HEMOSIL®

D-DIMER ASSAY PANEL

D-Dimer Testing

Current state, clinical utility and future outlook

Introduction by Dr. Carl-Erik Dempfle

IMPORTANT NOTE: The conclusions in this document based on references that pertain to off-label claims for use of D-Dimer are neither promoted nor validated by IL. As a company, we support the claims and intended uses agreed upon with the respective regulatory agencies that clear and approve our regulatory submissions.

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Glossary

ACS	Acute coronary syndrome
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CT	Computed tomography
CV	Coefficient of variation
D-DU	D-Dimer unit
DIC	Disseminated intravascular coagulation
DVT	Deep vein/venous thrombosis
EDTA	Ethylenediaminetetraacetic acid
ELFA	Enzyme-linked fluorescent assay
ELISA	Enzyme-linked immunosorbent assay
FbDPs	Fibrin degradation products
FDP	Fibrin degradation products or fibrinogen degradation products
FEU	Fibrinogen-equivalent unit
FgDPs	Fibrinogen degradation products
HAMA	Human anti-mouse antibodies
HIT	Heparin-induced thrombocytopenia
ISTH	International Society for Thrombosis and Hemostasis
JMHW	Japanese Ministry for Health and Welfare
mAbs	Monoclonal antibodies
MRI	Magnetic resonance imaging
NPV	Negative predictive value
PAI-1	Plasminogen activation inhibitor - 1
PE	Pulmonary embolism/embolus
PPV	Positive predictive value
PT	Prothrombin time
PTP	Pre-test probability
RF	Rheumatoid factor
RLUs	Relative light units
SFC	Soluble fibrin complexes
TAT	Thrombin-antithrombin complex
TF	Tissue factor
tPA	Tissue plasminogen activator
UEDVT	Upper extremity deep vein/venous thrombosis
VTE	Venous thromboembolism
XDP	Cross-linked fibrin degradation products

The Value of D-Dimer Testing in Differentiating Clinical Conditions

By Prof. Dr. med. Carl-Erik Dempfle
Department of Medicine,
University Hospital of Mannheim,
Theodor Kutzer Ufer
Mannheim, Germany

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The D-Dimer assay is a useful tool in the evaluation of patients for conditions associated with fibrin generation. In the majority of cases, the presence of elevated D-Dimer levels in the blood indicates intravascular fibrin formation, whereas low levels of D-Dimer exclude acute intravascular fibrin formation. In current clinical practice, D-Dimer assays are used to exclude the presence of specific medical conditions associated with *in vivo* fibrin formation; monitor the intensity of coagulation activation; and estimate general vascular risk.

As components of diagnostic algorithms, D-Dimer assays are used routinely to exclude the presence of acute deep vein thrombosis (DVT) and pulmonary embolism (PE), and also to exclude the presence of acute aortic dissection (1-3). Low levels of D-Dimer also exclude the presence of disseminated intravascular coagulation (DIC) whereas elevated D-Dimer levels support the diagnosis of DIC. In this context, the D-Dimer level may also serve as a prognostic marker, that is, elevated D-Dimer levels are frequently observed in patients with malignancy and may indicate an adverse prognosis.

In addition, D-Dimer assays assist in the differential diagnosis of suspected cardiovascular conditions; (4) for example, the differential diagnosis of patients presenting in the ER with acute chest pain symptoms. Under these circumstances the D-Dimer assay may distinguish between conditions with intravascular fibrin formation (e.g., aortic dissection, aortic aneurysm, or pulmonary embolism) versus conditions without intravascular fibrin formation (e.g., acute coronary syndrome or pneumothorax).

In their role as monitoring devices, D-Dimer assays quantitatively measure or monitor coagulation activation in patients with DIC, patients undergoing anticoagulant therapy, and patients susceptible to venous thromboembolism (VTE) recurrence after the cessation of anticoagulation therapy.

Beyond their role in the exclusion of VTE, the measure of D-Dimer concentrations in the blood can be applied to the investigation of many clinical indications. For example, elevated D-Dimer blood levels may serve as markers for tumor-associated, intra- or extravascular fibrin formation, an indicator of pregnancy complications associated with coagulation activation. Conversely, low blood concentrations of the D-Dimer antigen may exclude the presence of acute heparin-induced thrombocytopenia (HIT). As time progresses, the clinical application of the D-Dimer assay continues to expand into new clinical realms.

This article is intended as an introduction to the biochemistry, pathophysiology, and clinical application of D-Dimer assays, and the assay systems currently available for quantitative measurement of D-Dimer.

D-Dimer and the Fibrin Cascade

The D-Dimer is a molecular motif present within fibrin complexes and a portion of fibrin degradation products (FDPs). The D-Dimer motif is formed by covalent linking between the D-domains of adjacent fibrin monomer units within a fibrin polymer. Formation of D-Dimer requires the action of thrombin and Factor XIIIa on fibrinogen.

The digestion of fibrin by plasmin results in the production of FDPs containing the D-Dimer motif of various sizes. Fibrin fragment D-Dimer is generated as a final degradation product in the course of fibrin proteolysis induced by plasmin, i.e., the end product of extensive fibrin proteolysis is fibrin fragment D-Dimer. Fibrin fragment contains two cross-linked D-domains of the fibrinogen protein (the precursor of fibrin).

D-Dimer assays detect the presence of the D-Dimer motif in fibrin derivatives (e.g., fibrin complexes and FDPs) present in blood or other biological fluids. In most clinical conditions, the antigenic signal is caused predominantely by the presence of plasmin-induced FDPs. However, the D-Dimer antigen is also found in patients with aplasminogenemia, a condition in which plasmin proteolysis does not seem to be a prerequisite for D-Dimer formation (5-7).

Fibrin Formation

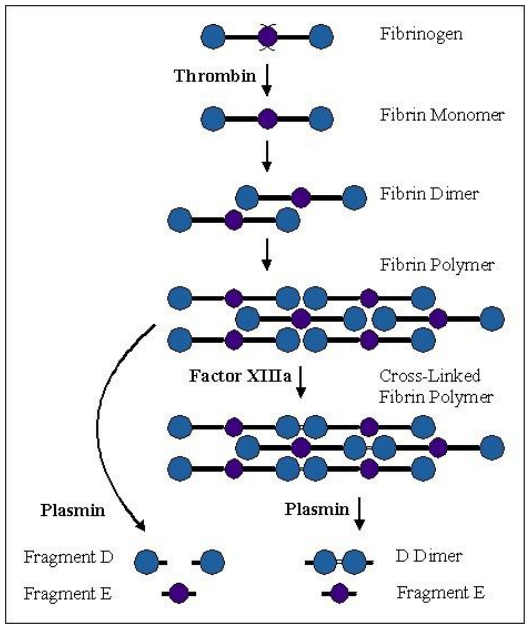
Fibrinogen is the soluble plasma glycoprotein precursor of fibrin. As illustrated in Figure 1, the protease thrombin cleaves short polypeptide fragments (fibrinopeptides) from the N-termini of the A alpha- and the B beta-chains of fibrinogen. The cleavage of these fibrinopeptides uncovers polymerization sites within the central E-domain of the resultant fibrin monomer. These sites interact with corresponding sites on the D-domains of adjacent fibrin monomers, leading to the formation of polymeric structures of fibrin monomer units that overlap by half. Further elongation leads to the assembly of fibrin protofibrils having lateral linkage with adjacent protofibrils, giving rise to fibrin strands. In the presence of calcium, thrombin also removes an activation peptide from Factor XIII, converting the Factor XIII molecule via conformational changes into Factor XIIIa. Factor XIIIa catalyzes the formation of covalent bonds between the D-domains of adjacent fibrin monomers within fibrin polymers. This covalent cross-linkage of the polymerized fibrin molecules stabilizes the fibrin polymer, increases tensile strength, and increases complex resistance to proteolysis.

Fibrinolysis

Fibrinolysis is the proteolytic digestion of fibrin by plasmin. Plasmin digests fibrin clots, circulating fibrin complexes, and also perhaps fibrinogen. The activation of plasminogen is linked to the presence of fibrin, which acts as a cofactor in plasminogen activation by tissue plasminogen activator (tPA). Also, the activation of plasminogen is highly regulated by the inactivation of tPA by plasminogen activation inhibitor (PAI-1), and the inactivation of plasmin by alpha-2-plasmin inhibitor. Consequently, plasmin proteolysis predominantly leads to the formation of fibrin degradation products. Plasmin proteolysis is initiated nearly in parallel to fibrin formation. Therefore, the presence of fibrin degradation products does not necessarily indicate proteolysis of an insoluble fibrin clot.

Fibrin proteolysis generates an array of soluble fragments of varying molecular weights, some of which contain the D-Dimer motif. Collectively, these fragments are referred to as FDPs, referring to the general mixture of products generated by proteolysis of fibrin as well as fibrinogen. Fibrinogen degradation products (FgDP) and fibrin degradation products (FbDP) may be distinguished by the presence or absence of the D-Dimer motif, which is only present within degradation products of (Factor XIIIa-cross-linked) fibrin. Those FDPs containing the D-Dimer motif are also sometimes referred to as XDPs (cross-linked fibrin degradation products). The fibrin fragment, D-Dimer, is the minimal structure containing two covalently linked D-domains (i.e., dimerized D-domains) (8, 9). Although Figure 1 depicts the D-Dimer motif in isolation, FDPs comprise a variety of oligomers containing the D-Dimer motif. Assays using monoclonal antibodies that are specific to the D-Dimer motif detect a variety of XDPs containing dimerized D-domains, including fibrin fragment D-Dimer, as well as a variety of structures with a higher molecular weight (10).

Figure 1: D-Dimer Formation and Dissolution
Cleavage of fibrinogen by thrombin yields self-polymerizing fibrin monomers; Factor XIIIa catalyzes the formation of covalent bonds between the fibrin polymers, creating the D-Dimer domains and insoluble blood clots. Plasmin digests the blood clot, yielding heterogeneous soluble fragments of varying molecular weights, including D-Dimer oligomers, D-fragments, and E-fragments.



In the clinical setting, the objective of measuring D-Dimer blood concentrations is the detection or exclusion of intravascular fibrin formation. Generally, the amount of XDPs released from intravascular clots is minimal compared with the large amount of fibrin compounds containing the D-Dimer motif related to fibrin formation in the flowing blood. In patients with acute VTE, D-Dimer levels rapidly decline after start of anticoagulant therapy, despite the fact that anticoagulant therapy enhances the proteolysis of the clot (leading to an increased release of clot-related FbDPs into the circulation). Thus, detection of D-Dimer is an indicator of ongoing intravascular fibrin formation rather than an indicator of clot dissolution. Figure 1 also depicts this plasmin digestion of soluble fibrin complexes into smaller, diverse, fragmented FDPs.

The D-Dimer Antigen

The D-Dimer antigen belongs to the group of epitopes specific to fibrin. During blood clot formation and dissolution, the biochemical and conformational alterations undergone by fibrinogen and fibrin create neo-epitopes that serve as unique targets for various monoclonal antibodies (11).

As shown in Figure 1, fibrinogen contains two D-domains separated by a central E-domain. In fact, fibrinogen consists of two dimers, each having three nonidentical chains–A alpha, B beta, and gamma–with the N-terminals of all six chains forming the center of the molecule (the E-domain) (7, 12). The protease thrombin cleaves the A alpha- and B beta-chains to release fibrinopeptides A and B, thereby creating neo-epitopes on the neo-N-terminals of these chains that are fibrin-specific (13, 14). This cleavage alters the conformation of the fibrin molecule as well as the structural alternation prompting the formation of additional neo-epitopes specific to fibrin. Additional sets of neo-epitopes are introduced by the polymerization of fibrin monomers and also by the degradation of fibrin by plasmin.

In most assayed clinical blood samples, the fibrin D-Dimer fragment (the D-Dimer motif in isolation) contributes only a small proportion of the D-Dimer signal (15). Overall, the collections of D-Dimer antigens assayed in clinical blood samples arise from a number of molecular sources, namely:

- soluble fibrin complexes containing D-Dimers;
- proteolytic XDPs of such soluble fibrin complexes;
- proteolytic XDPs of intravascular fibrin clots; and
- proteolytic XDPs of extravascular fibrin deposits.

Rather than emanating from fibrin D-Dimer fragments, the predominant proportion of the D-Dimer signal typically arises from cross-linked, soluble, fibrin complexes or high molecular weight XDPs (16). The relative proportions of D-Dimer antigens within any particular clinical blood sample depend on the location and intensity of fibrin formation. For example, the presence of acute intravascular fibrin formation results in a predominance of higher molecular weight fibrin complexes and XDPs. A high level of fibrinolytic activation gives rise to more extensive fibrin proteolysis, yielding a predominance of XDPs of lower molecular weight and a higher proportion of small fragments, such as fibrin fragment D-dimer. Proteolysis of intravascular clots generates a large array of XDPs and, by release of active thrombin from the clot, perhaps “new” soluble fibrin complexes. Proteolysis of extravascular fibrin also releases an array of fibrin degradation products into the bloodstream, but mainly those of smaller molecular size.

D-Dimer Antibodies

D-Dimer assays use monoclonal antibodies (mAbs) reactive with neo-epitopes specific to fibrin. The majority of mAbs target the neo-epitopes formed during covalent bonding between the gamma-chains of the two fibrinogen D-domains (these covalent bonds linking adjacent fibrin monomer units in the fibrin polymerization process) (11, 17, 18). Thus, commercial D-Dimer assays utilize antibodies directed against the neo-epitopes created by the formation of the D-Dimers motif itself.

In general, mAbs utilized by current assays were developed by the immunization of mice with fibrin fragment D-Dimer, or mixtures containing fibrin D-Dimer fragments and other XDPs (19, 20). Given the antigenic products of mouse immunization, the antibodies having the greatest affinity for the D-Dimer motif are selected for inclusion in the D-Dimer assays. By definition, these antibodies are selected to have a low affinity for fibrinogen, FgDPs, and other fibrin compounds lacking the D-Dimer motif. Ideally, assays containing these mAbs selectively react with the D-Dimer motif of XDPs with a high degree of specificity, even within an abundant presence of fibrinogen and/or FDPs lacking the D-Dimer motif. Such mAbs react with the D-Dimer motifs in isolation (i.e., the fibrin fragment D-Dimer) as well as the D-Dimer motif as a component of larger oligomers (17, 18).

However, as mentioned previously, a high proportion of the fibrin complexes contain dimerized D-domains, which are also reactive with D-Dimer-specific monoclonal antibodies. **Although some assays display a preference for either low or high molecular weight XDPs, neither the molecular size nor the source of the D-Dimer antigen is disclosed by measurement of D-Dimer in blood.**

Primary Clinical Applications of D-Dimer Assays

The primary clinical application of D-Dimer assays is the exclusion of VTE (DVT or PE). A number of commercially available D-Dimer assays have received FDA clearance for this clinical application, although this clearance is restricted to usage within specific diagnostic algorithms. Certain D-Dimer assays are also cleared as an aid in the diagnosis of VTE and aid in the diagnosis of DIC.

Secondary clinical applications of D-Dimer assays include the prevention of VTE recurrence and the differential diagnosis of acute symptoms suggestive of VTE and/or specific cardiovascular conditions, such as acute coronary syndrome, acute aortic dissection, and aortic aneurysms.

This section provides a brief discussion of the role of D-Dimer assays in these clinical applications.

Exclusion of Thromboembolism (VTE or DVT/PE)

The importance of the D-Dimer assay in the exclusion of VTE in symptomatic patients has made it the most widely used marker of *in vivo* clotting activation. The diagnosis of VTE based on clinical findings alone is unreliable; therefore, objective testing of symptomatic patients is a necessity before the diagnosis or exclusion of VTE in those symptomatic patients (10). Patients with acute DVT or PE typically display elevated levels of D-Dimer antigen (21). Consequently, D-Dimer antigen levels below a predefined, assay-specific threshold value exclude acute proximal DVT or PE as the cause of the clinical symptoms (22).

Several available commercial D-Dimer assays display a sensitivity of close to 100% for the exclusion of acute, proximal, deep vein thrombosis or pulmonary embolism (23). For most D-Dimer assays, the threshold for exclusion of VTE is identical or close to the upper limit of the normal range. Therefore, in routine clinical use, patients with a D-Dimer level below the assay-specific threshold forgo diagnostic imaging when D-Dimer assaying is conducted in conjunction with clinical scoring algorithms that determine the pre-test probability (PTP) of the patient having thromboembolic disease.

In appropriate clinical settings, the use of a D-Dimer assay can preclude the necessity of imaging studies among patients suspected of VTE (10). As a general rule, patients presenting with obvious clinical symptoms of VTE, indicating a high probability of the diagnosis, would proceed directly to diagnostic imaging; whereas in all other patients, **measurement of D-Dimer as a first diagnostic step may considerably reduce the diagnostic workload for procedures such as compression ultrasound, contrast venography, or computerized tomography (CT).** Apart from reducing medical cost, the omission of diagnostic imaging in patients with a D-Dimer level below threshold reduces patient risk, as imaging studies can be invasive.

While D-Dimer concentrations below the predefined threshold virtually rule out the presence of thrombosis, D-Dimer concentrations above threshold may indicate the presence of a thrombotic condition or the presence of other clinical conditions capable of increasing D-Dimer concentrations. Being a general indicator of *in vivo* fibrin formation, D-Dimer assays have relatively low specificity for diagnosis of VTE. Elevated D-Dimer levels are caused by many other clinical conditions. Depending on the clinical context, D-Dimer assays typically display a diagnostic specificity ranging from 30% to 60%. Several independent factors give rise to this lack of specificity. D-Dimer plasma concentrations are elevated in elderly patients, in most patients with recent major surgery, in most patients with concurrent sepsis, and in many patients with disseminated malignancy, significant liver disease, or pregnancy. Thus, among patients with clinical conditions typically associated with increased *in vivo* fibrin formation that result in elevated blood concentrations of D-Dimer antigen, a relatively low proportion can be potentially excluded from further clinical workup by the D-Dimer assay alone, if the intention of testing is the exclusion of VTE.

Concerning the age-related increase in D-Dimer levels, as well as with pregnancy, the use of modified cut-off levels may restore some of the diagnostic specificity of the D-Dimer assay in VTE exclusion.

