VIDAS® D-Dimer Exclusion II™ (DEX2)

IVD

VIDAS® D-Dimer Exclusion II™ is an automated quantitative test for use on the instruments of the VIDAS® family for the immunoenzymatic determination of fibrin degradation products (FbDP) in human plasma (sodium citrate) using the ELFA technique (Enzyme Linked Fluorescent Assay).

VIDAS® D-Dimer Exclusion II™ 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.

VIDAS® D-Dimer Exclusion II™ is indicated for use in the HERDOO2 clinical decision rule (CDR) to assess the risk of recurrence of venous thromboembolism (VTE) in women with a first unprovoked VTE. Risk stratification by this CDR is an aid to guide the duration of oral anticoagulant therapy.

SUMMARY AND EXPLANATION

Fibrin degradation products (FbDP) are a highly heterogeneous group of soluble fragments that appear in the circulation as a result of two simultaneous physiological processes (1):

- Coagulation, resulting in the conversion of soluble fibrinogen into insoluble stabilized fibrin by the enzymes thrombin and factor XIIIa,
- Fibrinolysis, resulting in the dissolution of the fibrin clot by the enzyme plasmin. The D-dimer fragment is the terminal product of this process (2).

Although FbDP's vary in size, they are characterized by the presence of one or more D-dimer epitopes (1, 2). Therefore, FbDP's are collectively referred to as 'D-dimer'. Assays for D-dimer can be performed directly in plasma by virtue of monoclonal antibodies which are able to identify fibrin-specific epitopes without cross-reactivity with fibrinogen or its degradation products (1).

D-dimer reflects the presence of stabilized fibrin and this has made this marker a useful tool in the diagnosis of venous thromboembolism (VTE) (3). Quantitative D-dimer assays based on ELISA techniques have a high sensitivity for the presence of an occluding thrombus and, consequently, are particularly useful in excluding venous thromboembolism (4, 5).

In conjunction with assessment of clinical pretest probability, it is possible to safely rule out the diagnosis of deep vein thrombosis (DVT) and/or pulmonary embolism (PE) in suspected outpatients when the concentration of D-dimer is below a predefined cutoff (determined by rigorous clinical studies) (5-7). The clinical utility of D-dimer ELISA assays in the diagnostic work-up of suspected DVT or PE resides in the significant reduction in the number of imaging tests that are required, with a concomitant reduction in the total cost of diagnosis (8, 9).

For patients with suspected pulmonary embolism, the use of an age-adjusted cutoff compared to a single clinical cutoff, enables to reduce the number of imaging tests required (10).

D-dimer is not specific for DVT/PE and elevated levels are also observed in a variety of other conditions where activation of coagulation and fibrinolysis occurs (for example, surgery, trauma, infection, inflammation, pregnancy, cancer) (3). This makes D-dimer less useful for exclusion of DVT or PE in hospitalized patients due to the high proportion of comorbid conditions associated with elevated D-dimer levels (5, 11). Under certain conditions, lower than expected D-dimer results may occur giving rise to false-negatives. Therefore, it is not safe to use D-dimer for exclusion of DVT/PE in patients with high pre-test probability, long duration of DVT/PE symptoms (more than one week) or already under anticoagulant treatment (11, 12).

The VIDAS® D-Dimer Exclusion II™ assay is part of the HERDOO2 clinical decision rule that has been shown to identify women with unprovoked VTE who are at sufficiently low risk of a recurrent VTE event that they could discontinue oral anticoagulants after short-term therapy (13).

The HERDOO2 rule states that this is the case if none or only one of the following four criteria is present: 1) **H**yperpigmentation, **E**dema or **R**edness (HER) on examination of legs; 2) **D**-dimer ≥ 250 ng/mL; 3) **O**besity (body mass index ≥ 30 kg/m^2); 4) **O**lder age (≥ 65 years).

The International Society on Thrombosis and Haemostasis (ISTH) suggests that it is safe to discontinue anticoagulants if the risk of recurrent VTE is less than 5% one year after stopping anticoagulant therapy (14).

The REVERSE II study confirmed that the one-year VTE recurrence rate was below the level recommended by the ISTH, since the recurrence rate in women with a first unprovoked VTE and 0 or 1 HERDOO2 criteria was 3.0% in this study(15).

