EXPERIMENTAL PROTOCOL

Amendment 2.1

(Country-specific protocol amendment for US sites)

1. GENERAL INFORMATION

Title: ThRombosis exclUsion STudy for STA® - Liatest® D-Di XL **Brief title**: TRUST
Study product: STA® - Liatest® D-Di XL – an immuno-turbidimetric in vitro diagnostic (IVD) assay for the quantitative determination of D-dimer in venous plasma
Coordinating investigator authorized to sign the protocol: Christopher Kabrhel, MD, MPH, Massachusetts General Hospital, Boston, MA (US)
Centers: between 10 and 25 investigation centers in Europe, US and Canada
Participating investigators: Physicians at each center – site list will be provided separately
Sponsor: DIAGNOSTICA STAGO S.A.S 3 allée Thérésa, 92600 Asnières sur Seine, France **Representative of the sponsor authorized to sign the protocol:** Aurore Barthod-Malat, Market Access – Clinical Affairs Specialist
Monitoring, Data Management and Statistics: Donawa Lifescience Consulting S.r.l Piazza Albania 10 00153 Rome, Italy Tel: + 39 06 5782665 Fax: + 39 06 5743786

Revision History:

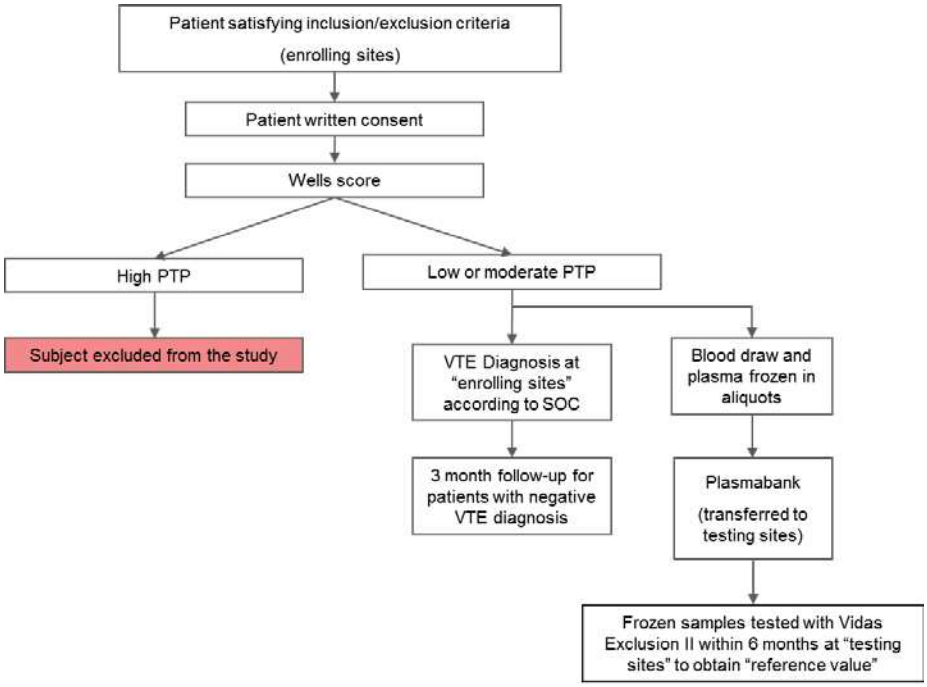
Version	**Description**	**Date**
1.0	First release	11 January 2018
1.1 Country specific amendment for France	Modifications specific to France requested by the central Ethics Committee (CPP Sud Méditerranée II): - §6.1. Clarification that screening logs may be used in the study but will not be mandatory - §6.7. Clarification of the timing of the 3 month follow-up - §14.7. and 14.8. Modifications related to GDPR regulation. Update of title of Donawa personnel involved in the study	08 August 2018
2.0	Number of evaluable reference–positive subjects to be enrolled increased from 100 to 115. Consequently, taking into consideration observed prevalence, number of suspected PE and/or DVT subjects expected to be enrolled increased from 2,848 to 3,538. The expected enrolment of US vs out of US patients was updated based on actual enrolment data. Statistical sections updated: - §9.5. Entire section updated - §9.6. NPV and Sensitivity testing hypothesis updated Typo correction throughout the protocol.	04 September 2019
2.1 Country specific amendment for US sites	- Revision of Section 8.1 to allow remote monitoring activities - Revision of Sec 14.1, to allow verbal consent procedure in clinical sites where local COVID-19 Infection Control Guidelines discourage transfer of hardcopy paper between research staff and patients (to minimize the risk of viral transmission) - Modifications of sections 9.5 to include completion of PE part of the study and update of sample size based on observed prevalence.	24 August 2020

PROTOCOL – SUMMARY
Study type: Prospective, non-randomized, non-interventional, multicenter diagnostic accuracy study in a standard of care setting.
Test product: STA® - Liatest® D-Di XL– an immuno-turbidimetric in vitro diagnostic (IVD) assay for the quantitative determination of D-dimer in venous plasma
Regulatory status of the product: EUROPE: The product is not CE marked US: The product is not approved for commercial use
Coordinating investigator: Christopher Kabrhel, MD, MPH, Massachusetts General Hospital, Boston, MA (US)
Centers: between 10 and 25 investigation centers in Europe, US and Canada.
Sponsor: DIAGNOSTICA STAGO S.A.S 3 allée Thérésa, 92600 Asnières sur Seine, France
Primary objective: To demonstrate the ability of STA® - Liatest® D-Di XL combined with a clinical PreTest Probability (PTP) to safely exclude pulmonary embolism (PE) and deep venous thrombosis (DVT) as measured by Negative Predictive Value (NPV) and sensitivity. **Primary endpoint:** For each "unlikely"* subject, Venous ThromboEmbolism (VTE) diagnosed dichotomously (positive/negative) by: • Reference Standard, which includes data obtained by imaging or follow-up, • Device (STA® - Liatest® D-Di XL) * *Subjects with low or moderate pre-test probability as determined by Wells score are collectively labeled "unlikely"*
Secondary objectives: 1. To estimate additional STA® - Liatest® D-Di XL accuracy parameters, including specificity and positive predictive value (PPV) 2. To constitute a plasma bank to be used for future studies with other DDi assays under development by Stago.

Study design

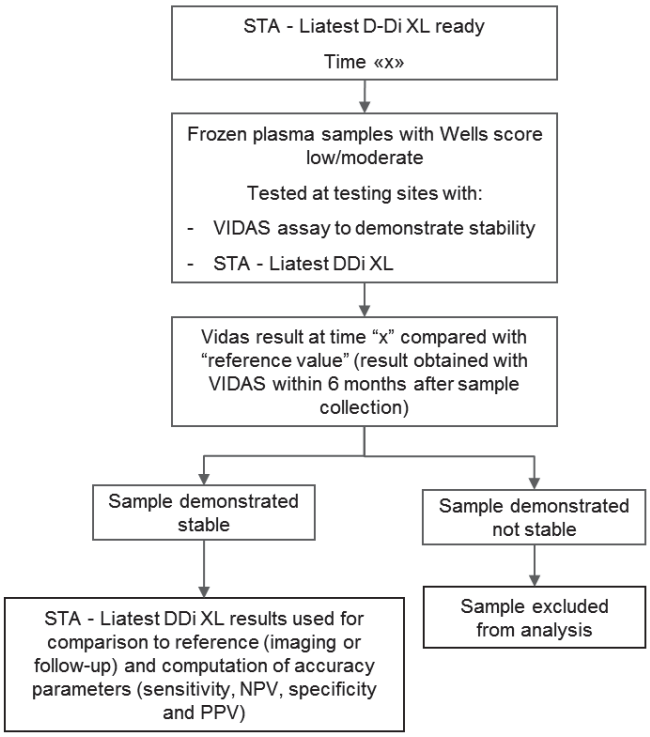
The study will be conducted in two phases:

1) The initial phase (see diagram below) will include sample and data collection from subjects suspected of VTE (PE and/or DVT) at participating sites ("enrolling sites"); subjects will be diagnosed and treated in accordance with routine standard of care (SOC). A test using VIDAS® D-Dimer Exclusion II™ (VIDAS) assay will be conducted on one of the frozen plasma aliquots. The result obtained (i.e. D-Di "reference value") will be used in the following phase to assess sample stability.



2) When the STA® - Liatest® D-Di XL product is ready for clinical testing (see diagram below), frozen samples from unlikely patients will be tested with VIDAS assay and with STA® - Liatest® D-Di XL. The results of the VIDAS assay will be used to assess samples stability. The results from "stable" samples will be used for comparison to reference (imaging or follow-up) and computation of accuracy parameters (sensitivity, NPV, specificity and PPV). STA® - Liatest® D-Di XL and stability testing will be performed at selected sites ("testing sites"). There will be at least 2 testing sites in the US and 2 in Europe.

Procedures for STA - Liatest DDi XL clinical testing



Main inclusion criteria: The study population will be recruited from prospective, consecutive, ambulatory outpatients (presenting at the emergency unit or outpatient clinic) suspected of having VTE.