Pre-Test Probability (PTP)

D-Dimer assays are used in conjunction with PTP scoring algorithms that stratify patients into probability groups (24). Sets of standardized scoring rules have been offered for the evaluation of the PTP for DVT and/or PE. These criteria have been validated in clinical trials and have also been reliable when used by other clinical groups. These scoring systems are based on criteria and risk factors derived from large databases stemming from studies in which a PTP model was incorporated into the clinical algorithm (25). Table 1 lists the clinical variables and corresponding scores of the "Wells Simplified Clinical Model for Assessment of DVT" (26), a PTP scoring system. Such a scoring system, used to rank patients into stratified risk groups, is also referred to as a clinical prediction rule (24).

Table 1: 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 venous 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 (nonvaricose)	1
Previously documented DVT	1
Alternative diagnosis at least as likely as DVT	−2

Given a cumulative score of ≥ 2, then the probability of DVT is 'likely.' Given a score of ≤ 1, then the probability for DVT is 'unlikely.' Alternatively, a score of < 1 has a low probability, a score of 1 or 2 an intermediate probability, and a score of > 2 a high probability. D-Dimer testing to be performed for exclusion of DVT only if Wells Score is < 2. Adapted from: Wells PS. Integrated strategies for the diagnosis of venous thromboembolism. J Thromb Haemost. *2007 Jul;5 Suppl 1:41-50. (25).*

D-Dimer measurement, in conjunction with a standardized assessment of PTP, is successfully used to exclude VTE without the need for diagnostic imaging, provided the threshold for the D-Dimer assay has been objectively established and validated for this purpose (10). The predefined thresholds used in current commercial D-Dimer assays for VTE exclusion are specific to each manufacturers' assay. These assay-specific thresholds were determined and validated by clinical trials in which patients with suspected acute venous thrombosis and/or pulmonary embolism were excluded by technical examinations, such as contrast venography for DVT and spiral computerized tomography for PE. Clinical trials used to validate D-Dimer assays should incorporate a "management study" design in which D-Dimer assays are used in conjunction with a PTP scoring algorithm with a reevaluation of clinical decisions (regarding the patient exclusion from diagnostic imaging) at a 3-month follow-up (10, 27, 28).

Threshold Validation by Management Studies

Figure 2 illustrates the procedural diagnostic process of a management study design. The management study design is characterized by the combination of D-Dimer assay outcomes combined with PTP rank stratification to determine which patients either undergo or forgo diagnostic imaging procedures. As illustrated in Figure 2, patients with D-Dimer antigen levels below threshold and a low or intermediate PTP

score do not require further VTE-related diagnostic imaging, while patients with positive D-Dimer findings and a low, intermediate, or high PTP score progress to diagnostic imaging. (Of course, any patient might undergo diagnostic imaging per discretion of the attending physician.)

Overall, for patients with D-Dimer levels within normal range, the presence of new or ongoing thrombosis is highly improbable (29). However, a quantitative D-Dimer assay should not be used to exclude VTE– especially in patients with high PTP (10). Therefore, in the management study design approach, all patients with a high PTP score undergo diagnostic imaging regardless of D-Dimer antigen outcomes. The primary utility of D-Dimer assays is the exclusion of thromboembolic disease wherein the probability of VTE is low or intermediate (10). **With low or intermediate PTP, the use of D-Dimer assays reduces the proportion of patients receiving diagnostic imaging (contrast venography, duplex ultrasound, computerized tomography, lung scintigraphy, etc.) by 30%-50% (30).**

For patients with a high PTP score, D-Dimer testing may be helpful in estimating the intensity of coagulation activation or monitoring the efficacy of anticoagulant therapy, but not for the exclusion of diagnostic imaging.

Figure 2: Typical Design of Management Study

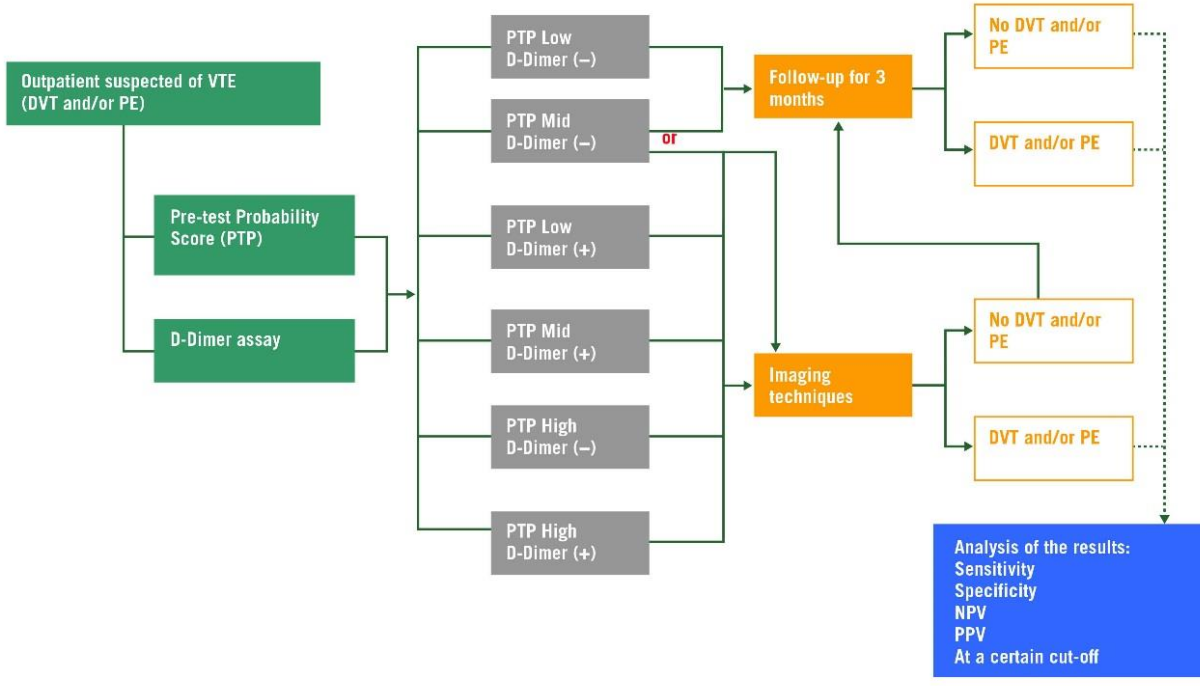


Table 2 and Table 3 summarize the sensitivity and negative predictive value (NPV) of HemosIL assay outcomes in management summaries.

Table 2: Summary of HemosIL Assay Sensitivity for DVT/PE Exclusion Performance in Management Studies

			DVT Sensitivity			PE Sensitivity		
Assay	**Multi-Center**	**n**	**All Samples**	**High PTP**	**Low + Moderate PTP**	**All Samples**	**High PTP**	**Low + Moderate PTP**
HemosIL D-Dimer & HemosIL D-Dimer 500 on ACL TOP	✓	632	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
HemosIL D-Dimer & HemosIL D-Dimer 500 on ACL ELITE	✓	629	100.0%	100.0%	100.0%	98.0%	100.0%	97.6%
HemosIL D-Dimer HS on ACL TOP	✓	668	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
HemosIL D-Dimer HS 500 on ACL TOP	✓	747	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Source: Table 12, Table 13, Table 18, and Table 22

Table 3: Summary of HemosIL Assay Negative Predictive Value (NPV) for DVT/PE Exclusion Performance in Management Studies

			DVT - Negative Predictive Value			PE - Negative Predictive Value		
Assay	**Multi-Center**	**n**	**All Samples**	**High PTP**	**Low + Moderate PTP**	**All Samples**	**High PTP**	**Low + Moderate PTP**
HemosIL D-Dimer & HemosIL D-Dimer 500 on ACL TOP	✓	632	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
HemosIL D-Dimer & HemosIL D-Dimer 500 on ACL ELITE	✓	629	100.0%	100.0%	100.0%	99.1%	100.0%	99.1%
HemosIL D-Dimer HS on ACL TOP	✓	668	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
HemosIL D-Dimer HS 500 on ACL TOP	✓	747	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Source: Table 12, Table 13, Table 18, and Table 22

Special Considerations

Detection of DVT Recurrence: D-Dimer assays may have clinical application in the evaluation of patients suspected of experiencing DVT recurrence. Ultrasound examination or contrast venography may not be able to identify new thrombotic material in the presence of residual thrombosis. However, should the patient have D-Dimer levels within the normal range, then the presence of new or ongoing thrombosis is highly improbable (29). D-Dimer may be measured during anticoagulant therapy in order to identify patients with insufficient response to anticoagulant therapy. Patients with malignant disease, especially adenocarcinoma or hematological malignancies, may display elevated D-Dimer levels as an indicator of

coagulation activation not controlled by the treatment. Vitamin K antagonists may fail to control malignancy-associated coagulation activation, and direct enzyme inhibitors or heparin-related compounds may be more effective.

Distal DVT: The clinical utility of D-Dimer testing among patients with suspected distal DVT differs from that among patients with suspected proximal DVT. Distal DVT appears to be associated with a lower level of coagulation activation and fibrin formation compared with proximal DVT. Also, many patients enter the diagnostic workup late, perhaps after the initial coagulation activation process has ceased. Patients with distal DVT exhibit a normal D-Dimer in 35% of evaluations (31). Therefore, D-Dimer antigen testing cannot be used to avoid ultrasound evaluation. All patients with suspected distal DVT require ultrasound evaluation (10).

Thresholds Not Applicable to All Thrombotic Events: The data regarding the use of clinical PTP and the D-Dimer test for VTE exclusion were developed in the clinical evaluations of suspected DVT and PE. Any application of these guidelines in evaluation of other thrombotic events is not recommended and may be misleading (10).

Prevention of VTE Recurrence

D-Dimer assays play a clinical role in the prevention of VTE recurrence after the cessation of anticoagulant therapy. Patients with VTE or PE typically receive anticoagulants for 3–12 months after the disease presentation (excepting those patients who remain on anticoagulants indefinitely, i.e., patients with documented recurrent VTE, antiphospholipid syndrome, or inherited antithrombin deficiency).

D-Dimer assays can be used to evaluate patients who have completed their course of anticoagulant therapy. At approximately 4 weeks after treatment has ceased, the detection of elevated D-Dimer levels indicates a high risk of VTE recurrence (32, 33). Reinitiation of anticoagulant therapy is recommended for such patients (34, 35), suggesting that D-Dimer testing after anticoagulation suspension for a first episode of unprovoked VTE could help identify patients with high risk of VTE recurrence and tailor the duration of treatment (35).

Differential Diagnosis of Acute Chest Pain Symptoms

The patient presentation of acute chest pain exemplifies a challenging differential diagnosis with dire consequences, a diagnosis necessarily accomplished under severe time restraints. Acute chest pain as a clinical symptom is not specific to any one medical condition. However, the D-Dimer assay may help differentiate between conditions in which fibrin formation is typically present or absent.

The most common reasons for acute chest pain and/or acute dyspnea are:

- acute coronary syndrome (including myocardial infarction and other myocardial ischemia);
- acute aortic dissection; and
- acute pulmonary embolism.

Less common conditions possibly presenting with acute chest pain include:

- pneumonia;
- pneumothorax; and
- esophageal lesions.

The role of the D-Dimer antigen in excluding PE is well known; however, D-Dimer assays may also play a role in differentiating acute coronary syndrome (ACS) from acute aortic dissection (4). Diagnostic differentiation of the two conditions is vital in terms of appropriate therapeutic intervention: whereas primary treatment of ACS includes heparin and potent platelet function inhibitors, these drugs are likely detrimental to patients with aortic dissection (36). Therefore, the diagnostic exclusion of aortic dissection is of the utmost importance before the initiation of drug administration. D-Dimer assays may have clinical utility in aiding this differential diagnosis as D-Dimer plasma concentrations may differentiate between the two conditions.

Acute Aortic Dissection

Aortic dissection is a life-threatening condition caused by the formation of a false lumen in the wall of the aorta. Clinical symptoms include severe chest and upper back pain, and tachycardia. Aortic dissection may induce ECG abnormalities and, if the aortic arch is affected, elevated troponin levels due to impaired coronary flow. Acute stroke may result from impaired carotid artery blood flow or embolization.

Clinical trials have shown that acute aortic dissection is associated with strongly elevated D-Dimer levels, whereas D-Dimer levels in patients with acute coronary syndrome are normal or only slightly elevated, and a normal range of D-Dimer antigen level excludes acute aortic dissection (1, 2). Of notable interest, the diagnostic sensitivity and specificity of D-Dimer assays in the exclusion of acute aortic dissection were found to be similar to their capability for the exclusion of VTE (3).

However, D-Dimer levels may decline below threshold as soon as the false lumen is occluded by thrombotic material. Given this circumstance, intravascular fibrin formation has stopped, allowing D-Dimer levels to drop below threshold (37), a limitation of this diagnostic approach.

As in other clinical conditions associated with acute intravascular fibrin generation, the time between onset of symptoms and blood sampling should be taken into consideration when evaluating the results. In conclusion, patients with acute severe chest or upper back pain and high levels of D-Dimer antigen should undergo further diagnostic procedures, such as CT scan or magnetic resonance imaging (MRI), for exclusion of acute aortic dissection, preferably before administration of anticoagulant drugs and antiplatelet agents.

Acute Coronary Syndrome (ACS)

ACS is a more common cause of acute chest pain than is acute aortic dissection. Patients with ACS display normal or slightly elevated D-Dimer levels, unless cardiopulmonary resuscitation procedures and/or defibrillations have been performed (2, 38). In patients with ECG or laboratory signs of myocardial ischemia, the D-Dimer assay increases patient safety by excluding acute aortic dissection or aortic aneurysm, which would pose a potential bleeding hazard upon administration of anticoagulants and platelet function inhibitor drugs.

Aortic Aneurysms

Patients with aortic aneurysms also typically display elevated D-Dimer levels. D-Dimer assays are not able to distinguish between aortic dissection and aortic aneurysm.

In summary, diagnostic imaging with CT or MRI represents the most efficient diagnostic approach for the diagnosis or exclusion of acute aortic dissection, acute pulmonary embolism, pneumonia, or carcinoma in patients presenting with acute chest pain and strongly elevated D-Dimer level of evidence.

Disseminated Intravascular Coagulation (DIC)

D-Dimer assays can be used as an aid in the diagnosis of DIC as well as a monitor of treatment efficacy. Patients with DIC tend to present with extremely high blood concentrations of the D-Dimer antigen that, for some D-Dimer assays, require the dilution of blood samples. Given the extremely high concentrations found in DIC patients, quantitative D-Dimer assays used for the diagnosis and monitoring of DIC are required to have a wide linearity range.

Rather than being a specific disease, DIC may result from a variety of clinical conditions. DIC is the consequence of the pathological activation of coagulation mechanisms induced by underlying medical conditions. Clinical conditions typically associated with DIC include (39):

- sepsis or severe infection of any etiology;
- trauma (e.g., polytrauma, neurotrauma, fat embolism);
- organ destruction (e.g., severe pancreatitis);

- disseminated malignancy (e.g., solid tumors, adenocarcinoma, myeloproliferative/lymphoproliferative malignancies);
- obstetrical complications (e.g., amniotic fluid embolism, abruptio placentae);
- vascular abnormalities (e.g., Kasabach-Meritt syndrome, large vascular aneurysms)
- severe hepatic failure; and
- severe toxic or immunologic reactions (e.g., snake bites, recreational drugs, transfusion reactions, transplant rejection).

In any particular patient, the nature of DIC and the character of the D-Dimer oligomers present in the blood may vary in accordance with the underlying medical condition(s). DIC may be associated with suppressed or enhanced fibrinolysis (40). In patients with the hypofibrinolytic phenotype, the D-Dimer antigen consists primarily of high molecular weight fibrin complexes due to a low degree of plasmin proteolysis. Hypofibrinolytic DIC frequently leads to microvascular occlusions that induce organ dysfunction (38, 41). This condition is typically observed in sepsis.

Conversely, hyperfibrinolytic DIC is characterized by low fibrinogen levels accompanied by high concentrations of fibrin D-Dimer, but also fibrinogen degradation products. Hyperfibrinolytic DIC is typically observed in promyelocytic leukemia (42) and amniotic fluid embolism (43) and in early stages after trauma (44). Very high levels of fibrinogen degradation products may interfere with the detection of D-Dimer in some assays, causing either overestimation of D-Dimer antigen (if the assay cross-reacts with fibrinogen degradation products) or underestimation (if fibrinogen degradation products interfere with binding of the D-Dimer motif to the monoclonal antibodies).

The therapeutic approach to DIC depends upon the underlying disease and observed laboratory abnormalities. Patients with low fibrinogen levels, or other signs of hyperfibrinolysis, receive fibrinogen concentrate plus antifibrinolytic drugs, such as tranexamic acid. Additional therapeutic options include treatment with coagulation factor concentrates (e.g., prothrombin complex concentrate, Factor XIII, or others), fresh frozen plasma, or other whole plasma preparations. In addition, platelet transfusions may be necessary in patients with bleeding due to low platelet count.

In patients with a hypofibrinolytic phenotype, short acting anticoagulants may be beneficial (45). In DIC patients a variety of other blood coagulation abnormalities typically exist alongside the excess concentrations of D-Dimer oligomers; therefore, the D-Dimer antigen is only one component of a diagnostic panel required for the diagnosis and characterization of DIC. DIC is diagnosed using scoring systems that typically include prothrombin time (PT) tests, platelet count, fibrinogen level, and a fibrin-related marker (either D-Dimer, FDPs, or soluble fibrin complexes). The most commonly used scoring systems for DIC are those of the International Society for Thrombosis and Hemostasis (ISTH DIC score) and the Japanese Ministry for Health and Welfare (JMHW DIC score) (39, 46-49). These DIC scoring systems are valuable prognostic markers. They provide guidance in the selection of interventions and also provide a method of risk stratification for patient placement within clinical trials. Apart from being a component of the diagnostic score system, D-Dimer levels may be used for treatment monitoring with a decline indicating successful treatment.

The D-Dimer Antigen in Other Clinical Conditions

Additionally, elevated D-Dimer blood levels may serve as markers for tumor-associated intra- or extravascular fibrin formation and specific complications of pregnancy associated with coagulation activation. Finally, D-Dimer assays may have a role in the exclusion of HIT.