PRINCIPLE

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR®), serves as the solid phase with an anti-FbDP monoclonal antibody adsorbed on its surface, and the pipetting device. The reagents in the single-use reagent strip are ready-to-use and pre-dispensed.

All of the reaction steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR® several times.

First the sample is taken by the SPR®, diluted and then cycled in and out of the SPR® several times. The antigen binds to the anti-FbDP immunoglobulins coated on the SPR®. Unbound components are eliminated during a washing step.

During the second step, the conjugate, which contains an alkaline phosphatase-labeled anti-FbDP monoclonal antibody, binds to the antigen coated on the SPR® to form a "sandwich".

Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR®. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory. The results can then be printed.

CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS:

60 DEX2 Strips	STR	Ready-to-use.
60 DEX2 SPRs 2 x 30	SPR®	Ready-to-use. Interior of SPRs coated with anti-FbDP monoclonal immunoglobulins (mouse).
DEX2 Calibrator: S1 Calibrator 2 x 2 mL (lyophilized)	S1	**Reconstitute with 2 mL of distilled water.** Wait for 5 minutes and then mix. **After reconstitution, the calibrator is stable for 28 days at 2-8°C or until the expiration date of the kit at -25 ± 6°C** (freeze immediately after reconstitution). 5 freeze/thaw cycles are possible. FbDP obtained from human plasma* diluted in glycine-albumin bovine buffer + preservatives. MLE data indicate the concentration in ng/mL (FEU) ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range).
DEX2 Controls: C1 Control 2 x 2 mL (lyophilized) C2 Control 2 x 2 mL (lyophilized)	C1 C2	**Reconstitute with 2 mL of distilled water**. Wait for 5 minutes and then mix. **After reconstitution, the controls are stable for 28 days at 2-8°C or until the expiration date of the kit at -25 ± 6°C** (freeze immediately after reconstitution). 5 freeze/thaw cycles are possible. FbDP obtained from human plasma* diluted in glycine-albumin bovine buffer + preservatives. MLE data indicate the confidence interval in ng/mL (FEU) ("Control C1 Dose Value Range") or ("Control C2 Dose Value Range").
DEX2 Diluent 1 x 5 mL (liquid)	R1	Ready-to-use. TRIS buffer (0.05 mol/L pH 7.4) + calf serum + preservatives.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar code printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, and antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The SPR® is coated during production with anti-FbDP monoclonal immunoglobulins (mouse). Each SPR® is identified by the DEX2 code. Only remove the required number of SPR®s from the pouch and **carefully reseal the pouch after opening.**

The Reagent Strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the DEX2 strip

Wells	Reagents
1	Sample well.
2 - 3 - 4	Empty wells.
5	Conjugate: alkaline phosphatase-labeled anti-FbDP monoclonal immunoglobulins (mouse) in TRIS buffer (0.05 mol/L pH 6.5) + horse serum + preservatives (400 µL).
6 - 7 - 9	Wash buffer: TRIS buffer (0.05 mol/L, pH 7.3) + chemical stabilizers + preservatives (600 µL).
8	Diluent: TRIS buffer (0.05 mol/L, pH 7.4) + calf serum + protein and chemical stabilizers + preservatives (600 µL).
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine* (DEA) (0.62 mol/L or 6.6%) pH 9.2 + 1 g/L sodium azide (300 µL).

* Signal Word: **DANGER**

Hazard statement

H318: Causes serious eye damage.

Precautionary statement

P280: Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Single-use pipette and/or micropipettes to dispense the appropriate volumes.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instrument of the VIDAS® family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only, by qualified laboratory personnel, in clinical laboratories.**
- This kit contains products of human origin. Source materials from which the controls and calibrator were derived were found negative when tested for HIV1, HIV2, HCV, and HBsAg. However, no known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - Latest Edition).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use the SPR®s if the pouch is pierced or if the dot sealing a SPR® has come unstuck.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the box label.
- Do not mix reagents (or disposables) from different lots.
- **Use powderless gloves**, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- **The substrate in well 10 contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.**
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instruments should be regularly maintained (refer to the User Manual for user and preventive maintenance operations).