Patients with DVT and PE will be analyzed as separate groups. Therefore, enrolment in each group will be monitored separately and will be stopped when the desired target per indication is reached. Subjects suspected of both DVT and PE will be included in both PE and DVT groups.

Number of Subjects

A sample size of N = 115 evaluable reference–positive subjects is needed to achieve at least 90% power to demonstrate sensitivity for PE and DVT separately. This will provide at least 80% power to demonstrate co-primary performance goals for PE and co-primary performance goals for DVT. The samples needed to obtain the number of positives will, due to low prevalence, yield a large number of negative subjects so that power for demonstrating NPV performance goals is about 100% in each indication.

Expected overall study enrolment – PE

Based on actual prevalence data obtained during enrolment, a total of at least 2,908 subjects were expected to participate in the PE study—with at least 2,206 from the US and at least 702 from OUS —to have 90% power to demonstrate STA® - Liatest® D-Di XL efficacy for PE.

At the time this protocol amendment is issued, a total of 3,054 PE patients were enrolled including 121 PE positive patients (uncleaned, non-final data). Thus, the PE data collection has been completed and data cleaning is in progress.

Expected overall study enrolment - DVT

Enrolment is still ongoing for DVT patients, for which the following estimated enrolments were calculated:

DVT estimated enrolment:
Based on actual prevalence data, a total of 1,870 subjects are expected to participate in the DVT study—with at least 1,032 from the US and at most 568 from OUS—to have 90% power to demonstrate STA® - Liatest® D-Di XL efficacy for DVT.

In summary, the two trials are expected to enroll a total of 4,924 subjects.

Note: some patients might have both PE and DVT indications and, therefore, contribute to both enrolment counts, hence the "at most" mention.

It is important to note that the above sample size is based on specific prevalence assumptions for DVT in the US and OUS. Blind sample size re-estimation has been specified for the DVT study separately to re-calculate sample size during the trial based on prevalence of reference-positive cases. Counting reference-positive will be done blind to STA® - Liatest® D-Di XL outcome.

Total expected duration of the study: around 48 months (from beginning of enrolment to site closure)

Total duration by subject: maximum duration of 4 months

Follow-up: All patients with low or moderate Wells score for whom VTE is excluded as per site standard of care (SOC) will be followed for 3 months to evaluate potential development of DVT and/or PE.

Statistical Considerations

Sample Size Justification: Presentation of the sample sizes noted in the synopsis above is based on meeting performance goals using two-sided, exact binomial 95% confidence intervals based on the following assumptions:

- US prevalence of reference-positive = 3.4% and 5.3% for PE and DVT respectively
- OUS prevalence of reference-positive = 5.7% and 8.1% for PE and DVT respectively
- D-Di XL sensitivity = 98% for both PE and DVT
- D-Di XL NPV = 99.6% for both PE and DVT

Statistical Hypotheses for Co-primary Endpoints: The following co-primary hypotheses are specified separately for PE and DVT:
H_0: NPV ≤ 0.95
H_1: NPV > 0.95

and

H_0: Sensitivity ≤ 0.90

H_1: Sensitivity > 0.90

Additionally, we will need to show that point estimates for NPV and are sensitivity ≥ 0.97 and ≥ 0.95 respectively in both indications.

TABLE OF CONTENTS

2. STUDY RATIONALE

2.1 JUSTIFICATION OF STUDY IN LIGHT OF CURRENT KNOWLEDGE

It is well known that D-dimer determination can be used in conjunction with a clinical pretest probability (PTP) assessment model to exclude pulmonary embolism (PE) and deep venous thrombosis (DVT) in outpatients suspected of PE or DVT [CLSI H59-A]. Assessment of the initial clinical probability is an essential step in the diagnostic management of PE and DVT [Wells 2000, 2003, Klok 2008, Ceriani 2010].

In this context, the quantitative D-dimer assay has its greatest utility for its negative predictive value (NPV) when applied to patients classified as "unlikely" or "low or moderate" clinical probability. In these patients, a D-dimer result below the exclusion threshold value suggests strongly against the presence of VTE, such that the diagnosis can be confidently excluded and no further diagnostic procedures for VTE (imaging) need to be performed [Perrier 1996, Kelly 2003, BCSH 2004, Goekoop 2007, Pasha 2009].

Diagnostica Stago (henceforth "Stago") is in the process of developing STA® - Liatest® D-Di XL, a new immuno-turbidimetric assay for the quantitative determination of D-dimer in venous plasma. STA® - Liatest® D-Di XL is an evolution of STA® - Liatest® D-Di, an assay approved in the United States (K964728, K141144, K162227) and Europe for use in conjunction with a clinical pretest probability (PTP) assessment model to exclude pulmonary embolism (PE) and deep venous thrombosis (DVT) in outpatients suspected of PE or DVT.

STA® - Liatest® D-Di XL is expected to have improved precision and analytical measuring range (AMR) compared to STA® - Liatest® D-Di. The new assay includes modifications:

- in the homogenization process of reactional medium containing D-dimer plasma and anti-D-dimer antibodies;
- of the signal processing and calibration both implemented via modifications of the system configuration, and addition of a blocking agent for rheumatoid factor.

STA® - Liatest® D-Di XL is currently undergoing development activities at Stago. Therefore this study will be conducted in two phases:

1) The initial phase will include sample and data collection from subjects suspected of VTE (PE and/or DVT) at participating sites ("enrolling sites"); subjects will be diagnosed and treated in accordance with routine standard of care (SOC) *(Note: SOC indicates that the diagnosis and treatment of VTE will be performed according to local practice; i.e. will not be influenced by this study).* Additionally, plasma samples will be collected and frozen in aliquots for later

testing with STA® - Liatest® D-Di XL and to constitute a plasma bank to be used for future studies with other DDi assays under development by Stago.

2) When the STA® - Liatest® D-Di XL product is ready for clinical testing, frozen samples from unlikely patients shown to be stable after freezing will be tested with STA® - Liatest® D-Di XL. The result obtained will be used for comparison to reference (imaging or follow-up) and computation of accuracy parameters (sensitivity, NPV, specificity and PPV). STA® - Liatest® D-Di XL and stability testing will be performed at selected sites ("testing sites"). There will be at least 2 testing sites in the US and 2 in Europe. Further details on study design can be found in section 4.2.

2.2 OVERALL RISK/BENEFIT ASSESSMENT

VTE diagnosis and treatment will be conducted in accordance with local standard of care (SOC) at each site.

The additional procedures required for this study are:

- Obtaining additional blood samples (i.e. around 12 ml of whole blood) from enrolled patients at the time of study entry so that plasma can be frozen for later testing with STA® - Liatest® D-Di XL. Plasma aliquots will be used for testing with STA® - Liatest® D-Di XL in this study and for the constitution of a plasma bank for Stago. The plasma bank will be used in future studies to evaluate new D-dimer test products to be used as improved diagnostic tools for DVT and/or PE (currently under development by Stago).
- Obtaining information through 3 months post diagnosis about the occurrence of DVT and/or PE (or other clinical outcomes such as death) in patients for whom VTE was ruled out at the initial visit (i.e. patients with low/moderate PTP score and D-Di negative or with negative imaging at the initial visit).
- For sites not using Wells Score to determine PTP score as part of the SOC for diagnosis of VTE, Wells score will be requested as a study specific procedure at study entry. The Wells score will be implemented in a manner fully consistent with the routine practice at the sites where Wells Score is not part of the SOC diagnosis of VTE (for further details please see section 6). However, at these sites, results of Wells score will *not* be used for diagnosis of VTE which will be performed according to SOC procedures at the site.

As a result, anticipated risks and benefits only include those that are associated with the routine assessment of patients presenting with symptoms suspicious of VTE. Participating in this study does not expose the patients to any additional risk.

3. STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVE AND ENDPOINT

Primary objective:

To demonstrate the ability of STA® - Liatest® D-Di XL combined with a clinical pretest probability (PTP) to safely exclude PE or DVT as measured by Negative Predictive Value (NPV) and sensitivity.

Primary endpoint:

For each "unlikely"[1] subject, VTE diagnosed dichotomously (positive/negative) by:

- Reference Standard, which includes data obtained by imaging or follow-up,
- Tested Device **(**STA® - Liatest® D-Di XL**)**

3.2 SECONDARY OBJECTIVES

Secondary objectives:

To estimate additional STA® - Liatest® D-Di XL accuracy parameters, including specificity and positive predictive value (PPV).

To constitute a plasma bank to be used for future studies with other DDi assays under development by Stago.