Malignancy

Thrombosis and DIC are common complications of cancer (50). While almost all types of cancer cells activate the coagulation system, the pathogenesis of the prothrombotic state in cancer is complex and likely multifactorial (50). First, tumor cells release procoagulants, including tissue factor (TF) and cancer procoagulant, an enzyme that activates Factor X, as well as inflammatory cytokines that stimulate other cells to release TF. Fibrin formation is also activated by the interplay between tumor cells, endothelial cells, and components of the blood (e.g., monocytes/macrophages, platelets) (50).

Second, anticancer therapies are also capable of promoting the thrombotic state. Surgery, chemotherapy, hormone therapy, radiotherapy, and indwelling central venous catheters (50) activate fibrin formation or promote thrombotic conditions. Overall, the relative contributive weights of these factors and their interplay have not been precisely elucidated. Moreover, hemostatic activity in some cancer patients develops contrary to expectations. For example, many patients with disseminated adenocarcinoma have chronic DIC with massively elevated D-Dimer levels, yet exhibit no signs of microvascular occlusion or organ dysfunction. Conversely, the predominant hemostatic feature of promyelocytic leukemia is a massive activation of fibrinolysis, prompting degradation of both fibrin and fibrinogen and leading to low plasma fibrinogen levels. Yet patients with acute promyelocytic leukemia (APL) are at high risk for the development of life-threatening thrombotic and hemorrhagic complications, particularly during induction chemotherapy (51, 52).

Cancer patients with elevated D-Dimer levels display an increased risk for deep venous thrombosis associated with surgery or chemotherapy (53, 54). Yet the use of D-Dimer assays in VTE exclusion among cancer patients is compromised by several factors. First, D-Dimer levels may be elevated in cancer patients in absence of thrombosis (24). The prevalence of patients with D-Dimer levels below threshold is lower than in patients without malignant disease (55-58). Second, the diagnostic algorithms for VTE exclusion have not been validated in cancer patients (24). Finally, the NPV of D-Dimer testing in the cancer patient population is lower as a consequence of higher DVT prevalence in cancer patients (24). Conversely, the use of D-Dimer testing in the exclusion of PE in cancer patients, using a higher threshold, seems feasible (24). In summary, **the use of quantitative D-Dimer assays in the exclusion of VTE in cancer patients might be possible, but such clinical applications and the use of higher cut-off values would require validation within clinical trials**.

In malignancy, beyond VTE exclusion, the D-Dimer antigen may serve as a marker for intra- or extravascular fibrin formation associated with malignancy itself as well as having prognostic value in certain malignant conditions. For example, clinical studies suggest that elevated D-Dimer levels in patients with colorectal cancer and non-small cell lung carcinoma malignancy indicate disease progression and adverse outcome (59-61). Therefore, D-Dimer may serve as a prognostic marker among these patient populations. Future studies might evaluate further the role of D-Dimer assays in this prognostic role.

Pregnancy and Complications of Pregnancy

Blood concentrations of the D-Dimer antigen increase continuously throughout pregnancy (62). D-Dimer is not efficient for exclusion of pregnancy-associated VTE if the upper limit of normal range (of nonpregnant individuals) is used as the threshold level (63), and PTP algorithms have not been validated for pregnant women (24). Available data concerning the use of higher cut-off levels in pregnancy are still insufficient for a general recommendation.

As a monitoring device, the D-Dimer assay might prove helpful in the monitoring of specific, high-risk patients with thrombophilia, with thromboembolic events, or requiring dose adjustment of heparin or other

anticoagulants. D-Dimer assays might also serve as a diagnostic tool in the complications of pregnancy associated with the activation of coagulation, such as preeclampsia, fetal death, amniotic fluid embolism, and HELLP syndrome (Hemolytic anemia, Elevated Liver enzymes, and a Low Platelet count) (43, 64-66). Given that heparin therapy appears to be beneficial in patients with HELLP syndrome, potentially preventing recurrence (67, 68), the D-Dimer assay may provide a means for initiating and monitoring of anticoagulant treatment.

The D-Dimer antigen might also serve as a fibrin-related marker, enabling the diagnosis and monitoring of this amniotic fluid embolism. Amniotic fluid embolism usually induces hyperfibrinolytic DIC characterized by a massive activation of fibrinolysis, low fibrinogen levels, and bleeding (43, 69-71). Treatment includes volume replacement, fibrinogen replacement, and replenishment of other coagulation factors (e.g., platelet concentrates and recombinant Factor VIIa) (72, 73). In such settings, the D-Dimer assay could be used to gauge the activation state of coagulation over time. Future studies may elucidate the role of D-Dimer testing in obstetric conditions. Table 4 summarizes the clinical capabilities of D-Dimer Assays.

Table 4: The Clinical Capabilities of D-Dimer Assays

D-Dimer assays are able to:
• Exclude clinical conditions associated with coagulation activation and fibrin formation • Distinguish between clinical conditions with and without coagulation activation • Provide prognostic information • Guide anticoagulant therapy • Identify patients with failure of anticoagulant therapy or severe side effects such as Heparin-induced thrombocytopenia (HIT)
D-Dimer assays are not able to:
• Identify the cause of coagulation activation • Provide information concerning the presence or absence of a clot or concerning clot dissolution

Heparin-Induced Thrombocytopenia

HIT is a severe immunological complication of heparin therapy that often leads to thromboembolic complications. HIT is typically characterized by a drop in platelet count 5–10 days after initiation of heparin treatment, accompanied by an extreme elevation in the concentration of fibrin-related markers, such as D-Dimer (74). Among the routine coagulation tests, only D-Dimer is informative for differentiating HIT from DIC (75). Although there are little published data, it seems that a normal range D-Dimer level excludes acute HIT, and a D-Dimer level within the ‘expected range’ for a specific patient population, e.g., postoperative patients, should prompt the search for alternative reasons for thrombocytopenia (75).

The presence of HIT also may be excluded by use of immunoassays for the detection of antibodies against heparin-platelet factor 4 complexes (high levels being indicative of thrombotic risk). Functional assays detecting heparin-induced platelet aggregation in vitro are helpful to support the diagnosis (74).

Advantages and Limitations

Advantages

The D-Dimer antigen assays possess several important advantages over other laboratory markers of *in vivo* coagulation activation and their respective assays. **A primary advantage to the D-Dimer marker is its large diagnostic "window" (76).** D-Dimer assays detect the D-Dimer motif within fibrin in the bloodstream ("soluble fibrin"), as well as XDPs released from fibrin clots or extravascular sources, and also are able to detect fibrin formation after the process of fibrin formation has ceased. This capability is important and beneficial within the framework of VTE exclusion because many patients present to the clinician or emergency room several days after onset of clinical symptoms. By this time, several other laboratory markers, specifically fibrinopeptide A, thrombin-antithrombin complexes (TATs), or soluble fibrin complexes (SFC) may have returned to normal range (77).

Another major advantage of the D-Dimer antigen assays is the possibility of using citrated plasma and other biological materials, such as whole blood containing fibrinogen (78). The formation of D-Dimer antigen involves the cleavage of fibrinogen and conversion of Factor XIII into Factor XIIIa by thrombin, followed by the linkage of covalent bonds between the D-domains via Factor XIIIa catalysis. Factor XIII activation is dependent upon the presence of calcium ions. The removal of calcium in the clinical sample by addition of citrate or ethylenediaminetetraacetic acid (EDTA) prevents Factor XIII activation, thereby preventing additional D-Dimer antigen formation within the sample, precluding any false D-Dimer elevations should any active thrombin remain in the sample.

A final advantage of D-Dimer is its robustness in regard to pre-analytical influences. Whereas TATs, soluble fibrin, fibrinopeptides A and B, and other coagulation activation markers may increase *ex vivo* as a consequence of coagulation activation induced by sample handling and storage, levels of D-Dimer antigen generally do not change. D-Dimer assays also possess favorable resistance to interference from hemoglobin, bilirubin, triglycerides, lipemia, Rheumatoid Factor (RF), and human anti-mouse antibodies (HAMA).

Limitations

It is important to remember that D-Dimer antigen results are a general marker for *in vivo* fibrin formation; therefore, **D-Dimer assays can only be interpreted in the context of each patient's clinical condition**. Clinical data regarding the use of PTP in conjunction with D-Dimer assays were developed in clinical trial evaluations of DVT and PE. Such data are likely to be invalid when applied to other thrombotic events (10).

In addition, D-Dimer assays have a greater sensitivity for proximal DVT and PE. Patients with distal DVT may have a D-Dimer below the threshold of VTE exclusion in 35% of cases. As such, D-Dimer assay results should not be used to preclude ultrasound evaluation (10).

D-Dimer levels decline rapidly after initiation of anticoagulant therapy. Therefore, blood samples obtained for VTE exclusion should be drawn no later than 24 hours after the start of anticoagulant therapy (10). It is possible this time limitation could be different for patients receiving a prophylactic versus a therapeutic dose, but no appropriate clinical trials have evaluated this concept.

For the exclusion of VTE, D-Dimer assays are best applied in settings in which no alternative causes of D-Dimer antigen elevations are present, including (10):

- fibrinolytic therapy within the previous 7 days;
- trauma or surgery within the previous 4 weeks;
- large hematoma;
- disseminated malignancies;
- DIC;
- sepsis, severe infections, pneumonia;

- liver cirrhosis;
- pregnancy;
- atherosclerotic vascular disease;
- sickle cell disease; and
- advanced age (> 60 years).

In addition, D-Dimer antigen values may fall below the cut-off value despite the presence of VTE under a number of circumstances, such as (10):

- coagulation activation is too weak to raise D-Dimer values above threshold;
- the elapsed time since the thrombotic event has allowed clearance of the D-Dimer from circulation; and
- the thrombotic event is a small, subsegmental PE (therefore, D-Dimer may not be appropriate to safely exclude upper extremity deep vein/venous thrombosis (UEDVT) because of D-Dimer values (10, 44).

Finally, the use of D-Dimer assays for VTE exclusion in some patient populations is inappropriate, namely:

- hospitalized patients with conditions previously listed (80% of whom have elevated D-Dimer levels); and
- pregnant and perinatal women who exhibit elevated D-Dimer values due to pregnancy (although the role of D-Dimer testing continues to be debated, generally, the use of the D-Dimer antigen to exclude VTE in pregnant women is not recommended, although adjusted threshold levels may restore the diagnostic specificity for VTE exclusion).

The Lack of D-Dimer Assay Standardization

The lack of standardization among D-Dimer assays remains an outstanding issue. The principal issue is the lack of an international reference preparation (10), and the development of a standard reference remains difficult (15). The inability to develop a standard reference preparation is complicated by two key issues.

First, the D-Dimer antigen as measured in plasma or whole blood represents a heterogeneous mixture of cross-linked fibrin compounds (79). This hematological profile of these heterogeneous XDPs is likely to be both patient-specific and incident-specific within each patient. The marked variety of D-Dimer oligomers within patient materials cannot be approximated by mixtures of purified fibrin fragments (10).

Second, the mAbs utilized in D-Dimer assays are not standardized either. Each detecting mAb owns a unique specificity (24) that varies between assays and manufacturers, and assays exhibit varying sensitivity to fibrin compounds of different molecular size. For example, some D-Dimer assays exhibit extremely limited reactivity with fibrin D-Dimer fragments.

Initial attempts to standardize D-Dimer assays used purified D-Dimer fragments as a calibrator. Later studies demonstrated that less interassay variation was achieved when pooled plasma samples derived from patients constituting a range of elevated D-Dimer conditions replaced the purified XDPs (15, 80). Although this approach improved interassay harmonization on average, important discrepancies remain outstanding for individual samples, particularly among samples having higher D-Dimer concentrations (81).

Current consensus holds that D-Dimer assays should be calibrated with serial dilutions of a sample containing a “physiological” mixture of cross-linked fibrin derivatives, such as pooled plasma from patients with DIC or acute VTE (80, 81). Attempts to prepare similar material in vitro have so far been unsuccessful. Calibration materials may include purified D-Dimer fragment; semipurified, high molecular weight, cross-linked fibrin products prepared from purified fibrin; or pooled plasma samples (10).

D-Dimer levels are assigned to the primary calibrator, by convention, using 0.5 µg/mL as the upper limit of normal range for the majority of assays. Some manufacturers base this calibration using FDPs derived from a defined amount of fibrinogen. The units of measurement associated with this type of calibration are fibrin-equivalent units (FEUs). Other manufacturers base the calibration on a defined amount of purified fibrin D-Dimer fragment. The units of measure associated with this type of calibration area are D-Dimer Units (D-DU). After such primary calibrations have been established, manufacturers and operators may utilize secondary assay-specific calibrators as well.

Owing to the unique performance characteristics of each particular assay, clinicians need to be aware of the heterogeneous nature of the D-Dimer antigen and the performance characteristics of the particular D-Dimer test in use at their institution. A growing body of evidence suggests that the D-Dimer assay can play a growing prognostic and/or diagnostic role across a growing range of clinical conditions. Further efforts in research, standardization, and communications regarding assay performance an expanded list of clinical applications will enable more effective utilization of this device (24).

Professor Carl-Erik Dempfle, an international expert on thrombosis and Hemostasis who has contributed to over 150 publications, is eminently qualified to critically evaluate all clinical and laboratory aspects of fibrinolysis and applications of D-Dimer testing.

Articles on his research have been published across a wide spectrum of medical journals, including *Cerebrovascular Diseases, Thrombosis Research, Blood, German Medical Science, Der Anaesthesist, the New England Journal of Medicine, Thrombosis and Haemostasis, the International Journal of Cardiology, Critical Care Medicine, Blood Coagulation & Fibrinolysis, Haemophilia, Hamostaseologie, Clinical Chemistry and Laboratory Medicine,* and the *Journal of Thrombosis and Haemostasis.*

Dr. Dempfle is currently Professor of Internal Medicine at the Faculty of Medicine in Mannheim, University of Heidelberg, and head of the Coagulation Center Mannheim, Germany. He is also an active member of the ISTH Subcommittee on disseminated intravascular coagulation (DIC), has served as Secretary of the International Fibrinogen Research Society (IFRS), is editor of *Thrombosis Research*, and acts as a reviewer for numerous scientific journals.

Instrumentation Laboratory wishes to thank Dr. Dempfle for providing his educational and technical expertise to present valuable insights into the diseases and conditions of hypercoagulability that can be diagnosed, excluded, and monitored with D-Dimer tests.

D-Dimer Assay Overview

IMPORTANT NOTE: The conclusions in this document based on references that pertain to off-label claims for use of D-Dimer are neither promoted nor validated by IL. As a company, we support the claims and intended uses agreed upon with the respective regulatory agencies that clear and approve our regulatory submissions.

The first monoclonal antibody D-Dimer assays, developed in the mid-1980s, were among the first clinical diagnostic assays based on mAb technology (10, 82). The current D-Dimer assays are a product of the exploration for laboratory markers that ascertain *in vivo* fibrin formation (83-85). Earlier laboratory tests could not differentiate between fibrinogen and fibrin degradation products (24). It was the advent of monoclonal antibodies, granting specific targeting of the unique neo-epitopes of D-domain, that granted hospital laboratories the capability of quantifying D-Dimer concentrations. The current selection of commercial laboratory D-Dimer assays ranges from manual to automated to point-of-care devices (86).

Standardization

Commercial D-Dimer assays lack standardization. The performance characteristics of D-Dimer assays are specific to each model, brand, or manufacturer, therefore different assays are likely to produce different numerical values given identical blood samples. It is imperative that clinicians understand the nature and performance characteristics of the particular D-Dimer assay used by their hospital laboratory (24).

Individual D-Dimer assays have different performance characteristics because the mAbs employed in each assay have unique specificity profiles relative to the D-Dimer antigen and other XDPs. The D-Dimer analyte itself is a heterogeneous population of diverse molecular compositions, and the industry lacks a standard international reference preparation that would enable the uniform calibration of assays. Moreover, assays differ in the tagging antibody, dilution of sample, incubation times, and detection limits, all contributing to differences in the normal values. [However, in the absence of an international standard, a manufacturer's assay can be calibrated according to the harmonization criteria proposed by W. Nieuwenhuizen and P. Meijer (80)].

Clinical Utility

In terms of clinical utility, not all D-Dimer assays have identical FDA clearance or validation for all possible clinical indications related to D-Dimer testing. For example, and perhaps most significant, not all D-Dimer assays are validated for VTE exclusion. Hence, D-Dimer assays are generally present at three levels of claims reflecting their approved clinical indications. These uses are: 1.) measurement of D-Dimer, 2.) aid in diagnosis, or 3.) VTE exclusion.

Regarding the measurement of the D-Dimer antigen, both quantitative and qualitative D-Dimer assays are capable of accomplishing this task–distinguishing between the presence or absence of the D-Dimer antigen at pre-specified thresholds. Qualitative D-Dimer assays are not used for VTE exclusion.

Ongoing confusion plagues the arena of D-Dimer testing. For example, there is a common misconception among some clinicians that the universal threshold value of all D-Dimer assays is 500 mg/L FEU. However, the descriptive reporting units (D-DU vs. FEU) and the corresponding units used to report magnitudes of weight and volume vary by assay model and/or manufacturer. In reporting D-Dimer outcomes, all laboratories are encouraged to report both the type of units and the respective threshold used for the VTE exclusion (10).

The claim for VTE exclusion is limited to quantitative assays that express antigen concentrations via numerical values that can be validated by recognized standards.

A D-Dimer assay, defined by the United States FDA as an "aid-in-diagnosis" device, may be used in conjunction with clinical indications. The aid-in-diagnosis claim does not require manufacturers to generate as much supporting data as for the claim of VTE exclusion. Yet, at a minimum, a threshold value for these devices has been validated, and vital aspects of device performance, such as NPV, have been demonstrated conclusively (10).

The requirements for satisfying a claim of VTE exclusion are the most stringent. D-Dimer assays FDA 510(k) cleared for VTE exclusion have demonstrated an NPV, sensitivity, and coefficient of variation (CV) – below a predetermined threshold – with sufficient criteria to exclude VTE in patients with a low or intermediate probability of VTE, as judged by a PTP scoring algorithm.