STORAGE CONDITIONS

- Store the VIDAS® D-Dimer Exclusion II™ kit at 2-8°C.
- **Do not freeze reagents, with the exception of the calibrator and controls after reconstitution.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR® pouch is correctly sealed and undamaged. If not, do not use the SPR®s.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPR®s and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SAMPLES

Sample type and collection

- Collect blood by clean venipuncture **in trisodium citrate** (0.109 mol/L / 3.2% or 0.129 mol/L / 3.8%) or **CTAD** (sodium citrate, theophylline, adenosine and dipyridamole), observing the correct anticoagulant to blood ratio.
- Refer to the tube manufacturer's recommendations for use.
- Collection of whole blood using a syringe is not recommended in order to avoid formation of microclots in the sample.

Sample preparation

The current WHO/DIL/LAB/99.1 document provides recommendations for sample preparation.
For use of sample tubes, refer to the tube manufacturer's recommendations for use.

The pre-analytical step, including the preparation of blood samples, is an essential first step when performing medical analyses.

In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.

Sample stability

Plasma samples separated from the pellet can be stored in aliquots at 2-8°C for 3 days. If longer storage is required, plasma can be frozen at -25 ± 6°C for 6 months, with up to 2 freeze/thaw cycles.
Thaw the plasma at 37°C when assaying.

Sample-related interference

Interference was studied according to the recommendations of the CLSI® document EP7-A2.

None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin, up to 300 µmol/L (monomer)),
- lipemia (after spiking samples with lipids, up to 30 g/L equivalent in triglycerides),

- bilirubinemia (after spiking samples with bilirubin, up to 537 µmol/L),
- rheumatoid factor: up to 400 IU/mL (international units per milliliter),
- human albumin: up to 60 g/L.

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

The influence of 51 analytes was also detected *in vitro*: no interference was observed.

Drug	Concentration tested	Drug	Concentration tested	Drug	Concentration tested
Acetaminophen	20 mg/dL	Cyclosporine A	0.4 mg/dL	Lithium chloride	14 mg/dL
Acetylsalicylic acid = aspirin	65 mg/dL	Dabigatran etexilate	18 mg/dL	L-Thyroxine	0.06 mg/dL
Allopurinol	4 mg/dL	Dextran 75	2500 mg/dL	Nicotine	0.1 mg/dL
Amikacin sulfate	10.4 mg/dL	Diazepam	0.5 mg/dL	Nifedipine	0.04 mg/dL
Ampicillin	5 mg/dL	Digoxin	0.0006 mg/dL	Penicillin G sodium salt	2500 U/dL
Apixaban	0.6 mg/dL	D-L methyl dopa hydrochloride	1.8 mg/dL	Pentobarbital	9 mg/dL
Ascorbic Acid (vitamin C)	6 mg/dL	Dopamine hydrochloride	0.1 mg/dL	Phenobarbital	10 mg/dL
Atenolol	1 mg/dL	Edoxaban	3.6 mg/dL	Phenytoine	5 mg/dL
Caffeine	6 mg/dL	Erythromycin	6 mg/dL	Primidone	4 mg/dL
Captopril	0.5 mg/dL	Ethanol	400 mg/dL	Propranolol hydrochloride	0.2 mg/dL
Carbamazepine	3 mg/dL	Ethosuximide	25 mg/dL	Rivaroxaban	1.2 mg/dL
Chloramphenicol	5 mg/dL	Furosemide	6 mg/dL	Theophylline	4 mg/dL
Chlordiazepoxide hydrochloride	1.1 mg/dL	Gentamicin sulfate	1 mg/dL	Urea	500 mg/dL
Chlorpromazine hydrochloride	0.2 mg/dL	Lithium heparin	300 U/dL	Uric acid	24 mg/dL
Cimetidine	2 mg/dL	Sodium heparin	300 U/dL	Valproic acid sodium salt	60 mg/dL
Cinnarizine	3 mg/dL	Ibuprofen	50 mg/dL	Verapamil hydrochloride	0.2 mg/dL
Creatinine	30 mg/dL	Lidocaine	1.2 mg/dL	Warfarin	1 mg/dL

INSTRUCTIONS FOR USE

For complete instructions, see the User Manual.

Reading VIDAS® PTC (Protocol Test Change) data and MLE data

When using the assay for the first time:

With the external instrument barcode reader, **scan the barcodes (PTC and MLE) in the following order:**

1. According to the instrument used, scan the PTC barcode(s) at the end of the package insert or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data in the kit or on the box label.

When opening a new lot of reagents:

With the external instrument barcode reader, scan the MLE data on the box label before performing the test.

Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User Manual).