4. INVESTIGATION PLAN

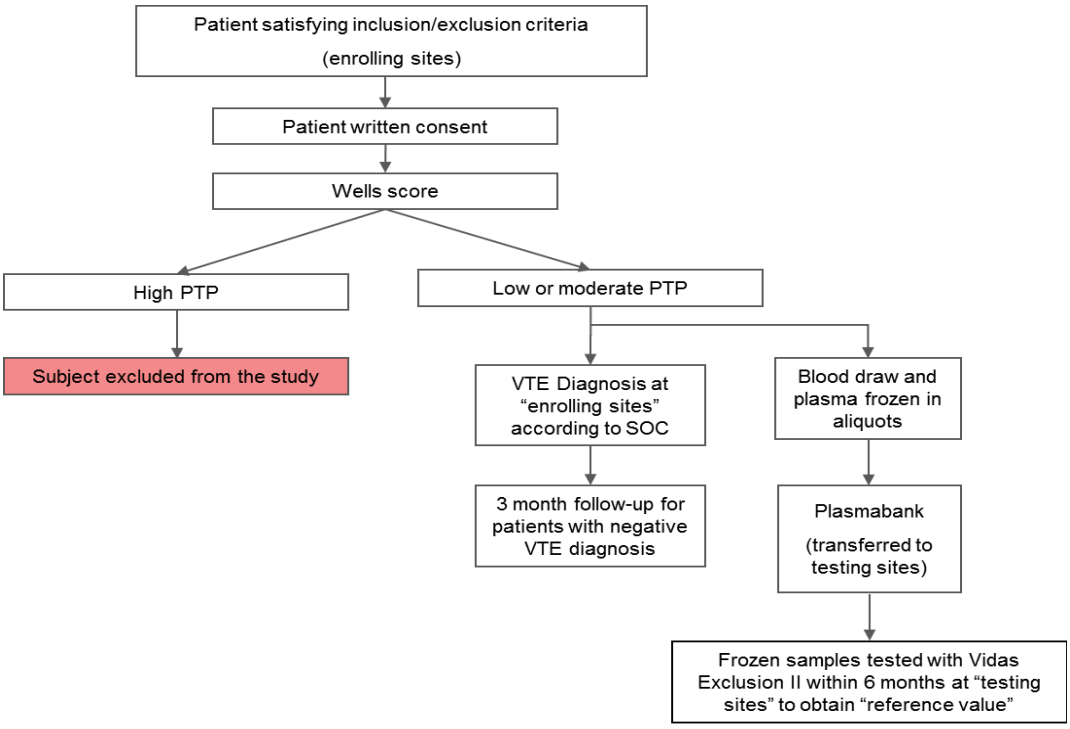
4.1 TYPE OF STUDY

This study is a prospective, non-randomized, non-interventional, multicenter, diagnostic accuracy study in a standard of care setting.

[1] *Subjects with low or moderate pre-test probability as determined by Wells score are collectively labeled "unlikely".*

4.2 STUDY DESIGN

The study design follows the diagrams shown below and is further described below.

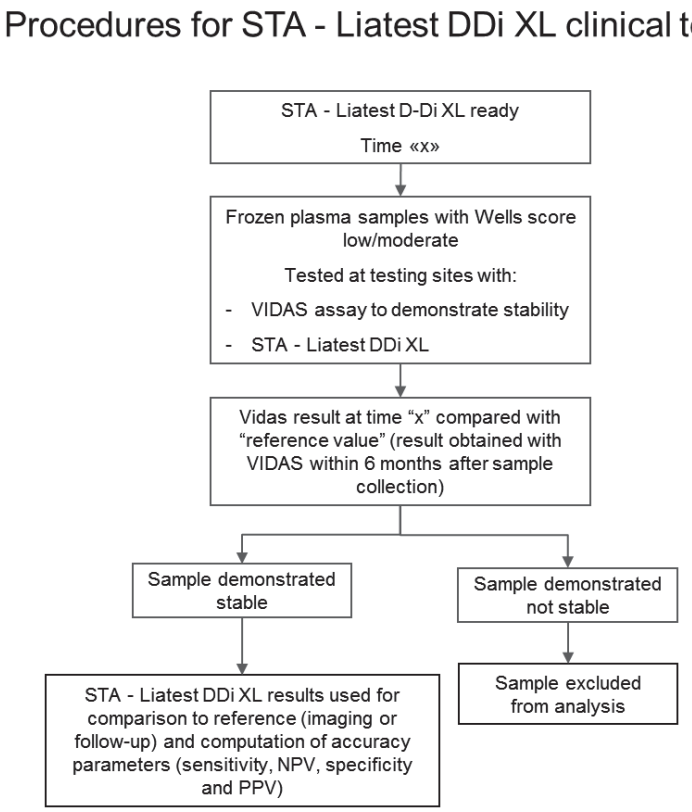


1. Subjects suspected of VTE (PE and/or DVT) will be diagnosed and treated in accordance with routine SOC. Sites not using the Wells score as part of SOC will be asked to do it specifically for the study. Additionally, for "unlikely" subjects (with low or moderate PTP), plasma samples will be collected and frozen in aliquots for later testing with STA® - Liatest® D-Di XL and for the constitution of a plasma bank (frozen plasma samples may be used in future studies to evaluate new D-dimer test products to be used as improved diagnostic tools for DVT and/or PE, currently under development by Stago).

2. A test using VIDAS® D-Dimer Exclusion II™ assay (bioMérieux SA, RCS LYON 673 620 399, 69280 Marcy-l'Etoile / France) will be conducted within 6 months on one of the frozen plasma aliquots at "testing sites": 6 months is a freezing time period not affecting sample stability as declared in the VIDAS package insert. The result obtained (i.e. D-Di value at reference time) will be used in the following phase (see bullet 3) to assess sample stability.

   Note that the VIDAS assay was chosen since it is known to provide the best precision among currently approved D-dimer assays [CAP survey 2014, 2015, 2016]. Consequently, using the VIDAS assay is expected to better assess plasma sample stability, which is most efficient for this trial since only stable samples will be included for subsequent testing with STA® - Liatest® D-Di XL.

3. When the STA® - Liatest® D-Di XL product will be ready, clinical testing will be done according to the following diagram.



Frozen plasma samples from "unlikely subjects" will be tested with VIDAS assay and with STA® - Liatest® D-Di XL within 4 hours from thawing from the same frozen sample. This measure with the VIDAS assay will be used to assess samples stability. A plasma sample will be considered stable if the result obtained relative to the first testing with the VIDAS assay ("reference value", see bullet #2) is within:

- +/- 0.15 µg/mL for DDi levels below or equal 1 µg/mL FEU (at first testing);
- +/- 15% for DDi levels above 1 µg/mL FEU (at first testing).

4. For samples determined stable according to defined criteria STA® - Liatest® D-Di XL result, combined with the result of the Wells Score assessment at the first visit, will be used for comparison to reference (imaging or follow-up) and computation of accuracy parameters (sensitivity, NPV, specificity and PPV).

It is important to note that VIDAS assay is used to test stability of frozen plasma samples with respect to D-dimer, *and is not meant to substitute for* STA® - Liatest® D-Di XL. In this context, our interest is in stability as a *characteristic of the sample* (not the device) and is best assessed by VIDAS assay, which is known to provide the greatest precision of cleared products and in this sense can be considered a "reference standard" for sample stability. Only after stability is established it is appropriate to evaluate D-dimer with the test device STA® - Liatest® D-Di XL.

Patients with DVT and PE will be analyzed as separate groups. Therefore, enrolment in each group will be monitored separately and will be stopped when the desired target per indication is reached.

4.3 SITE SELECTION

Investigators participating in the study will be assessed to be qualified by education, training and experience through pre-study visits, publication review and participation to similar studies. Each principal investigator will be contacted to assess what the routine procedure for the diagnosis of VTE is in his/her center. The site will be visited to determine available resources in terms of patient population, site staff and facility structure/organization, unless these information are accessible to Stago otherwise.

Stago plans to include in the study between 10 and 25 sites where patient enrolment will be performed and plasma samples banked frozen ("enrolling sites"). "Testing sites" will be in charge of testing samples, for initial testing with VIDAS assay and with STA® - Liatest® D-Di XL when assay is ready for clinical testing. There will be at least 4 "testing sites", 2 in Europe and 2 in the US. A "testing site" can also be an "enrolling site".

Testing of STA® - Liatest® D-Di XL will be performed by site staff blinded to imaging or follow up results. A procedure will be set up before testing starts to ensure that blinding is rigorously maintained at "testing sites".

4.4 STUDY SITES (CENTERS)

Between 10 and 25 investigation centers in Europe, US and Canada.

5. STUDY POPULATION

5.1 INCLUSION / EXCLUSION CRITERIA

INCLUSION CRITERIA

1. Patient is < 80 years old.
2. Patient presents at least one of these symptoms indicative of proximal DVT or PE:

- symptoms for **proximal DVT**: leg pain, tenderness (discomfort through palpation), leg swelling, and /or edema,
- symptoms for **PE**: hemoptysis, lung related chest pain, dyspnea.