Types of D-Dimer Assays

A large number of laboratory assays for D-Dimer are currently available, including automated laboratory assays and point of care assays (86). Currently available D-Dimer assays are not all identical because the D-Dimer antigen is present on different size degradation products; the monoclonal antibodies recognize different epitopes; and the assay format, assay calibration standards, and instrumentation vary (24). Clinicians and laboratory personnel need to work collaboratively in the selection of D-Dimer testing technology most suited for their specific institution and its patient population. In the selection of D-Dimer assays, a number of assay features require consideration:

- qualitative vs. quantitative;
- time to result;
- assay availability (the requirement to batch samples causes delay);
- acceptable precision in the vicinity of threshold values;
- units of measurement: FEU vs. D-DU; nanograms per milliliter (ng/mL) vs. micrograms per liter (µg/L) vs. micrograms per milliliter (µg/mL) vs. milligrams per liter (mg/L);
- validation of diagnostic threshold (manufacturer validation vs. site validation);
- intended use: VTE exclusion vs. aid-in-diagnosis; and
- clinical safety (noninvasive), time- and cost-efficiency.

Table 5 lists the common types of D-Dimer assays in current clinical use along with commercial examples of each.

Table 5: Common Types of D-Dimer Assays

Enzyme-Linked ImmunoSorbent Assay (ELISA)	Zymutest (HYPHEN BioMed)
Enzyme-Linked Fluorescent Assay (ELFA)	Vidas D-Dimer Exclusion (bioMérieux)
Latex-Enhanced Turbidimetric Immunoassay	HemosIL D-Dimer, D-Dimer HS, D-Dimer HS 500, and D-Dimer 500 (Instrumentation Laboratory)
Chemiluminescent Immunoassay	HemosIL AcuStar D-Dimer (Instrumentation Laboratory)
Whole-Blood Agglutination	SimpliRed (BBInternational)
Rapid Lateral Flow	Clearview Simplify (Inverness Medical)

Methodologies

ELISA AND ELFA

ELISA (enzyme-linked immunosorbent assay) and ELFA (enzyme-linked fluorescent assay), collectively referred to as quantitative sandwich assays, were the first immunoassays used to quantify D-Dimer plasma concentrations. In these immunoassays, the capture antibody–affixed to a plate or other solid phase–captured the D-Dimer antigen. The antigen-antibody complex was labeled with a tag–either chromogenic, fluorogenic, or chemiluminescent–which generated a signal proportional to the amount of D-Dimer present in the sample.

Quantitative sandwich assays have been developed utilizing many different combinations of solid phases, mAbs, and signal enzymes (10). ELISA methods were initially developed for research purposes; therefore, ELISA assays have been the reference standard for D-Dimer quantification (24, 78). Yet, in spite of their high sensitivity and specificity, conventional ELISA assays on microtiter plates can be expensive, labor intensive, and time consuming. Therefore, they can be impractical in most clinical situations where rapidly available results are needed. However, ELISA tests have been cleared by the FDA for VTE exclusion and are used worldwide for this purpose (24).

Latex/Microparticle Agglutination

The first generation of assays for clinical applications were of a microparticle agglutination design using latex beads coated with an antibody specific to a unique portion of the D-Dimer epitope that underwent conformational change upon covalent ligation by Factor XIIIa (24). The microparticle agglutination assay design is frequently used for the determination of D-Dimer (10), and has evolved through several modalities.

Latex agglutination assays rely on the presence of sufficient quantities of XDPs having the D-Dimer antigen motif to initiate agglutination (24). In current quantitative D-Dimer assays, the substrate consists of uniform latex particles coated with one or two specific antibodies. After mixing sample and immunochemical reagents together, the resultant binding (agglutination) generates a detectable signal. No separate process for the separation of bound from unbound tags is required. Depending on the method, the degree of agglutination is measured manually or automatically by detecting the amount of light scattering induced by the agglutinated microparticles. The intensity of light scattering depends on the size of the microparticles, and the progressive agglutination of microparticles changes the amount of scattered light as the reaction proceeds (10). In actual practice, manufacturers utilize a number of different variations on this basic design scheme.

Manual Microparticle Agglutination

Assays based on the manual microparticle agglutination design are semiquantitative. In manual methods, the sample and the antibody-coated latex beads are typically mixed on a test card or slide. Given sufficiently high concentrations of D-Dimer, the agglutination caused by the immunochemical reaction forms macroscopic clumps of substrate and D-Dimer oligomers. The slides or test cards are viewed macroscopically by a technologist or other clinician using a direct light source to determine the presence or absence of agglutination. Naturally, such manual assay methods are subject to a high degree of interobserver variability (10). Therefore, it is no surprise that recent evaluations clearly demonstrate that assays based on this methodology are inappropriate for clinical use in VTE exclusion (10).

Automated Microparticle Agglutination

Automated microparticle agglutination was developed to measure the rate at which agglutination occurred in the presence of D-Dimer antigen. The automated latex agglutination assays were developed to be used with specialized automated analyzers and coagulometers (10, 24, 78). Typical analysis time is 10 minutes plus centrifugation time (10).

In the automated process, the latex microparticles, coated with mAbs specific for the D-Dimer motif, are incubated with plasma, and the degree of agglutination is detected automatically. Given a suspension of microparticles alone, the wavelength of the light is greater than the diameter of the latex microparticles, and light absorption by the microparticle suspension is minimal. After the addition of plasma to the suspension, the aggregates of agglutinated microparticles gain diameters greater than the wavelength of the light and thereby increase light absorbance proportional to the amount of D-Dimer present in the sample.

Naturally, these assays are subject to the same variability that all D-Dimer assays share. The specificities of mAbs are not identical and likely interact differently with high- and low-weight fibrin degradation products (just as patient samples are heterogeneous mixtures). Additionally, these assays differ in calibration and reference preparation. As such, each assay is likely to have a distinct sensitivity, and outcomes are likely to vary by assay model and patient sample.

Automated Microparticle Agglutination plus Turbidimetry

D-Dimer assays that add turbidimetry to the method of automated microparticle agglutination represent the state-of-the-art technology, and are commonly employed today. Cleared by the FDA for VTE exclusion, latex turbidimetric methodologies are used routinely in laboratories and emergency rooms worldwide (10, 24). Turbidimetry measures the intensity of a beam of light transmitted through a suspension of agglutinated, antibody-coated microparticles. Alternately, nephelometry, which measures intensity of light angled away from the initial direction of the beam, is employed as the measure of turbidity (10).

Whole-Blood Agglutination

Whole-blood agglutination tests for D-Dimer antigen evaluation have been developed that do not require sophisticated laboratory instrumentation, yet have clinical utility for prompt clinical decision making regarding VTE exclusion (24). In the presence of sufficient concentrations of D-Dimer antigen, the red blood cells agglutinate. These assays may utilize whole blood or plasma, depending on the assay. Whole-blood agglutination tests are currently available as point-of-care devices (10).

In one commercially available method, blood cells are separated from the plasma by a filter within the test cartridge of the device. The plasma reacts with fluorescent antibody conjugates within the reaction chamber. After incubation, the mixture flows through a detection channel wherein the analyte and antibody conjugate are captured. The detected values of fluorescence proportionally reflect the concentration of D-Dimer within the sample.

Although whole-blood agglutination assays remain less sensitive than the other developed technologies, they demonstrate sufficient specificity to enable VTE exclusion in the correct clinical setting (24). Although the number of clinical studies documenting the performance of the whole-blood and plasma assays is

relatively small, the collective performance characteristics demonstrate a sensitivity for VTE of < 97% with an NPV of < 97%, depending on the method and the threshold (10).

Chemiluminescent Immunoassay

Currently, two commercial D-Dimer assays utilizing chemiluminescent methods are available. One method uses paramagnetic microparticles; the other method uses latex nanobeads.

The first approach uses paramagnetic microparticles as the solid phase that is coated with mAbs specific to the D-Dimer antigen. The clinical sample and the coated magnetic particles are combined in the reaction vessel. After magnetic separation and washing, the captured antibody labeled with isoluminol is incubated with the reagents. After a second magnetic separation and washing, two triggers are added and the resultant chemiluminescent reaction is measured by an analyzer system. The strength of the chemiluminescent reaction is directly proportional to the D-Dimer concentration in the sample.

The latex nanobeads method consists of three reactants: 1.) a biotinylated analyte receptor, 2.) a bead coated with streptavidin marked with photosensitive dye, and 3.) a second reagent bead coated with an analyte-specific binding partner marked with a chemiluminescent dye. When mixed, the three reactants combine with the analyte to form an immunocomplex. When the sample is illuminated by a specific wavelength of light, a chemiluminescent reaction is triggered by components of the bead-aggregate, with the reactive intensity measured by a fluorometric analyzer.

Quantitative D-Dimer for the Exclusion of VTE

Characteristics of the Ideal D-Dimer Assay

From a laboratory viewpoint, the ideal D-Dimer assay should have the following characteristics (78):

- quantitative performance;
- a wide linearity capable of providing accurate low-range values (i.e., patients with anticoagulant therapy) and high-range values (i.e., patients with disseminated intravascular coagulation);
- FDPs lacking the D-Dimer motif have no influence on assay outcomes;
- variations in fibrinogen concentration have no influence on assay results;
- continuous assay availability;
- results available in less than 15 minutes; and
- validation in appropriate clinical studies.

Quantitative D-Dimer assays may be utilized in the initial evaluation of patients suspected of having VTE (DVT and/or PE) since the exclusion of VTE cannot be made on clinical grounds alone (24). A single D-Dimer test alone does not have sufficiently high sensitivity and specificity to singularly exclude or diagnose VTE; therefore, the use of D-Dimer assays in conjunction with clinical information and PTP is imperative for proper patient management. Appropriate use of D-Dimer assays requires an understanding that the predetermined threshold cut-offs, and associated claims of indicated use, were established in management trials with patients suspected of VTE (DVT or PE).

CLSI Guidelines

Substantial guidance on the use of D-Dimer assays for VTE exclusion is provided by the Clinical and Laboratory Standards Institute (CLSI) (10). CLSI guidelines describe the use of D-Dimer assays in conjunction with PTP scoring in VTE exclusion; the proper collection and handling of the clinical sample; assays appropriate for D-Dimer analysis; methods to determine the threshold for VTE exclusion; interpretation of test results; and regulatory and accreditation requirements for use of exclusionary D-Dimer assays (10).

In the appropriate clinical context, quantitative D-Dimer assays enable clinicians to exclude suspected VTE in patients and proceed with patient management or other diagnostic procedures without the need to perform imaging studies, thereby conserving time and resources. Such clinical management is appropriate only when the particular D-Dimer assay in use has been validated for VTE exclusion, the determination has been made that D-Dimer elevations cannot be attributed to other causes, and the patient has been ranked into the low or intermediate PTP stratification by an appropriate scoring algorithm. Although hospitals are allowed to validate threshold levels and the efficacy of D-Dimer assays for VTE exclusion, the preferred method, per CLSI, is to use a commercial assay for which the manufacturer has performed the required studies to validate the cut-off value and that has been granted clearance by a regulatory agency with a specific Intended Use stating “for the exclusion of VTE” (10).

Highlights of CLSI Guidelines for Quantitative D-Dimer for the Exclusion of VTE

For the purposes of VTE exclusion, the CLSI guidelines suggest:

- the use of quantitative methods (e.g., sandwich assays and microparticle agglutination) is preferred over semiquantitative microparticle agglutination;
- the use of assays with a stated Intended Use of VTE exclusion only;
- the use of the cut-off value (threshold) identified by the manufacturer in the package insert;
- the use of assays with the recommended performance parameters in Low + Moderate PTP patients of:
  - NPV > 98%
  - 95% lower limit of the one-sided CI of the NPV > 95%
  - sensitivity > 97%
  - 95% lower limit of the one-sided CI of the sensitivity > 90%; and
- the practice of reporting assay outcomes by the type and magnitude of units recommended by the manufacturer without performing conversion of units.

All suitable precautions must be taken to ensure appropriate performance of D-Dimer testing.

D-Dimer testing has limited utility for VTE exclusion in unselected hospitalized patients (87). Coagulation activation resulting from systemic inflammation, such as sepsis, trauma, and rheumatologic disease, is also associated with elevated circulating D-Dimer levels. As such, D-Dimer antigen testing in patients afflicted with these clinical conditions can be far less effective in excluding the presence of VTE.

The efficacy of VTE exclusion for proximal DVT is greater than that for distal DVT. Up to 35% of patients with distal DVT will have normal D-Dimer antigen values; thus the D-Dimer assay test has limited use in evaluation of such patients. Therefore, all patients with suspected distal DVT require ultrasound evaluation (10).

FEUs vs. D-DUs—D-Dimer Units of Measure

Despite the widespread use of D-Dimer assays, many clinicians are confused by the variation in units, numerical results, and threshold values used by the different assays (10). The confusion arises from two areas of inconsistency.

First, as the D-Dimer assay evolved, two sets of units evolved to express D-Dimer levels: the D-Dimer unit (D-DU) and the fibrin-equivalent unit (FEU). The FEU is based on the amount of fibrinogen used for the preparation of XDPs containing the D-Dimer motif, which are used for the calibration of the respective D-Dimer assay. The D-DU is based on the amount of purified fibrin D-Dimer fragment present in the calibrator. As the molecular weight of the fibrinogen molecule is approximately twice that of a D-Dimer fragment, one FEU equals approximately two D-DUs. However, such direct multiplicative conversions of one unit of measure to the other are neither appropriate nor validated.

The second area of inconsistency in D-Dimer reporting is the variability of units used to express weight and volume. FEUs and D-DUs might be expressed as nanograms per milliliter (ng/mL), micrograms per liter (µg/L), micrograms per milliliter (µg/mL), or milligrams per liter (mg/L). This variability of the type and magnitude of the units is a source of confusion for both laboratories and clinicians (10).

Thus, ongoing confusion plagues the arena of D-Dimer testing. For example, there is a common misconception among some clinicians that the universal threshold value of all D-Dimer assays is 500 mg/L FEU. Rather, the descriptive reporting units (D-DU vs. FEU) and the corresponding units used to report magnitudes of weight and volume vary by assay model and/or manufacturer. In reporting D-Dimer outcomes, all laboratories are encouraged to report both the type of units and the respective threshold used for the VTE exclusion (10).

Transitioning to a New D-Dimer Test

Due to the unique performance characteristics exhibited by the different commercial D-Dimer assays, D-Dimer assay results are not, by definition, interchangeable. Therefore, the use of a single conversion factor between test results and consensus values at different D-Dimer levels is simply not possible.

As described previously and demonstrated by clinical application, individual test results with the same sample can vary by particular D-Dimer assays. Several published reports stress the importance of physician awareness in the difference between assays and the different cut-off values, and perhaps units of measure, used within a single institution (24).

Caution Advised

Both clinical guidelines and multi-center studies indicate caution in:

- selecting assay or study methods;
- appropriate attempting to convert D-Dimer assays from one unit to another (D-DU vs. FEU) using conversion multipliers;
- deriving conclusions from the evaluation of a small sample size;
- interpreting assay outcomes without proper knowledge of assay cut-off values; and
- dismissing appropriate and necessary clinical evidence when comparing performance outcomes between different assay or study methods.

Appropriate Approach

The appropriately structured approach for transitioning to a new D-Dimer assay includes quantitative method comparisons, qualitative method comparisons, analytical performance (quality control) testing, and clinical performance testing.

In the quantitative method comparison, the new D-Dimer assay is evaluated in comparison with the current method utilizing the same units to determine whether any correlations between the two methods exist. For this procedure to be valid, the results of the current test method must report in the same units of measure as established by the manufacturer. The unit cannot be a laboratory-validated conversion unit. A quantitative method comparison involves evaluating the slope and r value of the method comparison. Each laboratory should establish its own criteria for acceptability.

In the qualitative method comparison, the new assay method must be evaluated in comparison with a current method to establish, for each method, the rate of agreement for samples above and below the cut-off values. This comparison is typically performed using a 2x2 contingency table to divide the reference method results and test method results into subgroups; these subgroups are determined by quantitative value interpretations with respect to the cut-off value. Given these determinants, the positive agreement, negative agreement, and overall agreement can be calculated with respect to the reference method.

The clinical performance assessment compares the findings of the new D-Dimer assay versus the clinical diagnosis. This comparison tests the ability of the new assay method to evaluate the exclusion rate in reference to patients with a confirmed VTE diagnosis. A 2x2 contingency table is also used in this analysis wherein sensitivity, specificity, and NPV can be calculated with respect to a confirmed clinical diagnosis.

Using appropriate quality control materials, the users should also evaluate assay imprecision.

All samples used for the method comparison tests must be of the same sample type (citrated plasma, serum, whole blood, etc.); or in the case where the current method uses a different sample type, consist of matched sample, matrix pairs. For each method, the testing of the matched samples must be completed within a reasonable time frame.

Appropriate Assay Use

The intended uses of the assays must address the same indications intended for clinical use and be evaluated in a sample population representative of the intended use population. For example, assays validated only for use as an aid in the diagnosis of DVT and PE did not include patients suspected of DIC in their validation studies. Therefore, no patient suspected of DIC should be used in the comparison tests of these assays.

While any institution may, at its discretion, clinically validate an assay for off-label use, any comparison against a new method should be within the scope of the intended use of its current method. The manufacturer’s clinically validated cut-off for the exclusion of DVT or PE must be used in the data analysis. As with any evaluation, the criteria for acceptance should be defined by the laboratory prior to the evaluation. However, the preferred method is to use a commercial assay for which the manufacturer has performed the required studies to validate the cut-off value and that has been granted clearance by a regulatory agency with a specific Intended Use stating “for the exclusion of VTE” (10).

The HemosIL Solution

IMPORTANT NOTE: The conclusions in this document based on references that pertain to off-label claims for use of D-Dimer are neither promoted nor validated by IL. As a company, we support the claims and intended uses agreed upon with the respective regulatory agencies that clear and approve our regulatory submissions.