Calibration

Calibration, using **the calibrator** provided in the kit, must be performed each time a new lot of reagents is opened, after the MLE data have been entered. Calibration should then be performed **every 28 days**. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.

The calibrator, identified by S1, must be tested **in duplicate** (see User Manual).

The calibrator value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Kit controls

Two controls are included in each VIDAS® D-Dimer Exclusion II™. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. The instrument will only be able to check the control values if they are identified by C1 and C2.
Results cannot be validated if the control values deviate from the expected values.

Procedure

1. **Remove the kit from storage at 2-8°C and take out the required reagents. Carefully reseal the SPR® pouch and return the kit to 2-8°C. The reagents can be used immediately.**
2. Use one "DEX2" strip and one "DEX2" SPR® from the kit for each sample, control or calibrator to be tested. Make sure the storage pouch has been carefully resealed after the required SPR®s have been removed.
3. The test is identified by the "DEX2" code on the instrument. The calibrator must be identified by "S1", and tested in duplicate. If the controls are to be tested, they should be identified by "C1" and "C2" and tested singly.
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator, controls and samples using a vortex-type mixer in order to improve result reproducibility (for plasma separated from the pellet).
6. For optimum results, refer to all the paragraphs in the **SAMPLES** section.
7. Before pipetting ensure that samples, calibrators, controls and diluent are free of bubbles.
8. For this test, the calibrator, control and sample test portion is 200 µL.
9. Insert the "DEX2" SPRs and "DEX2" strips into their appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPR®s and the STRs match.
10. **Initiate the assay immediately** (refer to the User Manual). All the assay steps are performed automatically by the instrument.
11. Reclose the vials and return them to the recommended temperature after pipetting.
12. The assay results are available within 20 minutes. After the assay is completed, remove the SPR®s and STRs from the instrument.
13. Dispose of the used SPR®s and STRs into an appropriate recipient.

QUALITY CONTROL

Additional quality controls can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Once the assay is completed, the results are analyzed automatically by the computer. The results are calculated using the calibration curve that is stored by the instrument (4-parameter logistics model), and then printed. The D-dimer concentrations are expressed in ng/mL of Fibrinogen Equivalent Unit (FEU).
VIDAS® D-Dimer Exclusion II™ has been calibrated against an internal panel of human plasma, the concentrations of which have been determined using the VIDAS D-Dimer Exclusion kit (ref. 30 442). This reference (30 442) is no longer sold.
Samples with D-dimer concentrations greater than 10,000 ng/mL (FEU) can be retested after being diluted by 1/5 in the kit diluent. **If the dilution factor was entered when the worklist was created, the result is calculated automatically. If the dilution factor was not entered, multiply the results by the dilution factor to obtain the sample concentration.**

Description of clinical decision cutoffs

- **To exclude DVT and PE in combination with clinical pretest probability assessment.**

For deep vein thrombosis, the **cutoff level is 500 ng/mL.**
For pulmonary embolism, the **cutoff level** is **either 500 ng/mL**, or age-adjusted, as follows:

- **< 50 years: 500 ng/mL cutoff**
- **≥ 50 years: age x 10 ng/mL** (example: 650 ng/mL cutoff for 65 years)

With a decision cutoff of 500 ng/mL, a D-dimer result ≥ 500 ng/mL (FEU) is considered to be positive and a result < 500 ng/mL (FEU) is considered to be negative.

With an age-adjusted cutoff, a D-dimer result ≥ the age-adjusted cutoff is considered to be positive and a result < the age-adjusted cutoff is considered to be negative.

- **To aid in evaluating the VTE recurrence rate in women with a first unprovoked VTE, in order to guide the duration of oral anticoagulant therapy.**

The cutoff level used in the HERDOO2 clinical decision rule is **250 ng/mL**. Women with a first unprovoked VTE who are on oral anticoagulants are considered positive (1 point in the clinical decision rule) with a VIDAS® D-Dimer Exclusion II™ test result ≥ 250 ng/mL and negative (0 point in the clinical decision rule) with a test result < 250 ng/mL.

LIMITATIONS OF THE METHOD

- Interference may be encountered with certain plasmas containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history (clinical probability).
- Clinical performance data were determined on an outpatient population. Because D-dimer results are likely to be elevated in an inpatient population due to stasis, chronic illness, post-surgery and other non-specific conditions known to elevate D-dimer levels, the clinical utility of a negative result is not likely to be realized in an inpatient population. Therefore, clinical performance results should not be extrapolated to an inpatient population.
- Results below the detection limit of 45 ng/mL obtained with the VIDAS® D-Dimer Exclusion II™ kit are usually linked to poor pre-analytical conditions or incorrect instrument maintenance, and therefore, the assay must be repeated. Please contact your customer service for further assistance.