3. Patient provides written informed consent to participate in the study
4. Patient is willing to comply with specified follow-up evaluation at 3 months and can be contacted by telephone

EXCLUSION CRITERIA

1. Patient presenting with a condition that may be associated with increased D-dimer levels, even in the absence of VTE, such as:

- Fibrinolytic therapy within the previous seven (7) days,
- Disseminated intravascular coagulation
- Bone fracture or surgery (with general anesthesia longer than thirty (30) minutes) within the previous one (1) month,
- Deep hematoma diagnosed by imaging techniques within the previous one (1) month,
- Disseminated malignancies and active cancer (active cancer defined as: cancer for which therapeutic or palliative treatment is either ongoing at the time of enrolment or has stopped less than six (6) months before enrolment),
- Sepsis, severe infections, pneumonia within the previous 1 month,
- Known liver cirrhosis,
- Pregnancy or having delivered within the previous 1 month,
- Atherosclerotic vascular disease thrombosis within the previous 1month (e.g. myocardial infarction, stroke, coronary syndrome, peripheral artery disease stage III or IV),
- Sickle cell disease,

2. Patients presenting with a suspect thrombotic event related to catheter implantation
3. Ongoing therapeutic anticoagulants (curative and preventive regimen) started twenty four (24) hours or more before blood draw (except aspirin and platelet inhibitors)
4. Previous anticoagulant therapy stopped less than three (3) months before blood draw (except aspirin and platelet inhibitors)
5. Patients with previous DVT/PE occurred less than three (3) months from screening.
6. Suspect thrombotic events in other locations at screening, including distal to the knee and upper extremity DVT (based on standard of care examinations)
7. Patients with known tissue plasminogen activator (tPA) deficiency
8. Patient participating or who has participated within one month of enrolment in another investigational study
9. Major co-morbid condition(s) or other reasons that could limit the patient's ability to participate in the study or to comply with follow-up requirements, or impact the scientific integrity of the study.

6. STUDY PROCEDURES

6.1 SCREENING

Prospective, consecutive ambulatory outpatients (presenting to the emergency unit or outpatient clinic) suspected of having VTE will receive an explanation about the study, will be screened for eligibility using the inclusion and exclusion criteria (see Section 5.1). If the patient is eligible, he/she will be

offered the possibility to participate in the study and go through the informed consent process (see Section 14.1) These patients will then be evaluated using the Wells score and procedures according to SOC for VTE diagnosis at the site.

As patients eligible in this study require emergency procedures/tests to be performed, it is possible that D-Dimer test or VTE diagnosis are completed before the investigator has the possibility to obtain patient written consent to participate in the study. Therefore, **this procedure will not be considered a protocol deviation** as long as the Investigator is blinded to the result of the D-Dimer test or to final VTE diagnosis at the time of patient screening.

The relevant demographic characteristics, medical history and concomitant medication treatment of the enrolled patients will be reported.
Three months follow-up information may be recorded directly in the CRF, in this case the CRF will be considered as Source Document.
A register may be kept for:

- all screening failure patients, including reasons for non-inclusion
- patients screened and satisfying the criteria for inclusion/exclusion but for whom a final VTE diagnosis according to site's SOC is not available will be excluded from the study. These patients will be reported, as well as the reasons for their missing diagnosis.

6.2 VTE DIAGNOSIS AND STUDY PROCEDURES

At all sites, VTE diagnosis will follow SOC procedures for diagnosis and treatment.
For patients suspected of VTE, this may include imaging only, or D-Dimer test followed by imaging or PTP score + D-Dimer testing + imaging. For all patients for whom VTE is not confirmed by imaging at the initial visit, the study will require that a 3-month follow-up is performed.

- Irrespective of the procedures used locally for VTE diagnosis, sites will be asked to evaluate patients with symptoms consistent with VTE (DVT and/or PE) using the Wells score without knowledge of D-Dimer and/or imaging results. The Wells score result may or may not be used for VTE diagnosis depending on sites' SOC.
- About 12 ml of venous blood will be taken from unlikely subjects (see definition on page 10) in order to obtain at least 6 aliquots of 1 mL of patient plasma each: plasma aliquots will then be banked frozen at -70°C
- STA® - Liatest® D-Di XL will later be used on banked frozen samples to assess assay's accuracy relative to the reference standard (imaging or 3-month follow-up). Only samples determined stable using the procedure described in Section 4.2 will be included in the study.
- Samples may also be used in future studies to evaluate new D-dimer test products to be used as improved diagnostic tools for DVT and/or PE (currently under development by Stago).

Plasma from patients with high Wells score will not be collected in the study.

6.3 WELLS SCORE

All testing sites will follow the same PTP model: the Wells score[2] (see Appendix A).
In case of patients showing symptoms of both DVT and PE, both Wells scores will be determined.

Patients suspect of DVT must have a Wells score for DVT ≤ 2 to be eligible in the study
Patients suspect of PE must have a Wells score for PE ≤ 6 to be eligible in the study

For those investigation centers where the Wells score is *not* part of routine practice the questions for the Wells score will be answered by the treating attending physician, and the score will be calculated without knowledge of the D-dimer test and imaging results. This process will be exactly consistent with what would be routine practice at the site.

6.4 D-DIMER TESTING WITH STA® - Liatest® D-Di XL

STA® - Liatest® D-Di XL D-Dimer testing will be performed at "testing sites" and staff performing the assay will be blinded to reference diagnosis.

6.4.1. Instruments

STA® - Liatest® D-Di XL test will be performed at testing sites (see sec. 2.1) on frozen banked samples using Stago instruments from Max® generation of the following families: STA-R® family , STA Compact® family or STA –Satellite® family (currently under development – will be included if available at the time testing with STA® - Liatest® D-Di XL begins).
The use of these instruments with STA® - Liatest® D-Di XL will require updated software version including :

- Modification in the homogenization process of reactional medium containing D-dimer plasma and anti-D-dimer antibodies
- Modification of the signal processing and calibration curve.

Software changes will be implemented only for the STA® - Liatest® D-Di XL test and they will not impact the instrument configuration used for other D-Dimer assays.

If one of the above mentioned Stago analyzer is already routinely used at the testing site, at the beginning of the testing phase a full check of the instruments, including application of the new

[2]Wells PS. Integrated strategies for the diagnosis of venous thromboembolism. *J ThrombHaemost.* 2007;5(Suppl. 1):41-50

software version mentioned above, will be performed by Stago personnel. In addition, the performance of the instruments will be verified during site visits performed by Stago Technical personnel before study starts and will appear in the relevant validation report. The necessary quality control of the instruments should be performed by the laboratory personnel as per routine practice described in the instructions for use. This will be verified by the assigned monitor during monitoring visit.
The serial numbers and software versions will be recorded at the start of the study and included in the validation report.

If none of the above mentioned Stago analyzers are routinely used at the testing site, then at the beginning of the study Stago personnel will implement the Stago analyzer(s) at the site strictly following the process used by Stago to implement the analyzer in routine practice at clinical sites.

6.4.2. Reagents for STA® - Liatest® D-Di XL

The following reagents will be used in the study:

- STA® - Liatest® D-Di XL
- STA® - Owren-Koller (ref. 0360) / STA Diluent-Buffer (ref. 03138097)
- STA® - Cuvettes (ref. 38669)
- STA® - Cleaner Solution (ref. 0973) / STA Washing Solution (ref. 11447157)
- STA®- Mini Reducer (ref. 0797) / STA Reducer (ref. 11662996)
- STA®-Desorb U (ref. 0975) / STA Desorb U (ref. 11820192)
- STA® - D-Di Control
- STA® - D-Di Control Low.

6.4.3. VIDAS assay

VIDAS® D-Dimer Exclusion II™ (bioMérieux SA, RCS LYON 673 620 399, 69280 Marcy-l'Etoile / France, k112818) will be used to demonstrate sample stability (see sec. 4.2). As per package insert, the assay will be performed on instruments from the VIDAS family (VIDAS, miniVIDAS or VIDAS 3).
The package insert recommendations must be followed to perform D-dimer testing with VIDAS assay.

6.4.4. Collection and handling of patient samples

It is recommended to comply with CLSI Guideline H21-A5 Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays.

Blood samples will be collected at the initial visit using 3.2% trisodium citrate anticoagulant tubes, 1 volume of citrate for 9 volumes of blood. Plasma samples will then be centrifuged for 15 minutes at 2000-2500 g at room temperature. Alternative centrifugation conditions are allowed, provided that the

plasma is platelet poor (< 10000 platelets/mm^3) or that the adopted centrifugation conditions were shown not to affect D-dimer results.

Plasma will be distributed in freezing tubes in at least 6 aliquots of minimum 1 mL. Plasma will then be frozen and stored at each site at a temperature below - 70 °C in a freezer that does not have defrosting cycles.

6.4.5. STA® - Liatest® D-Di XL testing (for testing sites only)

Before testing a sample with STA® - Liatest® D-Di XL the sample's stability must be established as described in Section 4.2. Briefly, each sample from "unlikely" subjects will have been tested with VIDAS assay either fresh or after freezing within 6 months of initial collection (see diagram on sec. 4.2). The same sample will be tested with VIDAS assay and with STA® - Liatest® D-Di XL within 4 hours from thawing. If the sample does not meet stability criteria described in sec. 4.2. (+/- 0.15 µg/mL for DDi levels below or equal to 1 µg/mL FEU at first testing; +/- 15% for DDi levels above 1 µg/mL FEU at first testing), it will be excluded from the study.