The HemosIL D-Dimer Assay Panel

Rapid, Efficient and Clinically Validated–for Improved Patient Outcomes

Regarding D-Dimer assays, the prerequisites of any individual laboratory may differ due to their patient population, assay processing volume, or the necessity for concordance of units (i.e., D-DUs vs. FEUs) with legacy devices. Moreover, the clinical indications may require devices with specific linearity or interference profiles. The selection of D-Dimer assays offered by the HemosIL D-Dimer Assay Panel satisfies the requirements of virtually any clinical or hospital laboratory.

Accordingly, Instrumentation Laboratory addresses nearly every concern by offering five assays for the determination of D-Dimer levels in the Hemostasis or core laboratory:

- HemosIL D-Dimer;
- HemosIL D-Dimer HS;
- HemosIL D-Dimer 500;
- HemosIL D-Dimer HS 500; and
- HemosIL AcuStar D-Dimer.

For smaller laboratories, or laboratories with legacy devices utilizing D-DUs, HemosIL D-Dimer and HemosIL D-Dimer HS offer proven, accurate, and reliable solutions for D-Dimer testing utilizing D-DUs. HemosIL D-Dimer 500 is a proven, accurate and reliable alternative to HemosIL D-Dimer in that it uses the same technology and reagents while offering outcomes in FEUs.

HemosIL D-Dimer HS 500 assay may be a better alternative for laboratories processing greater volumes or reliant on the FEUs. HemosIL D-Dimer HS 500 assay has a wider linearity than HemosIL D-Dimer assay. Rather, HemosIL D-Dimer HS 500 has a linearity and interference profile comparable to that of HemosIL D-Dimer HS assay–both assays have a significantly improved interference profile compared with HemosIL D-Dimer relative to RF and HAMA–while providing outcomes in FEUs.

Of all assays provided by Instrumentation Laboratory, HemosIL AcuStar D-Dimer has the most dynamic linear range. This extensive linear range allows for the analysis of D-Dimer samples containing both very low and very high concentrations of D-Dimer antigen in the same run. The single-center management study demonstrated 100% sensitivity and 100% NPV for the HemosIL AcuStar D-Dimer, which is intended for the aid in the diagnosis of VTE.

In addition, all assays belonging to the HemosIL D-Dimer Assay Panel were designed to fulfill the parameters of an "ideal D-Dimer assay" as outlined by Dempfle *et al*; i.e., assays having the following characteristics:

- quantitative assessment;
- extensive linearity;
- processing time of < 15 minutes;
- exclusion efficacy demonstrated in appropriate clinical trials;
- immediate availability; and
- lack of influence from fibrinogen and FDPs.

As demonstrated by management studies, HemosIL D-Dimer, HemosIL D-Dimer HS, and HemosIL D-Dimer HS 500 assays can safely exclude the presence of DVT and PE when used in conjunction with a PTP score (27, 28).

FDA Clearance Status of the HemosIL D-Dimer Assay Panel

By using the HemosIL D-Dimer Assay Panel, you have the comfort of knowing all assays are FDA-cleared for the exclusion of VTE or as an aid in the diagnosis of VTE:

• HemosIL D-Dimer HS	FDA-cleared for VTE Exclusion
• HemosIL D-Dimer HS 500	FDA-cleared for VTE Exclusion
• HemosIL D-Dimer	FDA-cleared for VTE Exclusion
• HemosIL AcuStar D-Dimer	FDA-cleared as an Aid in the Diagnosis of VTE

In addition to these assays capable of VTE exclusion, HemosIL AcuStar D-Dimer assay is a chemiluminescent immunoassay that offers enhanced analytical sensitivity, an extensive dynamic range, and an 8-week onboard stability. HemosIL AcuStar D-Dimer assay reports outcomes in FEUs.

HemosIL AcuStar D-Dimer and HemosIL D-Dimer HS 500 assays are distinct in that they incorporate a ready-to-use liquid reagent (as opposed to a lyophilized reagent). The ready-to-use liquid reagent reduces preparation time and eliminates the variability in outcomes introduced by water source and operator pipetting technique.

Table 6 enables direct comparison of assay design and performance, including assay units, the cut-off value for VTE exclusion, technology of design, detection limit, linearity, precision, interference, stability, and calibration. All values for each assay were derived on ACL TOP systems, except HemosIL AcuStar D-Dimer assay–its values were derived on the ACL AcuStar system.

Table 6: HemosIL D-Dimer Assay Panel

	HemosIL D-Dimer	HemosIL D-Dimer 500*	HemosIL D-Dimer HS	HemosIL D-Dimer HS 500	HemosIL AcuStar D-Dimer
Instrument	ACL TOP	ACL TOP	ACL TOP	ACL TOP	ACL AcuStar
Indication	VTE Exclusion	VTE Exclusion	VTE Exclusion	VTE Exclusion	Aid in the Diagnosis of VTE
Units	D-DU (ng/mL)	FEU (ng/mL)	D-DU (ng/mL)	FEU (ng/mL)	FEU (ng/mL)
Cut-off for VTE	230 ng/mL (D-DU)	500 ng/mL (FEU)	230 ng/mL (D-DU)	500 ng/mL (FEU)	500 ng/mL (FEU)
Technology	Latex – 405 nm	Latex – 405 nm	Latex – 671 nm	Latex – 671 nm	Chemiluminescence
Detection Limit	69 ng/mL	150 ng/mL	21 ng/mL	146 ng/mL	6.51 ng/mL
Linearity (ng/mL)					
Without rerun	200–1,050	435–2,283	150–3,680	215–7,650	54.3–74,000
With rerun	n/a	435–11,413	150–69,000	215–128,000	54.3–1,110,000
Precision (Total %)					
Low					6.8%
Cut-off	7.7%	7.7%	7.0%	8.9%	5.4%
High			11.0%	9.5%	4.9%
Very High	4.5%	4.5%	7.0%	7.3%	5.6%
Interference					
Hemoglobin	100 mg/dL	100 mg/dL	500 mg/dL	500 mg/dL	500 mg/dL
Bilirubin	10 mg/dL	10 mg/dL	18 mg/dL	18 mg/dL	18 mg/dL
Triglycerides	1,500 mg/dL	1,500 mg/dL	1,327 mg/dL	1,327 mg/dL	1,250 mg/dL
Rheumatoid Factor	60 IU/mL	60 IU/mL	1,400 IU/mL	1,400 IU/mL	448 IU/mL
Onboard Stability	2 Days	2 Days	4 Days	1 Week	8 Weeks
Calibration Curve	Curve Generated by Instrument– 3 Dilutions, 4 Replicates	Curve Generated by Instrument– 3 Dilutions, 4 Replicates	Curve Generated by Instrument– 5 Dilutions, 4 Replicates	Curve Generated by Instrument– 5 Dilutions, 4 Replicates	Pre-calibrated Master Curve Working Curve– 2 Calibrators, 3 Replicates

* HemosIL D-Dimer 500 is not available in all countries.

Table 7 matches each assay with the corresponding instrument, VTE claim, units, VTE cut-off value, liquid reagent formulation, upper and lower linearity limits, stability, and controls.

Table 7: The HemosIL D-Dimer Assay Panel and Corresponding Instruments

	HemosIL D-Dimer	HemosIL D-Dimer 500*	HemosIL D-Dimer HS	HemosIL D-Dimer HS 500	HemosIL AcuStar D-Dimer
Units	D-DU	FEU	D-DU	FEU	FEU
Cut-off	230 ng/mL	500 ng/mL	230 ng/mL	500 ng/mL	500 ng/mL
Regulatory Claims	VTE Exclusion	VTE Exclusion	VTE Exclusion	VTE Exclusion	Aid in the Diagnosis of VTE
ACL 7000	✓				
ACL ELITE/ELITE Pro/8/9/10K	✓	✓			
ACL Futura/ACL Advance	✓	✓			
ACL TOP Family	✓	✓	✓	✓	
ACL AcuStar					✓
Formulation	Lyophilized	Lyophilized	Lyophilized	Liquid	Liquid
Onboard Stability	2 Days	2 Days	4 Days	7 Days	8 Weeks
D-Dimer Controls	HemosIL D-Dimer Controls (liq** or lyo formats)			HemosIL D-Dimer HS 500 Controls (liq** or lyo formats)	HemosIL AcuStar D-Dimer Controls

* HemosIL D-Dimer 500 is not available in all countries.
** Not currently 510(k) cleared.

In evaluating these assays for VTE exclusion, the FDA applied a key criterion. For both DVT and PE, the lower limit of the 95% confidence interval (CI %) of the NPV was > 95% for the low and moderate PTP patient groups. The use of this strict criterion established the capability of D-Dimer assays to safely exclude the presence of VTE in the low and moderate PTP patient groups–the patients among whom D-Dimer assays are most likely to impact patient management. Yet regulatory requirements for VTE exclusion vary by country. The United Kingdom, for example, requires an NPV of > 98%, whereas the United States requires an NPV of > 98% with the lower limit of the one-sided CI of the NPV being > 95% with a sensitivity of > 97% (10). As such, the consideration of local regulatory requirements might be necessary in the selection of D-Dimer assays.

Nevertheless, the aforementioned criterion applied by the FDA for VTE exclusion is applicable for D-Dimer testing throughout the line of instruments offered by Instrumentation Laboratory.

HemosIL D-Dimer and HemosIL D-Dimer 500 Assays

Simple, Proven and Efficient

Fully automated • Results in < 7 minutes

HemosIL D-Dimer and HemosIL D-Dimer 500 assays are fully automated, latex-enhanced immunoassays that quantify D-Dimer concentrations in human citrated plasma. Additionally, HemosIL D-Dimer is FDA-cleared for VTE exclusion in outpatients suspected of DVT and PE when used in conjunction with a clinical PTP assessment.

HemosIL D-Dimer and HemosIL D-Dimer 500 assays differ only in the units by which they quantify D-Dimer concentrations:

- HemosIL D-Dimer reports in D-DUs;
- HemosIL D-Dimer 500 reports in FEUs;

thereby, harmonizing D-Dimer measurements across divergent units of measurement. Together, these two assays exemplify the HemosIL strategy of providing two solutions ideal for networked systems or laboratories that require uniform VTE exclusionary cut-off values across multiple models of IL coagulation systems. No local conversion of test results is necessary (and, also, not advised). The two assays share identical assay architecture, reagent formulation, analytical performance, and clinical performance. Whereas the HemosIL D-Dimer assay maintains a 230 ng/mL D-DU cut-off value, the HemosIL D-Dimer 500 assay has been restandardized to provide a cut-off value of 500 ng/mL FEU.

- HemosIL D-Dimer is available on the ACL TOP Family, ACL ELITE/ELITE PRO/8/9/10K, ACL 7000, and ACL Advance instruments.
- HemosIL D-Dimer 500 is available on the ACL TOP Family, ACL ELITE/ELITE PRO/8/9/10K, and ACL Futura/ACL Advance instruments.

Principles of Assay Measurement

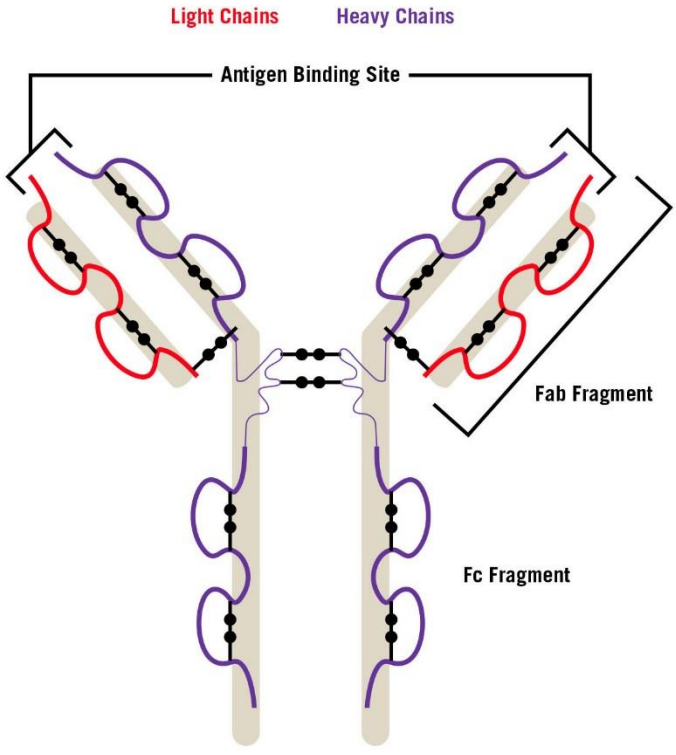
HemosIL D-Dimer and HemosIL D-Dimer 500 are latex-enhanced turbidimetric immunoassays.

The latex reagent of the assay is a suspension of polystyrene latex particles of uniform size coated with an mAb highly specific for the D-Dimer domain resident in FDPs or derivatives.

When the latex reagent, the reaction buffer, and plasma containing the D-Dimer antigen are combined into a mixture, the latex particles in suspension agglutinate. The degree of agglutination is directly proportional to the concentration of D-Dimer in the sample. The aggregates increase the turbidity of the suspension, and the change in turbidity is determined by measuring the decrease in the amount of light transmitted at 405 nm wavelength, thereby determining the concentration of D-Dimer antigens in the sample.

Figure 3 illustrates the monoclonal antibody highly specific for the D-Dimer domain that coats the polystyrene latex particles contained in the latex reagent of the HemosIL D-Dimer and HemosIL D-Dimer 500 assays.

Figure 3: Monoclonal Antibody Highly Specific for the D-Dimer Antigen



Analytical Performance

This section presents an overview of analytical performance in tables and brief descriptions of assay calibration, interference profiles, cross reactivity, and definitions for VTE exclusion cut-off values.

Overview of Analytical Performance

Tables 8 and 9 present and compare the analytical performance of the HemosIL D-Dimer and HemosIL D-Dimer 500 assays. While the two assays share identical assay architecture, reagent formulation, analytical performance, and clinical performance, they differ in that the HemosIL D-Dimer reports D-Dimer outcomes in D-DU (ng/mL) while the HemosIL D-Dimer 500 reports in FEU (ng/mL). Note that for each assay the values of some parameters differ when run on different instruments, while the values of several parameters appear to differ only because the two assays report in different units.

Table 8: Analytical Performance Comparison of HemosIL D-Dimer vs. HemosIL D-Dimer 500

	HemosIL D-Dimer	HemosIL D-Dimer 500
Cut-off for VTE (units)	230 ng/mL (D-DU)	500 ng/mL (FEU)
Technology	Latex – 405 nm	Latex – 405 nm
Linearity		
Without Rerun	200–1,050 ng/mL	435–2,280 ng/mL
Test Range with Rerun	200–5,250 ng/mL	435–11,400 ng/mL
Interfering Factors		
Hemoglobin	100 mg/dL	50 mg/dL
Bilirubin	10 mg/dL	5 mg/dL
Triglycerides	1,500 mg/dL	1,000 mg/dL
Rheumatoid Factor	May produce an overestimation	60 IU/mL
Time to Result	< 7 Minutes	< 7 Minutes

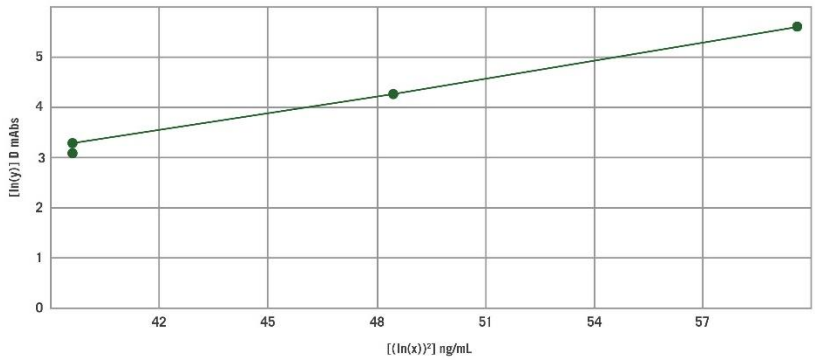
Table 9: Performance by Instrument: HemosIL D-Dimer and HemosIL D-Dimer 500

	HemosIL D-Dimer			HemosIL D-Dimer 500		
Instrument	ACL TOP Family	ACL Family	ACL Futura/ ACL Advance	ACL TOP Family	ACL ELITE/ PRO/ 8/9/10K	ACL Futura/ ACL Advance
Calibration Curve (Automatically generated by analyzers)	3 Dilutions 4 Replicates	3 Dilutions 4 Replicates	3 Dilutions 3 Replicates	3 Dilutions 4 Replicates	3 Dilutions 4 Replicates	3 Dilutions 3 Replicates
Precision (Total %)						
Plasma Pool	9.0%	11.7%	13.6%	9.0%	11.7%	13.6%
Low Control	7.7%	7.2%	4.9%	7.7%	7.2%	4.9%
High Control	4.5%	3.0%		4.5%	3.0%	
Interfering Factors						
Hemoglobin	100 mg/dL		50 mg/dL	100 mg/dL		50 mg/dL
Bilirubin	10 mg/dL		5 mg/dL	10 mg/dL		5 mg/dL
Triglycerides	1,500 mg/dL		1,000 mg/dL	1,500 mg/dL		1,000 mg/dL
Rheumatoid Factor	May produce an overestimation		60 IU/mL	May produce an overestimation		60 IU/mL
Onboard Stability	2 Days	ACL 7000 6 Hours ACL ELITE 1 Day	1 Week	2 Days	1 Day	1 Week

Calibration

Calibration of the HemosIL D-Dimer and HemosIL D-Dimer 500 assays is performed at three levels: 25%, 50%, and 100%. On the ACL TOP Family and ACL ELITE/ELITE PRO instruments, each calibration level is analyzed in quadruplicate with one result omitted, yielding three results. On the ACL Futura/ACL Advance instruments, the calibration is performed in triplicate. Figure 4 presents an example of a calibration curve on an ACL TOP Family instrument.

Figure 4: HemosIL D-Dimer/HemosIL D-Dimer 500 Calibration Curve on ACL TOP
An example of a calibration curve performed on ACL TOP Family instrument.



Interference

Both the HemosIL D-Dimer and HemosIL D-Dimer 500 assays possess favorable interference profiles. The interference profiles vary slightly by instrument.

In the ACL Family of instruments, these assays exhibit no significant interference from:

- hemoglobin < 50 mg/dL;
- bilirubin up to 5 mg/dL;
- lipids up to 1,000 mg/dL; and
- RF up to 60 IU/mL.