- HERDOO2 rule: for postmenopausal women (≥ 50 years) with 0 or 1 HERDOO2 criteria who discontinued oral anticoagulants (n=126), the recurrence rate was 5.7% (95% CI 2.6-10.9%) (15). Bearing in mind that the ISTH suggests that it is safe to discontinue anticoagulants if the recurrence rate is less than 5% one year after stopping the treatment (14), it is all the more important that clinicians take into consideration the clinical history of patients in this group before making any decisions.

REFEFRENCE VALUES

In a study carried out using 215 citrated plasma samples from blood donors, 90% of values were below 500 ng/mL (FEU).
It is recommended that each laboratory establish its own reference values from a rigorously selected population.

PERFORMANCE

Studies performed using VIDAS® D-Dimer Exclusion II™ gave the following results:

Measurement range

The measurement range of the VIDAS® D-Dimer Exclusion II™ assay is 45 ng/mL (FEU) to 10,000 ng/mL (FEU) (upper limit of quantification).

Linearity

Linearity was determined on plasmas according to the recommendations of CLSI® document EP6-A. The results obtained have shown that VIDAS® D-Dimer Exclusion II™ is linear from 45 to 10,000 ng/mL (FEU). However, for a few isolated samples, VIDAS® D-Dimer Exclusion II™ may not be linear due to the matrix.

Detection limit

Defined as the smallest concentration of D-dimer significantly different from the zero concentration with a risk α of 5%: **< 45 ng/mL (FEU).** The results of the detection limit were determined according to the standard CLSI® EP17-A.

Hook effect

No hook effect was found up to D-dimer concentrations of 400,000 ng/mL (FEU).

Specificity

Interference was studied according to the recommendations of the CLSI® document EP7-A2.

Cross reactant	Concentration (spiked samples)	Cross reactivity
Fibrinogen	≤10 g/L	No
Fibrinogen degradation products X	≤ 10 µg/mL	No
Fibrinogen degradation products Y	≤10 µg/mL	No
Fibrinogen degradation products D	10 - 100 µg/mL	Yes*

*: such high levels of fibrinogen degradation products D do not occur in the target population of suspected VTE patients. They may be observed when patients are subjected to therapeutic lysis with thrombolytic agents (16).

Note: The specificity of the two antibodies used in this assay has not been tested against fibrinogen degradation products E, therefore cross-reactivity cannot be ruled out.

Precision

Three samples were tested in duplicate in 40 different runs (2 runs per day) with 2 reagent lots at 3 sites (n=240).
The repeatability (intra-run precision), and between lot reproducibility were calculated using this protocol, based on the recommendations of CLSI® document EP5-A2:

		Repeatability		Between lot reproducibility	
Sample	Mean concentration ng/mL (FEU)	Standard deviation	CV (%)	Standard deviation	CV (%)
Sample 1	277.97	6.88	2.5	18.25	6.6
Sample 2	544.14	11.05	2.0	32.13	5.9
Sample 3	7,788.88	113.83	1.5	468.18	6.0

Comparison with another test method

Study 1:

328 fresh citrated plasmas from D-dimer samples coming from the laboratory routine activity of one European site and two North American sites, were tested using two lots of VIDAS® D-Dimer Exclusion II™ and five lots of VIDAS® D-Dimer Exclusion according to the recommendations of CLSI® document EP9-A2. The concentration range of plasma samples investigated is from 58.46 to 9,594.96 ng/mL (FEU). The following results were obtained:

- **Passing & Bablok regression and correlation coefficient (r):**

Sites		n	Slope	Intercept	r
Europe	**Site 1**	158	1.20	-41.96	0.991
North America	**Site 2**	43	1.15	-10.31	0.988
	Site 3	125	1.20	-43.18	0.989
All		326*	1.19	-34.92	0.987

* 2 statistical outliers were removed from the analysis.

