D-dimer
Endogenous substances	Hemoglobin (500 mg/dL)	104,7	92,0
	Triglyceride (3 g/dL)	97,2	93,9
	Bilirubin (20 mg/dL)	105,4	93,0
	Human albumin (60 mg/mL)	105,2	108,6
RF	Rheumatoid factor (400 IU/mL)	108,3	93,7
Pharmaceuticals	Acetaminophen (20 mg/dL)	103,1	101,8
	Acetylsalicylic acid (65 mg/dL)	105,5	102,7
	Allopurinol (4 mg/dL)	100,8	103,2
	Amikacin sulfate (10,4 mg/dL)	104,3	91,7
	Ampicillin-Na (5 mg/dL)	98,4	91,2
	Ascorbic acid (6 mg/dL)	103,2	94,2
	Atenolol (1 mg/dL)	102,3	99,4
	Caffeine (6 mg/dL)	100,1	95,1
	Captopril (0,5 mg/dL)	102,9	91,7
	Carbamazepine (3 mg/dL)	98,8	95,6
	Chloramphenicol (5 mg/dL)	104,4	105,9
	Chlorpromazine hydrochloride (0,2 mg/dL)	99,2	108,4
	Cimetidine (2 mg/dL)	96,2	93,1
	Cinnarizine (3 mg/dL)	103,7	98,2
	Creatinine (30 mg/dL)	98,2	105,9
	Cyclosporine (0,4 mg/dL)	99,3	96,5
	Dextran 75 (2500 mg/dL)	102,0	104,0

Interferent type	Interferent (Concentration tested)	% Recovery, Low D-dimer	% Recovery, High D-dimer
Pharmaceuticals	Digoxin (0,0006 mg/dL)	98,3	102,5
	D-L methyl dopa hydrochloride (1,8 mg/dL)	100,9	92,3
	Dopamine hydrochloride (0,1 mg/dL)	103,1	92,2
	Erythromycin (6 mg/dL)	104,2	100,4
	Ethanol (400 mg/dL)	105,2	102,0
	Ethosuximide (25 mg/dL)	100,3	100,0
	Furosemide (6 mg/dL)	105,0	99,8
	Gentamicin sulfate (1 mg/dL)	107,6	91,2
	Heparin Lithium salt (300 U/dL)	104,3	104,0
	Heparin sodium salt (300 U/dL)	101,6	94,6
	Ibuprofen (50 mg/dL)	106,1	96,0
	Lidocaine (1,2 mg/dL)	100,2	103,3
	Lithium chloride (14 mg/dL)	101,7	92,4
	Levothyroxine (0,06 mg/dL)	101,6	91,9
	Nicotine (0,1 mg/dL)	102,3	93,3
	Nifedipine (0,04 mg/dL)	103,5	100,1
	Penicillin G sodium salt (2500 U/dL)	103,1	95,9
	Phenytoine (5 mg/dL)	102,6	104,1
	Primidine (4 mg/dL)	99,6	102,2
	Propranolol hydrochloride (0,2 mg/dL)	106,7	93,4
	Theophylline (4 mg/dL)	100,1	100,0
	Urea (500 mg/dL)	101,6	98,2
	Uric acid (24 mg/dL)	108,7	94,6
	Valproic acid sodium salt (60 mg/dL)	99,4	94,5
	Verapamil hydrochloride (0,2 mg/dL)	102,1	100,5
	Warfarin (1 mg/dL)	105,2	108,5

Cross-reactivity

The following substances were evaluated for potential cross-reactivity with FREND™ D-Dimer at two concentration testing was done according to the CLSI guideline EP 07-A2. No significant cross-reactivity was found. It is confirmed that there is no effect on fibrinogen, FDP-X, FDP-D and FDP-E.

Cross-reactant	Cross-reactant concentration (ng/dL)	%Cross-reactivity	
		low	high
Fibrinogen	100,000,000	0,00002	0,00029
FDP-X	1,000,000	0,00432	0,03029
FDP-D	100,000	0,04490	0,15618
FDP-E	1,000,000	0,00185	0,04099

References

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Glossary of symbols

	Caution, warning, Consult accompanying documents
REF	Catalogue number/Reference number
LOT	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
EC REP	Authorized representative in the European Community
CE	CE marking
IVD	*In vitro* diagnostic medical device
	Temperature limitation
Σ n	Contains sufficient for ⟨n⟩ tests
	Do not reuse
	Do not use if package is damaged
Rx	For prescription use only
	Irritant

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