In the ACL TOP Family of instruments, these assays exhibit no significant interference from:

- hemoglobin up to 100 mg/dL;
- bilirubin up to 10 mg/dL; and
- triglycerides up to 1,500 mg/dL.

Nevertheless, sufficient concentrations of RF and/or HAMA in samples may induce elevated D-Dimer values, thereby yielding an increase in the number of false-positive outcomes.

Cross Reactivity and Specificity

The monoclonal antibody (MA-8D3) used in the D-Dimer latex reagent of HemosIL D-Dimer and HemosIL D-Dimer 500 has a high specificity for the D-Dimer antigen in the cross-linked FDPs generated by the fibrin proteolysis. Such cross-linked FDPs (also referred to as “XDPs”) manifest as oligomers of varying molecular weights.

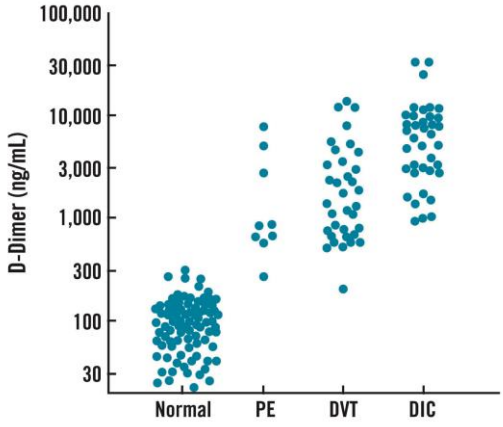
Conversely, this mAb exhibits low cross reactivity with FDPs lacking the D-Dimer motif, demonstrated experimentally by assaying plasma samples spiked with purified Fragments D and E in excess of 20 µg/mL.

Expected Values

As seen in Figure 5, samples from groups of normal subjects, patients suspected of VTE, and patients with DIC obtained at a hospital were tested with HemosIL D-Dimer assay. The cut-off values were > 230 ng/mL D-DU (> 500 ng/mL FEU) in patients with PE, DVT, and DIC; and < 230 ng/mL D-DU in normal subjects. Given these cut-off values, the distribution of outcomes are markedly different for normal subjects versus the patient groups with PE, DVT, and DIC.

Figure 5: D-Dimer Levels in VTE and DIC Patient Groups vs. Normal Subjects

In patients with PE, DVT and DIC, D-Dimer levels are > 230 ng/mL D-Dimer Units (D-DU).



Definition of Cut-off Level

In the HemosIL D-Dimer assay, the clinically validated cut-off value for VTE exclusion is 230 ng/mL D-DU. In the HemosIL D-Dimer 500 assay, the clinically validated cut-off value for VTE exclusion is 500 ng/mL FEU.

The values for the normal range were established by two studies. In the first study, 30 samples of healthy adult blood bank donors were run with several lots of HemosIL D-Dimer reagents and calibrator on instruments from the ACL Family and the ACL Futura/ACL Advance. A total of 30 samples were assayed in eight runs on each instrument, yielding a total of 240 results. Table 10 presents the outcomes.

Table 10: Normal Range Study of Healthy Blood Donors (n = 30)

HemosIL D-Dimer

System	n	Upper Normal Range
ACL TOP Family	231	232 ng/mL
ACL Family	240	255 ng/mL
ACL Futura/ACL Advance	240	278 ng/mL

HemosIL D-Dimer 500

System	n	Upper Normal Range
ACL TOP Family	231	504 ng/mL
ACL ELITE/ELITE PRO/8/9/10K	240	554 ng/mL
ACL Futura/ACL Advance	240	604 ng/mL

For these assays, these upper limits of the normal range are similar across multiple modes of IL coagulation instruments. Given these clinically and statistically validated standards, the local conversion of D-Dimer values from one unit to another is not required (or recommended).

Studies & Analysis

The analytical and clinical performance of HemosIL D-Dimer and HemosIL D-Dimer 500 assays have been evaluated in method comparison studies, a single-center outcome study, and a multi-center management study.

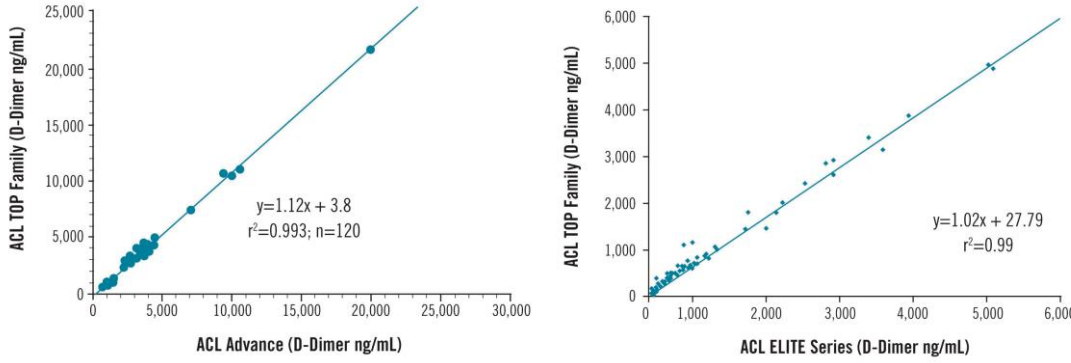
Method Comparison Studies

Two comparison studies of HemosIL D-Dimer have demonstrated excellent correlation and slope across methods.

A comparison study conducted with HemosIL D-Dimer compared assay outcomes on two pairs of IL coagulation instruments using samples obtained from outpatients suspected of VTE. First, the performance of HemosIL D-Dimer assay on the ACL TOP Family was compared with its performance on the ACL Advance. Second, the performance of HemosIL D-Dimer on the ACL TOP Family was compared with its performance on the ACL ELITE/ELITE PRO. As seen in Figure 6, these comparisons yielded excellent correlations and nearly identical slopes.

Figure 6: Method Comparison of HemosIL D-Dimer Across IL Instruments

A comparison study of HemosIL D-Dimer assay performance on the ACL TOP Family vs. the ACL Advance, and on the ACL TOP Family vs. ACL ELITE/ELITE PRO using samples from outpatients suspected of VTE.



A separate clinical study compared HemosIL D-Dimer to a commercially available D-Dimer ELISA. Samples from 67 patients were evaluated: 10 normal subjects, 38 patients with DIC, 9 patients with DVT, and 10 patients with various other disease states.

Elevated D-Dimer levels were observed in all the samples from clinically diagnosed DIC and DVT patients. Given these samples, the correlation coefficient (r) was 0.958 on the ACL TOP Family (n = 67) and 0.903 on the ACL Futura/ACL Advance (n = 58).

Clinical Performance Studies

Clinical performance studies of HemosIL D-Dimer and HemosIL D-Dimer 500 assays have consistently demonstrated superlative sensitivity and NPV in the exclusion of VTE across IL instruments. Specifically, the HemosIL D-Dimer and HemosIL D-Dimer 500 assays demonstrated:

- 100% sensitivity and 100% NPV on the ACL TOP Family;
- 100% sensitivity and 100% NPV in DVT patients on the ACL ELITE;
- 99.1% NPV for PE patients on the ACL ELITE; and
- 98% sensitivity for PE patients on the ACL ELITE.

Note: *Per FDA agreement, values derived on the ACL TOP Family are valid for the ACL Advance. The ACL TOP Family systems and ACL Futura/Advance are linear instruments; the ELITE/ELITE PRO/8/9/10K and ACL 6000/7000 are centrifugal instruments.*

Single-Center Outcome Study

A single-center outcome study utilizing HemosIL D-Dimer and HemosIL D-Dimer 500 assays was performed on approximately 300 frozen samples derived from patients suspected of PE or DVT. The frequency of VTE in this patient population was 26%. Positive samples were confirmed through standard imaging techniques. As presented in Table 11, both assays had 100% sensitivity and NPV on the ACL ELITE/ELITE PRO/8/9/10K and ACL TOP Family instruments (based on cut-off values of 230 ng/mL D-DU or 500 ng/mL FEU for HemosIL D-Dimer and HemosIL D-Dimer 500, respectively).

Table 11: HemosIL D-Dimer and HemosIL D-Dimer 500: Sensitivity, Specificity, and NPV in Single-Center Study

Instrument	n	Sensitivity (95% CI)	Specificity (95% CI)	Negative Predictive Value (95% CI)
ACL ELITE/ELITE PRO/8/9/10K	297	100% (95.2%–100%)	38% (31.4%–44.6%)	100% (95.7%–100%)
ACL TOP Family	294	100% (95.1%–100%)	36% (29.6%–42.6%)	100% (95.4%–100%)

Note: Values in parentheses are the 95% CI.

Multi-Center Management Study

A multi-center management study was performed at four hospitals on samples obtained from patients admitted consecutively to the emergency unit with suspected DVT or PE. In this study, assays were performed on two representative IL coagulation systems, as follows:

- an ACL TOP evaluated 632 samples
  - 302 patients suspected of DVT
  - 330 patients suspected of PE
- an ACL ELITE evaluated 629 samples
  - 298 patients suspected of DVT
  - 331 patients suspected of PE

Per standard management study procedures, all patients underwent PTP assessment using the Wells model and were classified as having a high, moderate, or low probability of DVT or PE. The following diagnostic algorithm was applied:

- patients with a negative D-Dimer test result and a low PTP score underwent no additional diagnostic testing and were evaluated at a 3-month follow-up.
- patients with a negative D-Dimer test result and a moderate PTP score either underwent immediate imaging evaluation or underwent follow-up at 3 months per physician decision.
- patients with a positive D-Dimer test result or a high PTP score underwent immediate imaging evaluations.

The findings on both instruments for DVT and PE are presented separately in Table 12 and Table 13, respectively. One patient with a moderate PTP score and a negative D-Dimer test result on the ACL ELITE was confirmed for PE through imaging techniques, although this sample was positive on the ACL TOP.

- On the ACL TOP, the overall prevalence of DVT was 19.5% (59/302).
- On the ACL ELITE, the overall prevalence of DVT was 20.5% (61/298).
- On the ACL TOP, the overall prevalence of PE was 15.2% (50/330).
- On the ACL ELITE, the overall prevalence of PE was 15.1% (50/331).

Table 12: HemosIL D-Dimer and HemosIL D-Dimer 500: Multi-Center Management Study on ACL TOP

DVT Performance			
	All Samples (n) (95% CI)	**High PTP (n) (95% CI)**	**Low + Moderate PTP (n) (95% CI)**
n	302	53	249
Sensitivity	100.0% (59/59) (93.9%–100.0%)	100.0% (27/27) (87.2%–100.0%)	100.0% (32/32) (89.1%–100.0%)
Specificity	41.6% (101/243) (35.3%–48.0%)	34.6% (9/26) (17.2%–55.7%)	42.4% (92/217) (35.7%–49.3%)
Negative Predictive Value	100.0% (101/101) (96.4%–100.0%)	100.0% (9/9) (66.4%–100.0%)	100.0% (92/92) (96.1%–100.0%)
Positive Predictive Value	29.4% (59/201) (23.2%–36.2%)	61.4% (27/44) (45.5%–75.6%)	20.4% (32/157) (14.4%–27.5%)
Prevalence	19.5% (59/302) (15.2%–24.5%)	50.9% (27/53) (36.8%–64.9%)	12.9% (32/249) (9.0%–17.7%)

PE Performance			
	All Samples (n) (95% CI)	**High PTP (n) (95% CI)**	**Low + Moderate PTP (n) (95% CI)**
n	330	24	306
Sensitivity	100.0% (50/50) (92.9%–100.0%)	100.0% (7/7) (59.0%–100.0%)	100.0% (43/43) (91.8%–100.0%)
Specificity	29.3% (82/280) (24.0%–35.0%)	17.6% (3/17) (3.8%–43.4%)	30.0% (79/263) (24.6%–36.0%)
Negative Predictive Value	100.0% (82/82) (95.6%–100.0%)	100.0% (3/3) (29.2%–100.0%)	100.0% (79/79) (95.4%–100.0%)
Positive Predictive Value	20.2% (50/248) (15.4%–25.7%)	33.3% (7/21) (14.6%–57.0%)	18.9% (43/227) (14.1%–24.7%)
Prevalence	15.2% (50/330) (11.5%–19.5%)	29.2% (7/24) (12.6%–51.1%)	14.1% (43/306) (10.4%–18.5%)

Table 13: HemosIL D-Dimer and HemosIL D-Dimer 500: Multi-Center Management Study on ACL ELITE

DVT Performance			
	All Samples (n) (95% CI)	**High PTP (n) (95% CI)**	**Low + Moderate PTP (n) (95% CI)**
n	298	54	244
Sensitivity	100.0% (61/61) (94.1%–100.0%)	100.0% (29/29) (88.1%–100.0%)	100.0% (32/32) (89.1%–100.0%)
Specificity	33.8% (80/237) (27.8%–40.2%)	24.0% (6/25) (9.4%–45.1%)	34.9% (74/212) (28.5%–41.7%)
Negative Predictive Value	100.0% (80/80) (95.5%–100.0%)	100.0% (6/6) (54.1%–100.0%)	100.0% (74/74) (95.1%–100.0%)
Positive Predictive Value	28.0% (61/218) (22.1%–34.4%)	60.4% (29/48) (45.3%–74.2%)	18.8% (32/170) (13.2%–25.5%)
Prevalence	20.5% (61/298) (16.0%–25.5%)	53.7% (29/54) (39.6%–67.4%)	13.1% (32/244) (9.1%–18.0%)

PE Performance			
	All Samples (n) (95% CI)	**High PTP (n) (95% CI)**	**Low + Moderate PTP (n) (95% CI)**
n	331	25	306
Sensitivity	98.0% (49/50) (89.4%–99.9%)	100.0% (8/8) (63.1%–100.0%)	97.6% (41/42) (87.4%–99.9%)
Specificity	41.3% (116/281) (35.5%–47.3%)	41.2% (7/17) (18.4%–67.1%)	41.3% (109/264) (35.3%–47.5%)
Negative Predictive Value	99.1% (116/117) (95.3%–100.0%)	100.0% (7/7) (59.0%–100.0%)	99.1% (109/110) (95.0%–100.0%)
Positive Predictive Value	22.9% (49/214) (17.4%–29.1%)	44.4% (8/18) (21.5%–69.2%)	20.9% (41/196) (15.4%–27.3%)
Prevalence	15.1% (50/331) (11.4%–19.4%)	32.0% (8/25) (14.9%–53.5%)	13.7% (42/306) (10.1%–18.1%)

Assay Kits

HemosIL D-Dimer and HemosIL D-Dimer 500 assay kits contain a latex reagent, the reaction buffer, and the calibrator:

- the latex reagent is a lyophilized suspension containing polystyrene latex particles coated with a mouse monoclonal antibody (MA-8D3) directed against the D-Dimer antigen, bovine serum albumin, buffer, stabilizers, and preservative.
- the reaction buffer is a phosphate buffer solution containing bovine serum albumin, stabilizers and preservative.

- the calibrator is a lyophilized solution of D-Dimer antigen partially purified from human fibrin digested with human plasmin; it contains bovine serum albumin, buffer, stabilizers and preservative.
- the controls kit is available separately. It contains two sets of control materials. The low-level control assesses precision and accuracy at borderline D-Dimer levels. The high-level control assesses precision and accuracy at abnormal D-Dimer levels.

Table 14: HemosIL D-Dimer and HemosIL D-Dimer 500 Assay Kit Components

Product	Part Number	Kit Configuration
D-Dimer	0020008500	4 x 3 mL Latex Reagent (lyo) 4 x 9 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer 500	0020301000	4 x 3 mL Latex Reagent (lyo) 4 x 9 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer Controls	0020008610	5 x 1 mL Low D-Dimer Control (lyo) 5 x 1 mL High D-Dimer Control (lyo)
D-Dimer Controls* (liquid)	0020013000	5 x 1 mL Level 1 D-Dimer Control (liq) 5 x 1 mL Level 2 D-Dimer Control (liq)

* Not currently 510(k) cleared.

HemosIL D-Dimer HS

Rapid and Cost-Efficient Solution for Enhanced Patient Care

- fully automated;
- results in < 5 minutes;
- minimal interference from RF and hemoglobin;
- high sensitivity, precision, and accuracy at low D-Dimer concentrations;
- enhanced linearity, granting precision and accuracy at extremely high D-Dimer concentrations; and
- 100% NPV for VTE exclusion (cut-off value of 230 ng/mL D-DU).

HemosIL D-Dimer HS assay is a fully automated, latex-enhanced immunoassay for the quantitative determination of D-Dimer concentrations in human citrated plasma. HemosIL D-Dimer HS assay is designed for use on the ACL TOP Family of instruments. HemosIL D-Dimer HS assay is FDA-cleared for VTE exclusion in outpatients suspected of DVT and PE when used in conjunction with a clinical PTP assessment.

As indicated by the designation “HS” (high specificity), the HemosIL D-Dimer HS assay was designed to reduce the inference from RF that typically causes false positive results. HemosIL D-Dimer HS also has an exceptionally high analytical sensitivity that confers greater accuracy and precision, and a wider linearity that allows the user to accurately measure D-Dimer concentrations in samples with high concentrations of D-Dimer antigen. The HemosIL D-Dimer HS has a 100% NPV for VTE exclusion at a cut-off value of 230 ng/mL (D-DU).

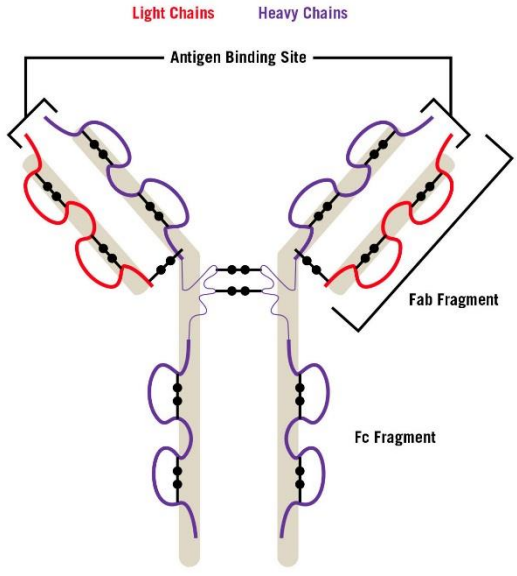
HemosIL D-Dimer HS assay provides a fast turnaround time and an extremely favorable interference profile, which greatly reduces the need for retesting, providing enhanced patient care in less time at lower cost.