Study 2:

378 citrated plasmas coming from the laboratory routine activity of one European site, were tested using two lots of VIDAS® D-Dimer Exclusion II™ and two lots of VIDAS® D-Dimer Exclusion according to the recommendations of CLSI® document EP9-A2. The concentration range of plasma samples investigated is from 46.70 to 9,913.53 ng/mL (FEU). The following results were obtained:

- **Passing & Bablok regression and correlation coefficient (r):**

Site 4	n	Slope	Intercept	r
Europe	374*	1.09	-29.35	0.991

* 4 statistical outliers were removed from the analysis

In conclusion, these two studies show **a mean slope of 1.14** between VIDAS® D-Dimer Exclusion II™ and VIDAS® D-Dimer Exclusion, with slopes between 1.09 and 1.19 depending on the lots.

Clinical performance

Clinical performance using the VIDAS® D-Dimer Exclusion II™ assay in conjunction with a patient's clinical pretest probability to exclude VTE (venous thromboembolism)

A study was performed using 315 frozen **clinically characterized** samples from patients from **previous prospective clinical studies** (17, 18).

The overall prevalence of VTE in the total population studied was 23.5% (74/315). The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) using a clinical cutoff of 500 ng/mL (FEU) are summarized below with the corresponding 95% confidence intervals (CI).

		Patients with suspected VTE		
		Low and intermediate pretest probability n= 303	**High** pretest probability n= 12	**All probabilities** n= 315
% Sensitivity (95% CI)	VIDAS® D-Dimer Exclusion II™	100% (94.2 - 100)	100% (73.5 - 100)	100% (95.1 - 100)
	VIDAS® D-Dimer Exclusion	100% (94.2 - 100)	100% (73.5 - 100)	100% (95.1 - 100)
% Specificity (95% CI)	VIDAS® D-Dimer Exclusion II™	35.7% (29.6 - 42.1)	N/A	35.7% (29.6 - 42.1)
	VIDAS® D-Dimer Exclusion	37.8% (31.6 - 44.2)	N/A	37.8% (31.6 - 44.2)
% NPV (95% CI)	VIDAS® D-Dimer Exclusion II™	100% (95.8 - 100)	N/A	100% (95.8 - 100)
	VIDAS® D-Dimer Exclusion	100% (96.0 - 100)	N/A	100% (96.0 - 100)
% PPV (95% CI)	VIDAS® D-Dimer Exclusion II™	28.6% (22.7 - 35.1)	100% (73.5 - 100)	32.3% (26.3 -38.8)
	VIDAS® D-Dimer Exclusion	29.2% (23.2 - 35.9)	100% (73.5 - 100)	33.0% (26.9 - 39.6)

* 95% CI calculated using the exact method

Agreement study between the two methods (VIDAS® D-Dimer Exclusion II™ and VIDAS® D-Dimer Exclusion) **and the clinical status** are presented below (n= 315 frozen samples):

			Clinical Status	
VIDAS® D-Dimer Exclusion II™	**VIDAS® D-Dimer Exclusion**	**Total samples**	**NEGATIVE (NO VTE)**	**POSITIVE (VTE)**
< 500 ng/mL	< 500 ng/mL	85	85	0
	≥ 500 ng/mL	1	1	0
≥ 500 ng/mL	< 500 ng/mL	6	6	0
	≥ 500 ng/mL	223	149	74
	Total samples	**315**	**241**	**74**

There is no significant difference at a risk level of 5% between the sensitivity and the specificity of the two assays.

A retrospective analysis using the age-adjusted cutoff was performed on patients suspected of pulmonary embolism (PE) with low and intermediate pretest probability. Among these patients, the observed prevalence of PE was 20.5% (62/303). The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) using the age-adjusted clinical cutoff are summarized below with the corresponding 95% confidence intervals (CI).

	Patients with suspected EP
	Low and intermediate pretest probability n=303
% Sensitivity (95% CI*)	100% (94.2 - 100)
% Specificity (95% CI*)	40.2% (34.3 - 46.5)
% NPV (95% CI*)	100% (96.3 - 100)
% PPV (95% CI*)	30.1% (24.2 - 36.7)

* IC: Confidence Interval

For the VIDAS® D-Dimer Exclusion II™ assay, the specificity using the age adjusted cutoff is significantly higher than the specificity using the clinical cutoff at 500 ng/mL (FEU). There is no significant difference at a risk level of 5%, between the sensitivity using the age adjusted cutoff and the sensitivity using the clinical cutoff at 500 ng/mL (FEU).