The package insert recommendations must be followed to perform D-dimer assays with the STA® – Liatest® D-Di XL. STA® – Liatest® D-Di XL is a pre-calibrated assay. The parameters of calibration are loaded in the analyzer with the bar-code printed on the bar-code insert.

Before testing, frozen plasma samples will be thawed in a water bath at 37°C for the needed time and mixed by reverse agitation before use.

D-dimer assay with STA® – Liatest® D-Di XL is performed using the instrument configuration set up by Stago (see 6.5.1). The assay will be run in singlicate.

STA® - D-Di Control and STA® - D-Di Control Low will be used to perform quality control for the STA® – Liatest® D-Di XL -dimer assay. Quality controls will be run according to instruction for use at least once a day or more if requested by local regulations.

D-dimer levels measured by STA® – Liatest® D-Di XL are expressed in fibrinogen equivalent units (FEU).
The STA® – Liatest® D-Di XL threshold is equal to 0.50 µg FEU/ml:

- the result is negative if sample level is inferior to 0.50 µg FEU/ml,
- the result is positive if sample level is superior or equal to 0.50 µg FEU/ml.

Testing of STA® - Liatest® D-Di XL will be performed at testing sites by site staff blinded to imaging or follow up results obtained at enrolling sites. A procedure will be set up before testing starts to ensure that blinding is rigorously maintained at each testing sites.

6.5 SAMPLE STABILITY AFTER FREEZING

As noted in Section 2.1, samples will be tested by VIDAS assay after freezing within 6 months of collection (6 months is a freezing time period not affecting sample stability as declared in the VIDAS package insert) and retested with the same assay at the time of testing with STA® - Liatest® D-Di XL.

Only samples meeting the acceptance criteria specified in Section 2.1 and for which associated QC results are in the QC ranges will be included in the primary efficacy analysis set (see sec. 9.4).

6.6 IMAGING STUDIES FOR VTE

Imaging techniques used for the study will include any technique used as a routine practice at the sites among which are: compression ultrasonography (CUS), ventilation/perfusion scan, helical computed tomography (CT) scan based on PIOPED II criteria, pulmonary angiogram and venography.
Technique(s) and equipment(s) used for imaging studies will be reported in medical records.
All imaging analyses and/or medical reports for each patient for the categories of patient for whom these data will be collected (see diagram in previous pages) will be archived.

6.7 FOLLOW UP

Patients for whom VTE was excluded as per SOC will be followed for 3 months to evaluate potential development of DVT and/or PE.

This follow-up will be performed by the clinician, the study coordinator or the nurse to obtain information regarding development of DVT and/or PE in the 3 months (i.e. 90 days) following Visit 1. The follow-up will have an allowed window of 1 month. Therefore it will take place in the period between 3 and 4 months(i.e. 90 to 120 days) after patient's first visit. The follow-up may be done using several methods according to site choice:

- Phone call to the patient,
- Medical record review if at enrollment patient states he/she does all its medical consultation at the investigation site and if he/she agrees that follow-up will be done by review of its medical record,
- Email to the patient asking the patient to call one site study representative if patient has given its consent to be contacted via email and server for email is secure,
- Call to family doctor in case patient is not answering the call.

The three-months follow-up information may be recorded directly in the CRF, in this case the CRF is considered as Source Document.

In addition, for female patients pregnant at the time of the follow up contact, all efforts will be done to determine if patient had an undetected pregnancy at visit 1, in which case the patient will be excluded from the study due to violation of an exclusion criterion.
If a patient cannot be contacted to collect follow-up information even beyond the 120 days from visit 1, he/she will be considered “lost to follow-up”. But before declaring that a patient is "lost to follow-up", the PI (or his/her team) must do his/her best effort to contact patients and attempts should be made via all available routes, and when necessary, patients should be traced through relatives and examination of State death records. A certified letter should be sent to the permanent address on file. The methods used to attempt to contact the patient should be noted.

6.8 AUTHORIZED ASSOCIATED TREATMENT

All the treatments judged necessary are allowed and left to the discretion of the investigator.

6.9 PROHIBITED ASSOCIATED TREATMENTS

No treatments are prohibited in this study.

7. DATA COLLECTION

All data described above will be recorded in the study Electronic Case Report Form (e-CRF) (see Section 8.2).

8. STUDY MONITORING AND DATA MANAGEMENT

8.1 STUDY MONITORING

Representatives of STAGO must be allowed to visit or remotely monitor all study site locations periodically to assess the data quality and study integrity. The sites will be monitored by Stago clinical personnel or by qualified clinical staff from a Contract Research Organisation (CRO) that will be selected by Stago. Monitors’ qualification, extent of Source Data Verification (SDV), timing and frequency of monitoring visits, possibility to perform remote monitoring, the essential documents to be reviewed and other study specific monitoring requirements will be addressed in a separate document that will be developed by STAGO and the CRO. Monitoring will include periodic review of the data to ensure that the investigators are in compliance with the protocol, the clinical trial agreement and the applicable regulations. Study records will be reviewed and compared against source documents and any discrepancies will be resolved.

Representatives of STAGO and/or the CRO will meet with the investigator prior to the initiation of the study in order to review the adequacy of the patient population, facilities, and equipment with respect to the needs of the study, and to familiarize the investigator with the study protocol.
At study initiation study monitors will visit the site in order to ensure that patients are being properly selected, that the methods described in the study protocol are understood and that data are being correctly recorded.

During the course of the study the monitors will either conduct on-site or remote review of the source documents and compare with the reported data into the electronic Case Report Forms (eCRF).Upon completion of the study the monitors will notify the site of closeout and a study closeout visit will be performed either on-site or remotely.

8.2 DATA ENTRY

All sites participating in this study will use e-CRF for all data.
The integrated procedures in production, development, test and maintenance will be thoroughly audited and fully documented in compliance with 21 CFR Part 11.
Checks for occurrence, probability, and coherence will be made simultaneously with, or as quickly as possible after, data entry. The investigator is warned of any errors detected and can take action immediately. In addition, monitors and data manager(s) can follow the quality of the data input in real time and perform check of forms on line. Each modification is made by clicking on the item, which generates a change that the investigator validates electronically.

- Certification of the data

The data signature can follow immediately after the data input, or after an email request for validation is sent to the center investigator. A formatted version of the data can be submitted to the center investigator for control and validation at any time.

- Security

Each individual with access to the eCRF will have a user name and a password associated with a profile that defines user access level (access denied, read-only access, read-write capability) on each function of the application. For example, center investigators have full access to their patient data, not those of the other centers but they can consult them.

8.3 DATA MANAGEMENT

The data management will be delegated to a CRO. The CRO will also be responsible for the development of the primary database for the study as well as the quality control of the database and confirming the overall integrity of the data.
Corrections are made through the electronic data capture tool, which generates an automated audit trail including date and timestamp, full name of the person making the correction and the original entry. The system also prompts the user to document the reason for change which is also retained in the audit trail.

9. STATISTICAL CONSIDERATIONS

9.1 OVERVIEW

The primary aim of this study is to demonstrate the ability of the STA® - Liatest® D-Di XL (Device) to exclude VTE in subjects with low or moderate pre-test probability (PTP) for each indication separately (PE and DVT), as quantified by NPV; subjects with low or moderate pre-test probability are collectively labeled “unlikely.” Additionally, this study aims to demonstrate the sensitivity of STA® - Liatest® D-Di XL in the “unlikely” population of the indications noted. Primary statistical testing and success criteria for this trial are taken from FDA approved CLSI guidelines.

Enrolment in the DVT and PE groups will be monitored separately. Ideally, recruitment rates will be similar in the two groups in which case analyses for both will be done at about the same time and a single regulatory submission will include both indications. If this is not the case, statistical analyses will be done separately and, if needed, submissions will be done separately as well. The statistical analysis plans (SAPs) for PE and DVT will be finalized and signed prior to data lock for the first indication completed, (should the indications differ in data lock times). This will enable using data from patients with both indications—i.e. patients suspected of having both PE and DVT—to be included in the analyses of the separate indications

9.2 DESIGN CONSIDERATIONS

This is a prospective, multi-center study which will recruit consecutive subjects suspected of VTE. The population of interest includes those subjects classified “unlikely” by the Wells score system as specified in the CLSI guideline.
Following are central elements of this trial's design:
Separate Statistical Testing of DVT and PE: Logistically this trial will be conducted as a single study. However data obtained will be analyzed separately for DVT and PE subjects. Trial design is identical for both indications and statistical analyses are almost identical.