Principles of Assay Measurement

The HemosIL D-Dimer HS assay is a latex-enhanced turbidimetric immunoassay.

The latex reagent of the assay is a suspension of polystyrene latex particles of uniform size coated with the F(ab')2 fragment of an mAb (MA-8D3) that is highly specific for the cross-linked D-Dimer domain in FDPs or derivatives (Figure 7). The use of the F(ab')2 fragment produces greater specificity in the detection of the D-Dimer antigen while avoiding interference from endogenous factors such as RF. HemosIL D-Dimer HS was tested for RF interference up to levels of 1,400 IU/mL and found not to demonstrate any RF interference. The assay also contains a blocking agent to HAMA.

Figure 7: The F(ab')2 Fragment of the MA-8D3 mAb
The Fc portion of the MA-8D3 antibody resides above the dotted line. Use of Fc portion alone eliminates RF interference up to concentrations of 1,400 IU/dL.



When the latex reagent, the reaction buffer, and plasma containing the D-Dimer antigen are mixed, the latex particles in the suspension agglutinate. The degree of agglutination is directly proportional to the concentration of D-Dimer in the sample. The aggregates increase the turbidity of the suspension. The degree of agglutination is determined by measuring the change in the turbidity of the suspension, i.e., by measuring the decrease of the light transmitted at 671 nm (the optimal wavelength for the particle size employed in this assay).

At this wavelength, interference from hemoglobin is minimal while the analytical sensitivity of the assay is increased, thereby providing better precision and accuracy for low concentrations of D-Dimer than HemosIL D-Dimer or HemosIL D-Dimer 500 assays. The extended linearity of the assay allows the measurement of very high concentrations of D-Dimer (up to 69,000 ng/mL with automatic rerun; such concentrations are common in clinical conditions such as DIC).

Analytical Performance

This section presents an overview of analytical performance, brief descriptions of assay calibration, the interference profile, expected values, and the definition of the VTE exclusion cut-off value.

Overview of Analytical Performance

Table 15 summarizes the analytical performance of the HemosIL D-Dimer HS assay on the ACL TOP Family of instruments.

Table 15: Analytical Performance: HemosIL D-Dimer HS on the ACL TOP Family

HemosIL D-Dimer HS	
Units	D-DU (ng/mL)
Cut-off for VTE	230 ng/mL D-DU
Technology	Latex – 405 nm
Detection Limit	21 ng/mL
Calibration Curve	5 Dilutions, 4 Replicates
Linearity	
Without Rerun	150 – 3,680 ng/mL
With Rerun	150 – 69,000 ng/mL
Precision (Total %)	
Plasma Pool	11.0%
Low Control	7.0%
High Control	7.0%
Interfering Factors	
Hemoglobin	500 mg/dL
Bilirubin	18 mg/dL
Triglycerides	1,327 mg/dL
Rheumatoid Factor	1,400 IU/mL
FDP	10 µg/mL
Onboard Stability	4 Days
Time to Result	< 5 Minutes

Calibration

The calibration curve is a spline curve constructed with four replicates of five D-Dimer HS levels automatically prepared by the ACL TOP Family analyzer. A cubic spline has no specification for r2; therefore, the user must verify the validity of the calibration curve by performing a quality control run. HemosIL D-Dimer Controls are recommended.

Interference Studies

The following substances induce no significant interference up to the stated concentrations:

- hemoglobin up to 500 mg/dL;
- bilirubin up to 18 mg/dL;
- triglycerides up to 1,327 mg/dL;
- RF up to 1,400 IU/mL; and
- FDPs up to 10 µg/mL.

HemosIL D-Dimer HS contains blocking agents against HAMA in the reaction buffer to mitigate interference of human anti-mouse antibodies.

Expected Values

In a normal range study performed with the HemosIL D-Dimer HS involving 234 samples obtained from individual blood blank donors, the upper limit of the normal range was established at 243 ng/mL D-DU for the population used in the study.

Definition of Cut-off Level

HemosIL D-Dimer HS has a clinically validated cut-off of 230 ng/mL D-DU for VTE exclusion.

Studies & Analysis

The analytical and clinical performance of HemosIL D-Dimer HS has been evaluated in method comparison studies, a single-center outcome study, and a multi-center management study.

Method Comparison Studies

The data obtained with HemosIL D-Dimer HS assays conducted on the ACL TOP in the aforementioned internal study (n = 100) were compared with those obtained with VIDAS D-Dimer (bioMerieux) tested in parallel (contemporary), as well as with results from historical VIDAS D-Dimer testing. At the cut-off value of 230 ng/mL D-DU, HemosIL D-Dimer HS demonstrated 100% NPV and 100% sensitivity – findings consistent with those of VIDAS D-Dimer. These data are presented in Table 16. These data show an excellent correlation with VIDAS D-Dimer, formerly considered the “gold standard” reference for VTE exclusion.

Table 16: HemosIL D-Dimer HS vs. Current and Historical VIDAS Findings

	HemosIL D-Dimer HS on ACL TOP	VIDAS Contemporary	VIDAS Historical
Number of Samples	100	100	100
Cut-off	230 ng/mL (D-DU)	500 ng/mL (FEU)	500 ng/mL (FEU)
Sensitivity (%) (95% CI)	100 (89.1–100.0)	100 (89.1–100.0)	100 (89.1–100.0)
Specificity (%) (95% CI)	44.1 (32.1–56.7)	37 (25.4–49.3)	38 (26.7–50.8)
NPV (%) (95% CI)	100 (88.4–100.0)	100% (86.3–100.0)	100 (86.8–100.0)

On the ACL TOP instrument, the findings of HemosIL D-Dimer HS were also compared with those of HemosIL D-Dimer in an analytical comparison study of 229 samples. This comparison revealed an excellent correlation (r = 0.973).

In a separate comparison study, the outcomes of 87 samples assayed by HemosIL D-Dimer HS on the ACL TOP were compared with those of the VIDAS D-Dimer Exclusion Assay. The comparison demonstrated a similar degree of correlation (r = 0.946).

Clinical Performance Studies

Clinical performance studies have demonstrated that HemosIL D-Dimer HS assay has 100% NPV and 100% sensitivity for VTE exclusion in patients suspected of DVT or PE.

Single-Center Outcome Study

In an external outcome study, 300 frozen samples from patients admitted consecutively to an emergency unit with suspected PE or DVT were evaluated by HemosIL D-Dimer HS on the ACL TOP and the VIDAS

D-Dimer Exclusion Assay. The findings for cut-off, sensitivity, specificity, NPV, and exclusion rate are presented in Table 17.

Of the 300 samples, 78 were confirmed as VTE positive (47 PE; 31 DVT) by standard objective tests and 222 were confirmed as negative by the HemosIL D-Dimer HS. The frequency of VTE in this population was 26%. Identical outcomes were provided by the VIDAS D-Dimer Exclusion Assay.

At a cut-off of 230 ng/mL D-DU, the HemosIL D-Dimer HS demonstrated 100% sensitivity, 100% NPV, no false-negative results, and consistency with the VIDAS D-Dimer Assay.

Table 17: HemosIL D-Dimer HS vs. VIDAS D-Dimer Assay on Frozen Samples (n = 300)

	HemosIL D-Dimer HS ACL TOP	VIDAS D-Dimer
Cut-off	230 ng/mL (D-DU)	500 ng/mL (FEU)
Sensitivity (%) (95% CI)	100 (95.4–100)	100 (95.4– 00)
Specificity (%) (95% CI)	46.8 (40.1–53.6)	34.7 (28.4–41.3)
NPV (%) (95% CI)	100 (96.5–100)	100 (95.3–100.0)
Exclusion Rate (%) (95% CI)	34.7 (29.3–40.1)	25.7 (20.7–30.6)

Multi-Center Management Study

The performance of HemosIL D-Dimer HS was evaluated in a multi-center management study performed at four hospitals. This study evaluated samples from 668 outpatients admitted consecutively to the emergency unit with suspected DVT (n = 307) or PE (n = 361). The findings of this study are presented in Table 18.

Per standard management study procedures, all patients underwent PTP assessment using the Wells model and were classified as having a high, moderate, or low probability of DVT or PE. The following diagnostic algorithm was applied:

- patients with a negative D-Dimer test result and a low PTP score underwent no additional diagnostic testing and were evaluated at a 3-month follow-up.
- patients with a negative D-Dimer test result and a moderate PTP score either underwent immediate imaging evaluation or underwent follow-up at 3 months per physician decision.
- patients with a positive D-Dimer test result or a high PTP score underwent immediate imaging evaluations.

Among this patient population, the overall DVT prevalence was 20.2% (62/307) and the overall PE prevalence was 16.1% (58/361). HemosIL D-Dimer HS on the ACL TOP demonstrated 100% sensitivity and 100% NPV at a cut-off of 230 ng/mL D-DU. These results demonstrate HemosIL D-Dimer HS can be used in conjunction with a PTP score to safely exclude VTE in outpatients suspected of DVT and PE.

Table 18: HemosIL D-Dimer HS Performance in Management Study (n = 668)

DVT Performance			
	All Samples	**High PTP**	**Low + Moderate PTP**
n	307	54	253
Sensitivity (%) (95% CI)	100.0 (62/62) (94.2–100.0)	100.0 (28/28) (87.7–100.0)	100.0 (34/34) (89.7–100.0)
Specificity (%) (95% CI)	38.4 (94/245) (32.2–44.8)	34.6 (9/26) (17.2–55.7)	38.8 (85/219) (32.3–45.6)
NPV (%) (95% CI)	100.0 (94/94) (96.2–100.0)	100.0 (9/9) (66.4–100.0)	100.0 (85/85) (95.8–100.0)

PE Performance			
	All Samples	**High PTP**	**Low + Moderate PTP**
n	361	28	333
Sensitivity (%) (95% CI)	100.0 (58/58) (93.8–100.0)	100.0 (10/10) (69.2–100.0)	100.0 (48/48) (92.6–100.0)
Specificity (%) (95% CI)	35.6 (108/303) (30.2–41.3)	16.7 (3/18) (3.6–41.4)	36.8 (105/285) (31.2–42.7)
NPV (%) (95% CI)	100.0 (108/108) (96.6–100.0)	100.0 (3/3) (29.2–100.0)	100.0 (105/105) (96.5–100.0)

Assay Kit

HemosIL D-Dimer HS contains a latex reagent, reaction buffer, and calibrator. The kit includes approximately 100 tests. A separate set of controls is available for use with the kit.

- The latex reagent is a lyophilized suspension of polystyrene latex particles coated with the F(ab')2 fragment of a mouse monoclonal antibody (MA-8D3) directed against D-Dimer containing bovine serum albumin, buffer, stabilizers and preservative.
- The reaction buffer is a phosphate buffer containing bovine serum albumin, stabilizers and preservative.
- The D-Dimer calibrator is a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers and preservative.
- The controls kit is available separately. It contains two sets of control materials. The low-level control assesses precision and accuracy at borderline D-Dimer levels. The high-level control assesses precision and accuracy at abnormal D-Dimer levels.

Table 19: HemosIL D-Dimer HS Assay Kit Components

Product	Part Number	Kit Configuration
D-Dimer HS	0020007700	3 x 2 mL Latex Reagent (lyo) 3 x 8 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer Controls	0020008610	5 x 1 mL Low D-Dimer Control (lyo) 5 x 1 mL High D-Dimer Control (lyo)

HemosIL D-Dimer HS 500

Simple, Proven and Efficient VTE Exclusion

- liquid, ready-to-use, fully automated assay;
- 7-day onboard stability; and
- excellent analytical sensitivity with superior accuracy and precision.

HemosIL D-Dimer HS 500 is a liquid, ready-to-use, fully automated, latex-enhanced immunoassay for the quantitative determination of D-Dimer concentrations in human citrated plasma. HemosIL D-Dimer HS 500 has been clinically validated and FDA-cleared for VTE exclusion using a cut-off value of 500 ng/mL FEU when used in conjunction with a clinical PTP assessment.

The assay provides enhanced analytical performance with 7-day onboard stability. The excellent precision and accuracy is assessed using dedicated HemosIL D-Dimer HS 500 controls. The HemosIL D-Dimer HS 500 assay is designed for use on the ACL TOP Family of instruments.

Principles of Assay Measurement

The HemosIL D-Dimer HS 500 assay is a latex-enhanced turbidimetric immunoassay.

The latex reagent of the assay is a suspension of polystyrene latex particles of uniform size coated with the F(ab')2 fragment of an mAb (MA-8D3) that is highly specific for the cross-linked D-Dimer domain in FDPs or derivatives. The use of the F(ab')2 fragment promotes greater specificity in the detection of the D-Dimer antigen while avoiding interference from endogenous factors such as RF. The HemosIL D-Dimer HS 500 was tested for RF interference up to levels of 1,400 IU/mL and found not to demonstrate any RF interference. The assay also contains a blocking agent to HAMA.

When the latex reagent, the reaction buffer, and plasma containing the D-Dimer antigen are mixed, the latex particles in the suspension agglutinate. The degree of agglutination is directly proportional to the concentration of D-Dimer in the sample. The aggregates increase the turbidity of the suspension. The degree of agglutination is determined by measuring the change in the turbidity of the suspension, i.e., by measuring the decrease of the light transmitted at 671 nm (the optimal wavelength for the particle size employed in this assay).

Analytical Performance

This section presents an overview of analytical performance in Table 20 and brief descriptions of assay calibration, the interference profile, and definition of the VTE exclusion cut-off value.

Overview of Analytical Performance

Table 20 summarizes the analytical performance of HemosIL D-Dimer HS 500 on the ACL TOP Family of instruments.

Table 20: Analytical Performance: HemosIL D-Dimer HS 500 on the ACL TOP Family

HemosIL D-Dimer HS 500 on ACL TOP	
Units	FEU
Cut-off for VTE	500 ng/mL
Technology	Latex – 671 nm
Detection Limit	203 ng/mL
Calibration Curve	5 Dilutions, 4 Replicates
Linearity	
Without Rerun	215 – 7,650 ng/mL
With Rerun	215 – 128,000 ng/mL
Precision (Total %)	
Plasma Pool	9.5
Low Control	8.9
High Control	7.3
Interfering Factors	
Hemoglobin	500 mg/dL
Bilirubin	18 mg/dL
Triglycerides	1,327 mg/dL
Rheumatoid Factor	1,400 IU/mL
FDP	10 µg/mL
Onboard Stability	7 Days
Time to Result	< 5 Minutes

Calibration

The calibration curve is constructed with five HemosIL D-Dimer HS 500 levels automatically prepared by the ACL TOP Family analyzer. Calibration levels of 20%, 6.25%, and 3.12% use the primary algorithm, and the undiluted calibrator at 100% uses the secondary algorithm. The 40% level may use either the primary or secondary algorithm.

The parameter table for HemosIL D-Dimer HS 500 includes the enabling of the measured result on the X-axis of the calibration curve, a procedure that precludes specific monotonic error. The regression type of the calibration curve on the ACL TOP Family is a cubic spline constructed from a set of piecewise, third-order polynomials passed through a set of control points (i.e., a smoothing function that connects the points of an equation). A cubic spline has no specification for r2, therefore the user must verify the validity of the calibration curve by performing a quality control run. HemosIL D-Dimer HS 500 Controls are recommended.

Interference Studies

The following substances induce no significant interference up to the stated concentrations:

- hemoglobin up to 500 mg/dL;
- bilirubin up to 18 mg/dL;
- triglycerides up to 1,327 mg/dL;
- RF up to 1,400 IU/mL; and
- FDP up to 10 µg/mL.

HemosIL D-Dimer HS 500 assay contains blocking agents against HAMA in the reaction buffer to mitigate interference of human anti-mouse antibodies.

Expected Values

A normal range study was performed using 138 samples from individual blood bank donors. As evaluated on an ACL TOP Family instrument, the upper limit of normal range for the HemosIL D-Dimer HS 500 assay was 500 ng/mL FEU.

Definition of Cut-off Level

The HemosIL D-Dimer HS 500 assay has a clinically validated cut-off value of 500 ng/mL FEU for VTE exclusion. As an International Standard for D-Dimer antigen detection is not currently available, the IL House Standard has been assigned according to the harmonization criteria proposed by W. Nieuwenhuizen and P. Meijer (80), and results for HemosIL D-Dimer HS 500 are expressed in ng/mL FEU.

The use of FEUs by HemosIL D-Dimer HS 500 assay aligns the device with other assays in the market that uniformly present units in FEU and utilize a 500 ng/mL (FEU) cut-off for VTE exclusion.

In reporting results, the analyzers belonging to the ACL TOP Family display “ng/mL” when reporting HemosIL D-Dimer HS 500 outcomes; however, the acronym “FEU” does not display in the software as part of the units. In a similar fashion, outcomes for the HemosIL D-Dimer and HemosIL D-Dimer HS are also reported “ng/mL,” but these units represent (D-DU). Again, the acronym “D-DU” does not display in the software as part of the units.

Studies & Analysis

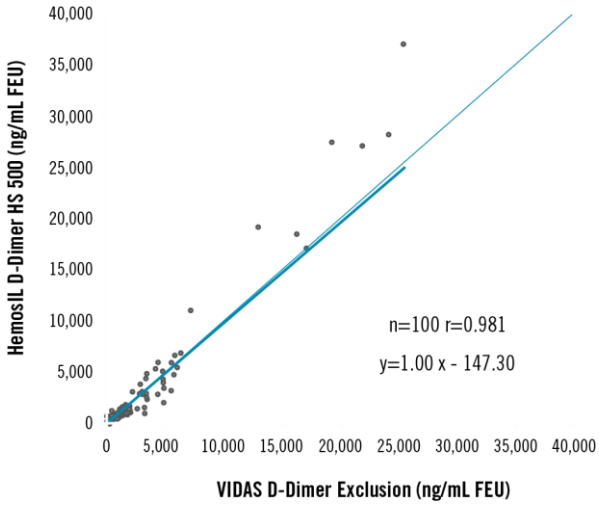
The analytical and clinical performance of HemosIL D-Dimer HS 500 has been evaluated in a method comparison study, a single-center outcome study, and a multi-center management study.