Clinical performance using the VIDAS® D-Dimer Exclusion assay in combination with a patient's pretest probability

❖ **To exclude DVT**

Frozen samples collected from patients enrolled in **a multi-center prospective cohort study** (19) were used to validate the diagnostic utility of VIDAS® D-Dimer Exclusion to exclude a diagnosis of deep vein thrombosis (DVT).
Consecutive eligible outpatients (n= 556) with a first suspected DVT episode were evaluated at three hospitals during the course of the study. Using the Wells model to estimate the probability of DVT, patients were classified as having a high, intermediate or low pretest probability (PTP) of DVT (20).

The VIDAS® D-Dimer Exclusion assay was performed without knowledge of the PTP assessment results and the clinical outcome of the patients from which the samples were derived. A clinical cutoff level of 500 ng/mL (FEU) was used.

A D-dimer result ≥ 500 ng/mL (FEU) was considered positive and a result < 500 ng/mL (FEU) was considered negative.
During the study, patients were grouped according to PTP. Patients with a negative D-dimer test result and a low or intermediate PTP of DVT underwent no further diagnostic testing and were followed up for 3 months for development of DVT. Patients with a positive D-dimer test result or high PTP underwent serial compression ultrasonography.
The overall prevalence of DVT among the total population studied was 10.1% (56/556). One sample from the original study was not tested due to volume limitations. The sensitivity, specificity and negative predictive value (NPV) of the VIDAS® D-Dimer Exclusion assay using a clinical cutoff of 500 ng/mL (FEU) are summarized below with the corresponding 95% confidence intervals (CI).

	Patients with suspected DVT			
	Low pretest probability n= 295	**Intermediate** pretest probability n= 189	**High** pretest probability n= 71	**All probabilities** n= 555
% Sensitivity (95% CI)	100% (81.5% – 100%)	100% (80.5% – 100%)	100% (83.9%– 100%)	100% (93.6%– 100%)
% Specificity (95% CI)	39.7% (33.9%– 45.7%)	26.7% (20.3% – 34.0%)	16.0% (7.2% – 29.1%)	32.9 % (28.8% – 37.2 %)
% NPV (95% CI)	100% (96.7% – 100%)	100% (92.3% – 100.0%)	100% (63.1% – 100%)	100% (97.8% – 100%)

❖ **To exclude PE**

The diagnostic utility of VIDAS® D-Dimer Exclusion to exclude a diagnosis of PE was validated in **a multi-center prospective cohort study** using samples from 1,290 consecutive patients presenting to the emergency department with suspected PE (21). Of these 1,290 patients enrolled, 325 were eventually excluded for a total of 965 patients included in the final analysis.
All patients enrolled were classified, using the Geneva score, as having a high, intermediate or low pretest probability (PTP) of PE (22).
D-dimer results ≥ the cutoff value of 500 ng/mL (FEU) were considered positive, and results < 500 ng/mL (FEU) were considered negative.

All patients with negative D-dimer test results received no treatment and underwent no further diagnostic testing. Patients with positive D-dimer test results were further evaluated using ultrasound, helical CT scan, and/or angiography. These patients were then treated based upon the results of the additional diagnostic testing. All patients were followed up for a period of three months to evaluate any possible venous thromboembolic events (DVT or PE) and episodes of bleeding.

The overall prevalence of PE among the total population studied was 23.0% (222/965). The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) using a clinical cutoff value of 500 ng/mL (FEU) are summarized below with the corresponding 95% confidence intervals (CI).

	Patients with suspected PE		
	Low and intermediate pretest probability n= 891	**High** pretest probability n= 74	**All probabilities** n= 965
% Sensitivity (95% CI)	100% (97.7% - 100%)	100% (94.3% - 100%)	100% (98.4% - 100%)
% Specificity (95% CI)	37.6% (34.0 % - 41.2%)	45.5% (16.7% - 76.6%)	37.7% (34.2% - 41.3%)
% NPV (95% CI)	100% (98.7% -100%)	100% (47.8% - 100%)	100% (98.7% - 100%)
% PPV (95% CI)	25.8% (22.4% - 29.5%)	91.3% (82.0% - 96.7%)	32.4% (28.9% - 36.1%)

A retrospective analysis using the age-adjusted cutoff was performed on patients suspected of pulmonary embolism (PE) with low and intermediate pretest probability. Among these patients, the observed prevalence of PE was 17.8% (159/891). The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) using the age-adjusted clinical cutoff, are summarized below with the corresponding 95% confidence intervals (CI)