Blinding of follow-up data: All “unlikely" subjects excluded at Visit 1 by SOC D-dimer negative or by negative imaging will be followed for occurrence of VTE for up to three months. To minimize possible bias during follow-up:

- Testing of STA® - Liatest® D-Di XL will be performed by testing site staff blinded to imaging or follow up results. A procedure will be set up before testing starts to ensure that blinding is rigorously maintained at testing sites
- Stago will be exposed to recruitment rates and count of reference-positive cases for the purpose of blind sample size re-estimation. Company will be blinded to all other data (including, of course, device outcome). A standard operating procedure (SOP) will be developed to ensure that this in fact is the case.
- Company and designated statisticians will be blind to efficacy data throughout the trial. The electronic data capture (EDC) provider will create a file accessible to data management and statistics that includes all study data excluding Device result (level of D-dimer) and Device diagnosis (positive or negative). This will enable counting reference-positive cases blind to outcome, which is needed for blind sample size re-estimation. The provider will create a separate file that is accessible to the provider only, in which subjects' D-dimer results are entered. Files will be merged at data lock, after the final SAPs have been completed and signed. The procedure will be drawn up in an SOP.

STA® - Liatest® D-Di XL testing for the purpose of this trial will be done on banked samples collected from subjects at Visit 1.

Special efforts will be made by PI (or his/her team) to obtain follow-up clinical outcome for subjects who could not initially be reached by the standard follow-up procedures. Such efforts may include attempts to trace subjects through relatives and examination of State death records.

Ensuring Requisite Power: The co-primary hypotheses in this trial relate to NPV and Sensitivity. To obtain requisite power for both co-primary endpoints a minimal number of VTE positive cases (for testing sensitivity) and D-Di negative cases (for testing NPV) is needed. Our computations indicate that having a sufficient number of VTE positive cases for testing sensitivity will, due to low VTE positive prevalence, yield a sufficient number of D-Di negative cases as well; when power for sensitivity is near the desired 90%, power for NPV is near 100%. Thus, determination of sample size is driven by the trial’s requirement for sensitivity.
The trial will conclude after enough VTE positive cases have been recruited to achieve requisite power.

As noted in Section 4.2 blood samples will be tested for stability using the VIDAS D-dimer assay and only those determined stable will be included in this study (and tested with STA® - Liatest® D-Di XL). It is important to note that:

a. VIDAS® D-Dimer Exclusion II assay was chosen because its precision is highest of all FDA approved assays in the indication. Greater precision is expected to yield a better assessment of stability since measurement error (random variation in outcome) may cause sample to be wrongly classified as "unstable".
b. A blood sample that has been frozen and thawed, and shown stable by testing (within acceptable error) is equivalent to the fresh sample. Consequently, testing stable frozen/thawed samples with STA® - Liatest® D-Di XL is equivalent to having tested the sample fresh.

9.3 ENDPOINTS

Primary Efficacy
For each "unlikely" subject, VTE diagnosed dichotomously (positive/negative) by:

- Reference Standard, which includes data obtained by imaging or follow-up,
- Device

9.4 ANALYSIS SETS

The Primary efficacy analysis set will consist of all "unlikely" VTE subjects for whom there are valid observed values on both STA® - Liatest D-Di XL and Reference Standard (imaging or follow up). This includes "unlikely" subjects suspected of PE, or DVT or both; the latter will be included in both of the separate PE and DVT analysis sets. "Unlikely" D-dimer negative subjects having imaging result at Visit 1 will have imaging as reference. Device result is considered valid if it is not missing, its associated QC results are in the QC ranges and is deemed stable after thawing according to criteria provided in Section 4.2. Subjects with major protocol deviations likely to affect outcome will be excluded by blind review[3]. In line with CLSI guidelines, distal DVT subjects are not included in the primary efficacy analysis set for the DVT indication.

9.5 SAMPLE SIZE CONSIDERATIONS

Presentation of sample size in this study is based on demonstrating STA® - Liatest® D-Di XL effectiveness in "unlikely" subjects for VTE meets the following performance goals as provided in Table 1A of CLSI guideline (H-59A p. 28):

- Sensitivity

[3] U.S. Dept. of HHS, FDA. Guidance for industry: E9 statistical principles in clinical trials. 1998; Section 5.2.1, pp. 28 –

- Lower 95% two-sided confidence interval (LCL) ≥ 0.90
- Point Estimate ≥ 0.95

- NPV
  - LCL 95% two-sided ≥ 0.95
  - Point Estimate ≥ 0.97

While performance goals (PGs) are the same for PE and DVT, power must be calculated separately for each because of different prevalence and accuracy assumptions for PE+ and DVT+. The accuracy assumptions are based on observed results in the studies submitted to FDA[4]. Notwithstanding, blind sample size re-estimation (i.e. counting of positive cases during the trial) will be employed to achieve requisite power. For both PE and DVT sample sizes we assume that at about 70% of reference-positive cases will be from the US and the rest (about 30%) from out of US (OUS).

PE: Power for Meeting PGs

Presentation of sample size is based on obtaining a enough reference-positive cases to achieve 90% power to meet the LCL PG for sensitivity (≥ 0.90), which will necessarily:

- Enable success on the point estimate PG for sensitivity, since the minimum true-positive rate needs to meet the LCL PG, given the sample size reported below, is the same as the minimum needed for the point estimate PG.
- Yield enough reference-negative patients for near 100% power to meet the PGs for NPV, due to low prevalence of positive cases.

Based on the counting of positive cases (allowed by this protocol) to the date of amendment V2.1, we assume:

- US prevalence of reference-positive = 3.4%
- OUS prevalence of reference-positive = 5.7%

Based on historical data, we assume:

- D-Di XL sensitivity = 98%
- D-Di XL NPV = 99.6%

Given the above, N = 115 reference-positive subjects are needed to have at least 90% power to demonstrate efficacy on both sensitivity and NPV[5]. Given at least N = 75 in the US (65%), 2,206 "unlikely" US subjects will be needed (75/0.034). Given N = 40 OUS (35%) at most1, 702 "unlikely" OUS subjects will be needed (40/0.057).

[4] In these studies, DVT results demonstrate higher accuracy parameters than PE. For the purpose of computing power in this study we assume the PE accuracy observed for both indications.
[5] Achieving requisite power for sensitivity, rather than NPV, drives the sample size

At the time this protocol amendment is issued, a total of 3,054 PE patients were enrolled including 121 PE positive patients (79 in US and 42 OUS - uncleaned, non-final data), allowing to have 90% power to demonstrate D-Di XL efficacy for PE.The PE data collection has been completed and data cleaning is in progress.

DVT: Power for Meeting PGs

Presentation of sample size is based on obtaining enough reference-positive case to achieve 90% power to meet the confidence interval PG for sensitivity (≥ 0.90), which will necessarily provide:

- The same power to meet the point estimate PG, since the minimum true-positives needed to meet the confidence interval PG is the same as that needed to meet the point estimate PG.
- A sufficient number of reference-negative patients for near 100% power to meet the PGs for NPV, due to low prevalence of positive cases

Based on data previously submitted to FDA, we assume:

- US prevalence of reference-positive = 5.3%
- OUS prevalence of reference-positive = 8.1%
- D-Di XL sensitivity = 98%
- D-Di XL NPV = 99.6%

Given the above, N = 115 reference-positive subjects are needed to have 90% power to demonstrate efficacy. Given at least N = 69 in the US (60%), at least 1,032 “unlikely” US subjects will be needed (69/0.053). Given N = 46 OUS (40%) 568 “unlikely” OUS subjects will be needed (46/0.081).

In summary, a total of 1,870 subjects are expected to participate in the DVT part of the study—at least 1,032 from the US and at most 568 from OUS—to have 90% power to demonstrate D-Di XL efficacy for DVT.

9.6 STATISTICAL ANALYSIS

Overview

The data will be summarized in tables listing the mean, standard deviation, median, minimum, maximum and number of subjects for continuous data or in tables listing count and percentage for categorical data, where appropriate. Data listing by subject will be provided.

The effects of noncompliance, dropouts, and possible covariates, will be assessed to determine the impact on the general applicability of results from this study.

All statistical analyses will be performed and data appendices will be created using the SAS® system.

Subject Disposition

Subject disposition will be tabulated; the number of enrolled, exposed, prematurely terminated and completed subjects will be summarized overall and by Device status.

A list of dropouts will be prepared including reason for discontinuation, and time of discontinuation.

Primary Analysis

Primary analysis will be conducted using Primary efficacy analysis set and will be done separately for PE and DVT.

Co-primary Analysis I

NPV will be computed in the usual manner as follows:

NPV = TN/(TN+FN)

Where,

TN = No. of true negative subjects

FN = No. of false negative subjects

We will test NPV using the following hypotheses:

H_0: NPV $\leq$ 0.95

H_1: NPV > 0.95

An exact binomial confidence interval will be used, where success will be declared if the two-sided 95% LCL is at or above the PG specified.

Additionally, we require: Point estimate of NPV $\geq$ 0.97.

Co-primary Analysis II

Sensitivity will be computed in the usual manner as:

Sensitivity = TP/(TP+FN)

Where,

TP = No. of true positive subjects

FN = No. of false negative subjects

We will test Sensitivity using the following hypotheses:
H_0: Sensitivity ≤ 0.90
H_1: Sensitivity > 0.90

An exact binomial confidence interval will be used, where success will be declared if the two-sided 95% LCL is at or above the PG specified.