	Patients with suspected EP
	Low and intermediate pretest probability n=891
% Sensitivity (95% CI*)	98.1% (94.6 - 99.6)
% Specificity (95% CI*)	45.4% (41.8 - 49.0)
% NPV (95% CI*)	99.1% (97.4 - 99.8)
% PPV (95% CI*)	28.1% (24.5 - 31.9)

* CI: Confidence Interval

For the VIDAS® D-Dimer Exclusion II™ assay, the specificity using the age-adjusted cutoff is significantly higher than the specificity using the clinical cutoff at 500 ng/mL (FEU). There is no significant difference at a risk level of 5%, between the sensitivity using the age-adjusted cutoff and the sensitivity using the clinical cutoff at 500 ng/mL (FEU).

Clinical performance of the VIDAS® D-Dimer Exclusion assay and the VIDAS® D-Dimer Exclusion II™ assay in conjunction with a patient's pretest probability to exclude PE (Pulmonary Embolism)

A multi-center prospective study (19 hospitals) was conducted, including 3,324 patients with suspected PE (10). The prevalence of PE among the studied population was 19% (631/3324).
D-dimer was measured using the VIDAS® D-Dimer Exclusion and VIDAS® D-Dimer Exclusion II™ assays on 1,345 patients with a non-high or unlikely pretest probability of PE.
Patients with a non-high or unlikely pretest probability of PE and a D-dimer result below their age-adjusted cutoff, received no treatment and underwent no further diagnostic testing. Failure rate in these patients was assessed by a 3-month follow-up period with all suspected venous thromboembolic events and deaths adjudicated by an independent committee.
In this study, for the VIDAS® D-Dimer Exclusion and the VIDAS® D-Dimer Exclusion II™ assays, the PE exclusion rate increased significantly from 31.4% (423/1345) with the cutoff at 500 ng/mL, to 41.1% (553/1345) with the age-adjusted cutoff, which represents a relative increase of 30.7%. For the VIDAS® D-Dimer Exclusion and the VIDAS® D-Dimer Exclusion II™ assays, the 3-month thromboembolic failure rate in patients with D-dimer concentrations > 500 ng/mL but < the age-adjusted cutoff, was 0.0% (95% CI: [0.0 – 2.9]).

Clinical performance of the VIDAS® D-Dimer Exclusion assay and the VIDAS® D-Dimer Exclusion II™ assay in the context of the use of the HERDOO2 clinical decision rule

The HERDOO2 clinical decision rule was validated during an international prospective multi-center clinical study (44 hospitals) (15). 2,785 patients having suffered from unprovoked VTE and having received oral anticoagulants for 5 to 12 months, were recruited (1,572 men and 1,213 women). The HERDOO2 score was calculated for the women in order to differentiate between those with a high or low risk of recurrent VTE. D-dimer measurement was performed using the VIDAS® D-Dimer Exclusion or VIDAS® D-Dimer Exclusion II™ assay while the patients were still taking anticoagulants.

Thanks to the HERDOO2 clinical decision rule, 591 of the 631 women classified as having a low risk of recurrence were able to discontinue anticoagulant therapy. The one-year VTE recurrence rate in women having suffered from unprovoked VTE and having stopped oral anticoagulant therapy after 5 to 12 months was 3.0% (CI 95%: [1.8 ; 4.8]). This recurrence rate is lower than the recommended 5% defined by the ISTH (14).

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable material following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

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INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

LIMITED WARRANTY

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REVISION HISTORY

Change type categories:

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	14219E	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS
2015/06	14219F	Technical	CONTENT OF THE KIT (60 TESTS) – RECONSTITUTION OF REAGENTS INSTRUCTIONS FOR USE
2017/03	21967A	Technical	REFERENCE PRODUCT SUMMARY AND EXPLANATION PRINCIPLE CONTENT OF THE KIT (60 TESTS) - RECONSTITUTION OF REAGENTS MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED WARNINGS AND PRECAUTIONS SAMPLES INSTRUCTIONS FOR USE QUALITY CONTROL RESULTS AND INTERPRETATION LIMITATIONS OF THE METHOD REFERENCE VALUES PERFORMANCE LITERATURE REFERENCES LIMITED WARRANTY

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