Additionally, we require: Point estimate of Sensitivity ≥ 0.95.

Success for DVT will be declared if the four DVT PGs have been met.
Success for PE will be declared if the four PE PGs have been met.

Covariate Analyses, Including Poolability
The effect of the following covariates will be examined on Device accuracy:

- Age (measured continuously),
- Gender,
- Sites within US and OUS separately (enrolling sites with at least 20 subjects will be included separately. Sites with a smaller number of subjects will be combined into a single, "other" site). This is a test of site poolability within US and OUS separately.
- Region (US, OUS), which is a test of poolability of the US and OUS populations,
- PTP (measured by validated and standardized test).

Effect of covariates will be assessed for each covariate individually using the following logistic regression model:
Device = Cov + Reference Standard + Cov*Reference Standard

Where,
Device – D-dimer XL result (dichotomous)
Reference Standard– Diagnosis by imaging or follow-up (dichotomous)
Cov – Relevant covariate (categorical or continuous, depending on covariate)

Additional potential covariates will be compared between US and OUS and, if found significant, will be analyzed using the model described above.

In this model we are interested in the Cov*Reference interaction, whereby a significant result indicates that Device performance relative to Reference Standard is affected by the covariate.

Secondary Analysis

The following will be provided for Primary analysis sets:

- Specificity,
- Positive Predictive Value,
- Positive and negative likelihood ratios.
- Prevalence of reference positive cases

9.7 INTERIM ANALYSIS

There will be no interim analysis.

10. ADVERSE EVENTS REPORTING

In the US the study meets the requirements for exemption as defined in the Investigational Device Exemption regulations (21 CFR Part 812.2(c)). As such, CFR Part 812.150 (a) (1) and (b) (1), which describe reporting requirements of the Investigators and of the Sponsor to IRBs and FDA in relation to adverse device effects, is not applicable.

In the EU, national regulations will be followed regarding adverse event reporting for IVD studies, if any.

11. PROTOCOL DEVIATIONS

A protocol deviation is defined as an event where the exact instructions contained in this protocol, the clinical study agreement or the applicable regulations have not been followed. Deviations are classified by occurrence, i.e., sporadic vs. repeated and seriousness, i.e., major vs. minor.

Major deviations may impact subject safety, alter the risk/benefit ratio, compromise the validity of the study data, and/or affect subjects' willingness to participate in the study. Minor deviations do **not** impact subject safety, compromise the validity of the study data, or affect subjects' willingness to participate in the study. STAGO (with the support of the selected CRO) will perform blind review of deviations and will determine which are major deviations. Subjects with major deviations will be excluded from the study.

Investigators are required to obtain prior approval from the sponsor before initiating deviations from the protocol, except where necessary to protect the life or physical well-being of a subject in an emergency. Such approval will be documented in writing and maintained in study files. Prior approval is generally not expected in situations where unforeseen circumstances are beyond the investigator's control; however, the event is still considered a deviation and must be recorded in the subject eCRF.

Protocol deviations will be reported to the sponsor using suitable forms, regardless of whether medically justifiable, pre-approved by the sponsor or taken to protect the subject in an emergency.
Investigators must also adhere to procedures for reporting study deviations to IRB/EC in accordance with their specific IRB/EC reporting policies and procedures.
The monitor will discuss deviations with relevant site personnel, and will document them according to the study monitoring plan. If needed, a Note to File will be issued and filed in the relevant file and a copy sent to the sponsor.
A summary report classifying and summarizing the deviations per type will be issued and reviewed by the sponsor personnel.
Deviations will be summarized and included in the study report. Assessment and discussion of their potential impact / lack of impact on study results will be addressed.

12. STUDY SCHEDULE

- Estimated study period for recruitment: 42 months
- Study period per patient: maximum duration 4 months
- Estimated total duration of the study: 48 months
- First patient inclusion: June 2018

13. DISCONTINUATION OF STUDY

Once a patient has been enrolled in the study he/she may withdraw his/her consent to participate in the study at any time without prejudice. Participation in this study is entirely voluntary.

Stago may decide to stop the study at a specific site at any time for the following reasons:

- Inability of the investigator to include patients on schedule
- Major protocol deviations
- Incomplete or false data.

14. ETHICAL AND LEGAL ASPECTS

14.1 SUBJECT CONSENT

Complete detailed information will be given to the patient. Prior to enrolment in the study, the investigator must obtain the patient informed consent. In general the informed consent should be documented in writing. . However, in relation to the infection control guidelines related to the COVID-19 pandemic, verbal consent procedure is allowed at clinical sites where:

- competent IRB / EC has authorized the verbal consent procedure (a waiver of the requirement to obtain a signed consent form has been released by IRB/EC before implementation of verbal consent procedure)

AND

- verbal consent procedure is adequately documented by Investigator, in accordance with local regulation and IRB / EC requirements

Informed consent procedure (including assent) for minors will be conducted in compliance with local regulations and/or EC/IRB requirements.

14.2 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance, among the others, with the US Code of Federal Regulation Title 21 Parts 50 (Informed Consent), 54 (Financial disclosure by clinical investigators), 56 (Institutional Review Board) and 812.119 (Disqualification of a Clinical Investigator). In Europe, in addition to the above, the study will be performed in compliance with the In Vitro Diagnostic (IVD) European Directive 98/79/EC and in compliance with European IVD Regulation (EU) 2017/746 of 5 April 2017, as soon as it will come into force.
With regards to study management and conduct, ISO 14155 (most recent version) and ICH-GCP will be followed to the extent they can be used for a study with an IVD product. The study will be conducted in compliance with the protocol. The protocol and any amendments and the informed consent form will have to obtain Institutional Review Board or Ethics committee (IRB/EC) approval prior to initiation of the study.
Study personnel involved in this study will be qualified by education, training, and experience to perform their respective task(s).
This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g. loss of medical license).
Systems with procedures that assure the quality of every aspect of the study will be implemented.

14.3 INSURANCE

All subjects participating in the study will be covered by an insurance policy taken out by STAGO, which is in accordance with applicable laws and/or regulations.

14.4 STUDY FUNDING

This study is funded by:
DIAGNOSTICA STAGO S.A.S
3 allée Thérésa,
92600 Asnières sur Seine
France

14.5 INSTITUTIONAL REVIEW BOARD / INDEPENDENT ETHICS COMMITTEE

In the US the study meets the requirements for exemption as defined in the Investigational Device Exemption regulations (21 CFR Part 812.2(c)). Therefore, only approval by the applicable Institutional Review Board (IRB) is required prior to study start.
Before study initiation, the investigator must have obtained written and dated approval from the IRB/EC for the protocol, the patient informed consent form, subject recruitment materials/process (e.g. advertisements), and any other written information to be provided to subjects.

14.6 PROTOCOL AMENDMENTS

The investigator or sponsor should provide the IRB/EC with reports, updates and other information (e.g. amendments) according to regulatory requirements or institution procedures of each country. Protocol amendments, except where necessary to eliminate an immediate hazard to subjects, will be issued by STAGO. Agreement from the investigator must be obtained for all protocol amendments and amendments to the patient informed consent form (or information/non-opposition document, as allowed by local regulations). The IRB/EC must be informed of all amendments and give written approval, which must be provided to STAGO.

14.7 CONFIDENTIALITY

All records identifying directly or indirectly the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, including the European Regulation 2016/679 dated 27 April 2016 and effective as of 25 May 2018 (hereafter the “GDPR”),will not be made publicly available.
In accordance with GDPR, the Sponsor is the “Controller” and the participating sites are “Processors”. The sites ensure the security and confidentiality over the personal data related to the subjects by taking appropriate technical and organizational measures, as described below.
Subject names will not be sent to STAGO. Only the patient number (and initials as allowed by local regulations) will be recorded in the e-CRF, and if the patient’s name appears on any document, it must be obliterated before a copy of the document is supplied to STAGO. The subjects will be informed as

detailed in Section 14.1 that representatives of STAGO, the study CRO, EC/IRB, or regulatory authorities may inspect their records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. If the results of the study are published, the subject's identity will remain confidential. The investigator will retain a secure list to enable the patients' records to be identified.

14.8 ARCHIVING OF DATA

The investigator should arrange for the archiving of the study documentation file and the raw data from the hospital for the longest of the following period of time:

- At least 15 years after completion/discontinuation of the study.

The hospital shall ensure the security and confidentiality of the study documentation file and the raw data from the hospital – and will not intentionally modify such data during this period.

14.9 USE OF DATA AND PUBLICATION

This study will be registered on the site www.clinicaltrials.gov.

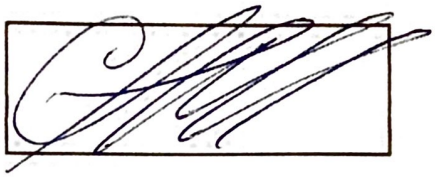
All data and results and all intellectual property rights to the data and results derived from the study will be the property of STAGO, who may utilize the data in various ways, such as for submission to government regulatory authorities or disclosure to other investigators. The investigator, whilst free to utilize data derived from study for scientific purposes, must discuss any publication with STAGO prior to release and obtain written consent of STAGO on the intended publication. STAGO recognizes the right of the investigator to publish the results upon completion of the study. However, the investigator must send a draft manuscript of the article or abstract to STAGO in order to obtain approval prior to submission of the final version for publication. This will be reviewed and approval will not be withheld unreasonably. In case of a difference of opinion between STAGO and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties.

All publication or communication (oral or written) will respect the international requirements: "Uniforms requirements for Manuscripts Submitted to Biomedical Journals" (http///www.cma.ca/publications/mwc/uniform.htm).

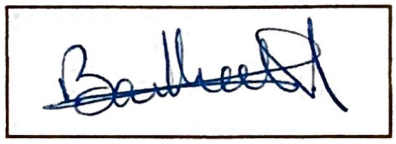
15. DATE AND SIGNATURE

This protocol has been read and approved on the date noted in the header.

Coordinating Investigator
Christopher Kabrhel, MD, MPH,
Massachusetts General Hospital,
Boston, MA (US)



For the sponsor
BARTHOD-MALAT Aurore
Market Access Clinical Affairs Specialist
DIAGNOSTICA STAGO



Investigator's Statement and Signature

Prior to enrolling patients in this Study, the Principal Investigator must obtain written approval of the protocol from his/her Institutional Review Board/ Ethics Committee (IRB/EC). This approval must be in the Principal Investigator's name and a copy sent to STAGO along with the IRB/EC-approved patient informed consent form (or information / non-opposition document, if allowed by local regulations) and the signed clinical trial agreement. Additionally, the Principal Investigator must sign the declaration below:

I have read this protocol and agree to adhere to its requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the test product and the conduct of the study.

I will conduct the Study in accordance with the Declaration of Helsinki, Good Clinical Practices for studies with *in vitro* diagnostics and with all applicable local regulations for studies with *in vitro* diagnostics.

Principal Investigator's Signature	Date
Principal Investigator's Printed Name	
Site Name	Site #

16. REFERENCES

British Committee for Standards in Haematology. The diagnosis of deep vein thrombosis in symptomatic outpatients and the potential for clinical assessment and D-dimer assays to reduce the need for diagnostic imaging. Br J Haematol 2004; 124: 15–25.

College of American Pathologists, Survey and Anatomic Pathology Programs, Coagulation, CGL-A 2014, CGL-A 2015, CGL-A 2016

CLSI document H59-A Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease; Approved Guideline

Ceriani E, Combescure C, Le Gal G et al: Clinical prediction rule for pulmonary embolism: a systematic review and metanalysis. J ThrombHaemost 2010, in press.

Goekoop RJ, Steeghs N, Niessen RW et al: Simple and safe exclusion of pulmonary embolism in outpatients using quantitative D-Dimer and Wells' simplified decision rule. ThrombHaemost 2007, 97: 146 – 50.

Kelly J, Hunt BJ. A clinical probability assessment and D-dimer measurement should be the initial step in the investigation of suspected venous thromboembolism. Chest 2003; 124: 1116–9.

Klok FA, Kruisman E, Spaan J et al: Comparison of the revised Geneva score with the Wells rule for assessing clinical probability of pulmonary embolism. J ThrombHaemost 2008, 6: 40 – 4.

Pasha SM, Klok FA, Snoep JD et al: Safety of excluding acute pulmonary embolism based on an unlikely clinical probability by the Wells rule and normal D-Dimer concentration: a meta-analysis. Thromb Res 2009, in press.

Perrier A, Bounameaux H, Marabia A, et al. Diagnosis of pulmonary embolism by a decision analysis-based strategy including clinical probability, D-dimers levels and ultrasonography: a management study. Arch Intern Med 1996; 156: 531–6.

Stein PD, Woodard PK, Weg JG et al: Diagnostic Pathways in Acute Pulmonary Embolism: Recommendations of The PIOPED II Investigators. Am J Med 2006, 119: 1048 – 55.

Wells PS, Anderson DR, Rodger M, et al. Derivation of a simple clinical model to categorize patients probability of pulmonary embolism: increasing the models utility with the SimpliRED D-dimer. ThrombHaemost 2000; 83: 416–420.

Wells PS, Anderson DR, Rodger M, et al. Evaluation of D-dimer in the Diagnosis of Suspected Deep-Vein Thrombosis. N Engl J Med 2003, 349: 1227 – 35.

Wells PS. Integrated strategies for the diagnosis of venous thromboembolism. J ThrombHaemost. 2007;5(Suppl. 1):41-50.

Woodmans B., Girardot O., Blanco M.J., Colesse G., Gourmelin Y.: "Stability of coagulation proteins in frozen plasma" Blood Coagulation Fibrinolysis, 12, 229-236, 2001

17. DEFINITIONS

Deep vein thrombosis (DVT) – an intravenous thrombus in a deep vein, usually in the proximal legs or pelvis, but may also occur in an upper extremity.

Exclusion of venous thromboembolism (VTE) – a claim that can be applied to any method, the results of which can reliably exclude VTE; **NOTE:** Regarding a D-dimer reagent, studies must demonstrate that, below a predetermined threshold, the NPV, sensitivity and coefficient of variation (CV) at the threshold have sufficient power to exclude VTE when the test is applied to patients judged to have low or moderate probability of VTE determined using a "Pre-Test Probability" scoring algorithm.

Negative predictive value (NPV) – the likelihood that an individual with a negative test result does not have the disease or other characteristic that the test is designed to detect;varies with the prevalence of the disease in the population tested; **NOTE 2:** From predictive value theory, the proportion of all negative tests that are true negatives.

Pretest probability (PTP) – the a priori probability of a particular clinical state before results of a diagnostic test are known; **NOTE 1:** This probability may be obtained by estimates of the prevalence of a disease in a population or by a clinical estimate of the probability of a disease existing in a given patient; **NOTE 2:** An evaluation of clinical features (history, signs, and symptoms) that collectively assist in predicting the outcome of a test or procedure. For D-dimer, levels are described as low, intermediate or moderate (unlikely) and high (likely). Intermediate and moderate are used interchangeably.

Proximal DVT – intravenous thrombus occurring in the iliac, femoral or popliteal veins. These veins are proximal to the popliteal fossa but do not include superficial (subcutaneous) veins.

Pulmonary embolism/embolus (PE) – a material that travels in the venous bloodstream until it lodges in the constriction of the pulmonary arterial system. The most common is a thrombus that forms a thromboembolism.

Sensitivity (Se) (of a measuring system) - quotient of the change in an indication of a measuring system and the corresponding change in a value of a quantity being measured (ISO/IEC Guide 99); **NOTE:** From predictive value theory, the proportion of subjects with a condition who have a positive test.

Threshold – an value above or below which a specific action is indicated. **NOTE:** In the case of D-dimer, the level below which VTE can be excluded in the appropriate clinical setting.

Venous thromboembolism (VTE) – A thrombus (blood clot) or embolus in the venous circulation.

18. ABBREVIATIONS AND ACRONYMS

CUS	compression ultrasonography
DVT	deep vein thrombosis
EDC	electronic data capture
FEU	fibrinogen equivalent units
NPV	negative predictive value
PE	pulmonary embolism
PI	Principal Investigator
PTP	pre-test probability
SAP	statistical analysis plan
SDV	source data verification
SOC	standard of care
SOP	standard operating procedure
VTE	venous thromboembolism
e-CRF	electronic case report form

19. APPENDICES

19.1 APPENDIX A

Wells simplified clinical model for assessment of DVT

Clinical variable	Score
Active cancer (treatment ongoing or within previous 6 months or palliative)	1
Paralysis, paresis, or recent plaster immobilization of the lower extremities	1
Recently bedridden for 3 days or more, or major surgery within the previous 12 weeks requiring general or regional anesthesia	1
Localized tenderness along the distribution of the deep vein system	1
Entire leg swollen	1
Calf swelling at least 3 cm larger than that on the asymptomatic leg (measured 10 cm below the tibial tuberosity)	1
Pitting edema confined to the symptomatic leg	1
Collateral superficial veins (non-varicose)	1
Previously documented DVT	1
Alternative diagnosis at least as likely as DVT	-2

DVT. *< 1, probability of DVT is low, moderate is 1 or 2, and high is > 2.

Eligible patients for D-dimer testing must have a Wells score for DVT ≤ 2.

Wells clinical model for assessment of PE

Clinical variable	Score
Clinical signs and symptoms of DVT (minimum of leg swelling and pain with palpation of the deep veins)	3
An alternative diagnosis is less likely than PE	3
Heart rate greater than 100	1.5
Immobilization or surgery in the previous four weeks	1.5
Previous DVT/PE	1.5
Hemoptysis	1
Malignancy (ongoing treatment, treated in the last 6 months or palliative)	1

PE. *< 2, probability of PE is low, moderate is 2-6, and high is > 6.

Eligible patients for D-dimer testing must have a Wells score for PE ≤ 6.