Method Comparison Study

Although no international standard exists for D-Dimer assays, new assays on the market are most often compared with the VIDAS D-Dimer method, which is considered by some regulatory agencies to be the “gold standard” reference.

A total of 100 samples from outpatients who presented to the emergency department with suspected VTE were analyzed with HemosIL D-Dimer HS 500 and VIDAS D-Dimer. Of these 100 samples, 28 samples were confirmed positive for PE, while the remaining 72 were confirmed as negative. When compared with the VIDAS D-Dimer, HemosIL D-Dimer HS 500 demonstrated an excellent Passing and Bablok agreement: a slope of 1.00 and Pearson correlation coefficient of r = 0.981. Figure 8 depicts the correlation of HemosIL D-Dimer HS 500 assay with VIDAS D-Dimer.

Figure 8: Correlation: HemosIL D-Dimer HS 500 vs. VIDAS D-Dimer Exclusion Assay



Clinical Performance Studies

Single-Center Outcome Study

An outcome study was performed at the University of Leuven on 295 frozen samples from patients admitted consecutively to an emergency unit with suspected PE or DVT. The frequency of VTE among this population was 25.4%.

Of the 295 samples, 75 were confirmed as VTE positive by standard imaging techniques (47 PE; 28 DVT), and the remaining 220 were confirmed as negative. HemosIL D-Dimer HS 500 demonstrated 100% sensitivity and 100% NPV for VTE exclusion. The results of this study, determined with a cut-off level of 500 ng/mL FEU, are presented in Table 21.

Table 21: HemosIL D-Dimer HS 500 in Single-Center Study (n = 295)

Instrument	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)
HemosIL D-Dimer HS 500 on ACL TOP Family	295	100.0 (95.2–100.0)	42.3 (35.7–49.1)	100.0 (96.1–100.0)

Multi-Center Management Study

A multi-center management study was performed at four hospitals on 747 frozen samples from outpatients with suspected PE (n = 346) or DVT (n = 401).

Per standard management study procedures, all patients underwent PTP assessment using the Wells model and were classified as having a high, moderate, or low probability of DVT or PE. The following diagnostic algorithm was applied:

- patients with a negative D-Dimer test result and a low PTP score underwent no additional diagnostic testing and were evaluated at a 3-month follow-up.
- patients with a negative D-Dimer test result and a moderate PTP score either underwent immediate imaging evaluation or underwent follow-up at 3 months per physician decision.
- patients with a positive D-Dimer test result or a high PTP score underwent immediate imaging evaluations.

A total of 90 samples were confirmed positive for DVT (22.4%) and 52 samples were confirmed as positive for PE (15.0%) by standard objective tests. The remaining 605 samples were confirmed as negative. At 3-month follow-up, none of the patients with negative D-Dimer testing results had developed DVT or PE.

In this population, HemosIL D-Dimer HS 500 assay demonstrated 100% NPV and 100% sensitivity for DVT and PE with a greater than 95% lower limit of the 95% CI for patients in the Low + Moderate PTP group. In summary, HemosIL D-Dimer HS 500, in conjunction with PTP assessment, was clinically validated to exclude VTE using a cut-off value of 500 ng/mL FEU. The outcomes of this study are presented in Table 22.

Table 22: HemosIL D-Dimer HS 500 in Multi-Center Management Study (n = 747)

DVT Performance			
	All Samples	**High PTP**	**Low + Moderate PTP**
n	401	79	322
Sensitivity (%) (95% CI)	100.0 (90/90) (96.0–100.0)	100.0 (45/45) (92.1–100.0)	100.0 (45/45) (92.1–100.0)
Specificity (%) (95% CI)	42.1 (131/311) (36.6–47.8)	32.4 (11/34) (17.4–50.5)	43.3 (120/277) (37.4–49.4)
NPV (%) (95% CI)	100.0 (131/131) (97.2–100.0)	100.0 (11/11) (71.5–100.0)	100.0 (120/120) (97.0–100.0)

PE Performance			
	All Samples	**High PTP**	**Low + Moderate PTP**
n	346	24	322
Sensitivity (%) (95% CI)	100.0 (52/52) (93.2–100.0)	100.0 (9/9) (66.4–100.0)	100.0 (43/43) (91.8–100.0)
Specificity (%) (95% CI)	48.3 (142/294) (42.5–54.2)	33.3 (5/15) (11.8–61.6)	49.1 (137/279) (43.1–55.1)
NPV (%) (95% CI)	100.0 (142/142) (97.4–100.0)	100.0 (5/5) (47.8–100.0)	100.0 (137/137) (97.3–100.0)

Assay Kit

HemosIL D-Dimer HS 500 contains a latex reagent, reaction buffer, and calibrator. The kit includes approximately 100 tests. A separate set of controls is available for use with the kit.

- The latex reagent is a suspension of polystyrene latex particles coated with the F(ab')2 fragment of a mouse monoclonal antibody (MA-8D3) directed against D-Dimer containing bovine serum albumin, buffer, stabilizers and preservative.
- The reaction buffer is a hepes buffer containing bovine serum albumin, stabilizers and preservative.
- The D-Dimer calibrator is a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers and preservative.
- The controls kit is available separately. It contains two sets of control materials. The low-level control assesses precision and accuracy at borderline D-Dimer levels. The high-level control assesses precision and accuracy at abnormal D-Dimer levels.

Table 23: HemosIL D-Dimer HS 500 Assay Kit Components

Product	Part Number	Kit Configuration
D-Dimer HS 500	0020500100	3 x 4 mL Latex Reagent (liq) 3 x 6 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer HS 500 Controls	0020500200	5 x 1 mL Low D-Dimer Control (lyo) 5 x 1 mL High D-Dimer Control (lyo)
D-Dimer Controls* (liquid)	0020013000	5 x 1 mL Level 1 D-Dimer HS Control (liq) 5 x 1 mL Level 2 D-Dimer HS Control (liq)

* Not currently 510(k) cleared.

HemosIL AcuStar D-Dimer

Simple, Proven and Efficient for Aid in the Diagnosis of VTE

- unmatched simplicity;
- ready-to-use, cartridge-based, pre-calibrated assays;
- extended, 8-week onboard stability;
- optimal for analysis 24 hours/day, 7 days/week; and
- FDA-cleared as an aid in the diagnosis of VTE.

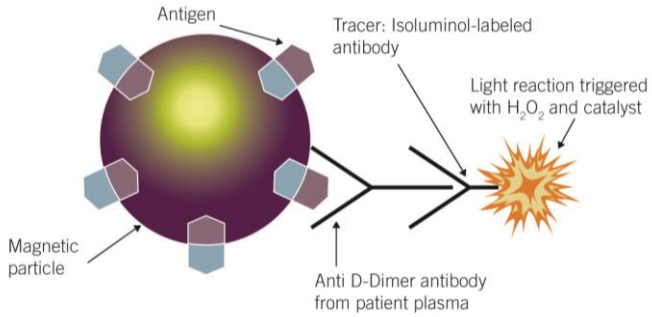
HemosIL AcuStar D-Dimer assay is the first fully automated, chemiluminescent D-Dimer assay for the quantitative determination of D-Dimer in human citrated plasma. HemosIL AcuStar D-Dimer assay has been FDA-cleared as an aid in the diagnosis of VTE and clinically validated at a cut-off of 500 ng/mL FEU. The wide linearity of HemosIL AcuStar D-Dimer allows the assay of samples containing either very low or very high D-Dimer concentrations without the need for reruns. The design of the ACL AcuStar cartridge allows extended stability of the reagent after opening. The onboard stability of the cartridge on the ACL AcuStar is 2 months. HemosIL AcuStar D-Dimer assay was designed for the ACL AcuStar™ instrument.

Principles of Assay Measurement

HemosIL AcuStar D-Dimer assay is a two-step immunoassay that quantifies D-Dimer in human citrated plasma using magnetic particles as solid phase and a chemiluminescent detection system.

In the first step, the sample, magnetic particles coated with the anti-D-Dimer antibody, and the assay buffer are combined. In this suspension, fibrin soluble derivatives containing the D-Dimer domain bind to the anti-D-Dimer antibody coating the magnetic particles. After magnetic separation and washing, incubated in the second step, an anti-XDP antibody labeled with isoluminol is added. After a second magnetic separation and washing, two triggers are added. The resultant chemiluminescent reaction is measured as relative light units (RLUs) by the ACL AcuStar detection system. This process is illustrated in Figure 9. The RLUs are directly proportional to the D-Dimer concentration in the sample. Due to the washing steps involved in the assay, optical interferences have virtually no effect on HemosIL AcuStar D-Dimer outcomes.

Figure 9: AcuStar D-Dimer Particle Diagram

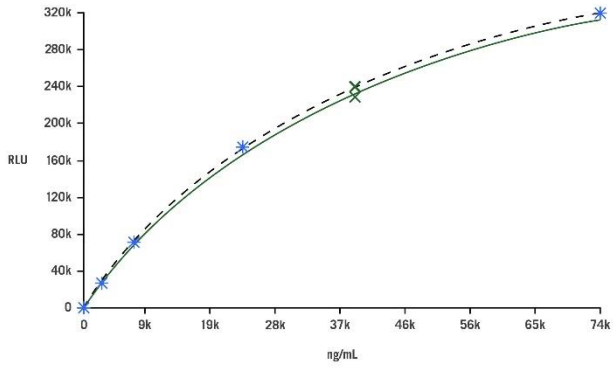


Calibration Curve

The HemosIL AcuStar D-Dimer calibration curve on the ACL AcuStar instrument, as shown in Figure 10, utilizes a 4-Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined, lot dependent, and uploaded into the instrument via the cartridge barcode. The ACL AcuStar instrument automatically prepares eight D-Dimer levels (0; 75; 230; 500; 2,300; 7,400; 23,000; and 74,000 ng/mL). The predefined Master Curve is transformed into a new, instrument-specific Working Curve upon measurement of two calibrators. Calibration levels for the Working Curve are 500 ng/mL and 37,000 ng/mL. The concentration values of the calibrators are uploaded via the calibrator tube barcode.

The user must verify the validity of the calibration curve on the ACL AcuStar instrument by running quality control. HemosIL AcuStar D-Dimer Controls are recommended.

Figure 10: HemosIL AcuStar D-Dimer Calibration Curve on the ACL AcuStar



Analytical Performance

This section presents an overview of analytical performance in Table 24 and brief descriptions of assay calibration, the interference profile, expected values, and the definition of the VTE exclusion cut-off value.

Overview of Analytical Performance

Table 24 summarizes the analytical performance of HemosIL AcuStar D-Dimer on the ACL AcuStar System.

Table 24: Analytical Performance: HemosIL AcuStar D-Dimer on the ACL AcuStar

HemosIL AcuStar D-Dimer on the ACL AcuStar System	
Units	FEU
Cut-off for VTE	500 ng/mL
Technology	Chemiluminescence
Detection Limit	6.51 ng/mL
Calibration Curve **(Automatically generated by analyzers)**	0, 75, 230, 500, 2300, 7400, 23000, 74000 ng/mL
Linearity Without rerun With rerun	 54.3 – 74,000 ng/mL 54.3 – 1,100,000 ng/mL
Precision (Total %) Plasma Pool Low Control High Control Very High Control	 5.4 6.8 4.9 5.6
Interfering Factors Hemoglobin Bilirubin Triglycerides Rheumatoid Factor	 500 mg/dL 18 mg/dL 1,250 mg/dL 448 IU/mL
Onboard Stability	2 months
Time to Result	26 minutes for 1st result, 1 minute thereafter

Interference Studies

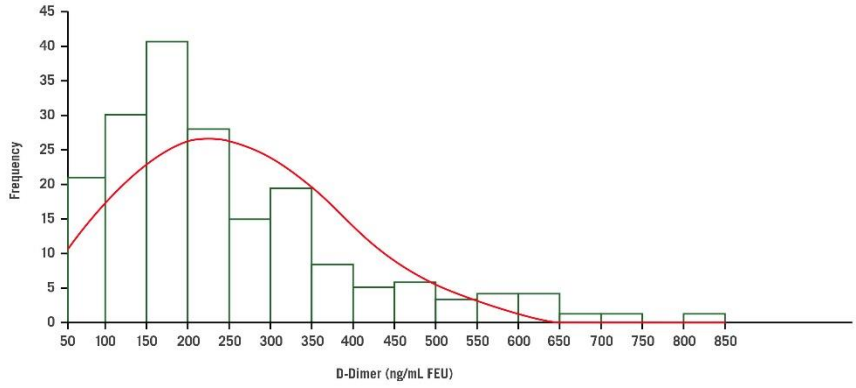
The following substances induce no significant interference up to the stated concentrations:

- hemoglobin up to 500 mg/dL;
- bilirubin up to 18 mg/dL;
- triglycerides up to 1,250 mg/dL;
- RF up to 450 IU/mL; and
- HAMA up to 1 µg/mL.

Expected Values

A normal range study was completed using 189 citrated plasma samples obtained from apparently healthy blood bank donors. The D-Dimer concentration distribution in the normal population did not follow a normal distribution, therefore nonparametric statistics were applied. The upper limit of normal range was established at 630 ng/mL FEU based on the 95% reference interval limit of the distribution. This data supports an upper limit of normal range for HemosIL AcuStar D-Dimer on the ACL AcuStar as 630 ng/mL FEU, as depicted in Figure 11.

Figure 11: Upper Limit of Normal Range for HemosIL AcuStar D-Dimer Assay



Definition of Cut-off Level

Results for the HemosIL AcuStar D-Dimer assay are expressed in ng/mL FEU. The cut-off is 500 ng/mL (FEU) as an aid in the diagnosis of VTE. The HemosIL AcuStar D-Dimer assay has been standardized to both the VIDAS D-Dimer Exclusion and HemosIL D-Dimer HS 500 assays.

The cut-off for the HemosIL AcuStar D-Dimer assay was clinically validated in an external, single-center management study involving patients suspected of VTE.

Studies & Analysis

The analytical and clinical performance of the HemosIL AcuStar D-Dimer assay has been evaluated in method comparison studies, a management study, and a clinical study.

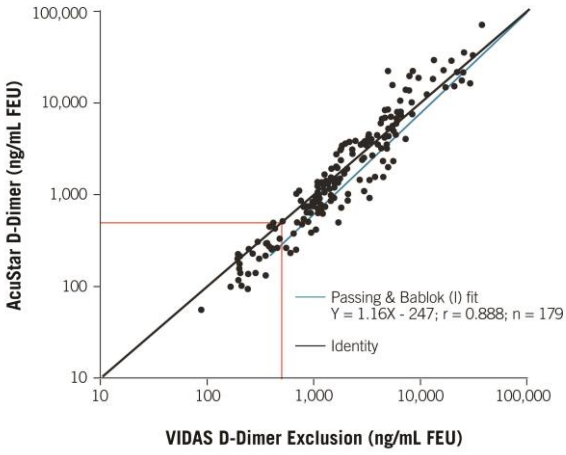
Method Comparison Studies

A multitude of method comparisons were performed wherein the HemosIL AcuStar D-Dimer assay was regressed against the HemosIL D-Dimer HS assay on the ACL TOP and the VIDAS D-Dimer Exclusion Assay.

HemosIL AcuStar D-Dimer vs. HemosIL D-Dimer HS: A method comparison study compared the results of the HemosIL AcuStar D-Dimer assay with those of the HemosIL D-Dimer HS assay on an ACL TOP Family system involving patients with VTE and other conditions: 5 patients with DVT, 7 patients with PE, 2 patients with DVT and PE, and 88 patients with other various disease states (n = 102). The correlation (r) with the HemosIL AcuStar D-Dimer assay on the ACL AcuStar was 0.895.

HemosIL AcuStar D-Dimer vs. VIDAS D-Dimer Exclusion: An internal method comparison study compared the assay results of the HemosIL AcuStar D-Dimer versus VIDAS D-Dimer Exclusion. The HemosIL AcuStar D-Dimer exhibited good correlation along the measuring range (slope: 1.16, intercept: -247, r: 0.888), as depicted in Figure 12. Note: The linearity of the VIDAS method is limited to a maximum of 10,000 ng/mL FEU.

Figure 12: Correlation of HemosIL AcuStar D-Dimer vs. VIDAS D-Dimer Exclusion



HemosIL AcuStar D-Dimer vs. ELISA: An additional clinical study compared HemosIL AcuStar D-Dimer assay with a commercially available ELISA assay that had been cleared for VTE exclusion by the FDA. Samples from 100 patients were evaluated: 76 normal subjects, 9 patients with DVT, and 15 patients with PE. The correlation (r) of this assay with the HemosIL AcuStar D-Dimer assay on the ACL AcuStar was 0.986.

Management Study

A single-center management study was performed to support the intended use claims of HemosIL AcuStar D-Dimer as an aid in the diagnosis of VTE. In this management study, a total of 344 frozen samples from patients with suspected PE or DVT were evaluated.

Of the 344 samples, 97 were confirmed positive for VTE (64 PE; 33 DVT) by standard objective tests and the remaining 247 were confirmed as negative. Table 25 summarizes the assay findings as determined by a cut-off value of 500 ng/mL FEU.

In this population with a VTE prevalence of 28.2% VTE, HemosIL AcuStar D-Dimer assay demonstrated 100% sensitivity and 100% NPV and correctly classified 68.0% of the samples.

Table 25: HemosIL AcuStar D-Dimer in Single-Center Management Study (n = 344)

Instrument	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)
HemosIL AcuStar D-Dimer	344	100.0 (96.3–100.0)	100.0 (97.3–100.0)	55.5 (49.0–61.8)

Clinical Study

An internal, blinded clinical study of samples obtained from patients suspected of VTE evaluated the clinical performance of various assays including HemosIL AcuStar D-Dimer assay. The samples were obtained from 108 normal subjects and 42 patients with VTE (n = 150), yielding a VTE prevalence of 28.0%. In this evaluation, the current cut-off values for each assay were used to assess sensitivity, specificity, NPV, positive predictive value (PPV), agreement, and exclusion rates of the assays with the corresponding 95% CI.

Given a cut-off value of 500 ng/mL (FEU), the HemosIL AcuStar D-Dimer exhibited 100% sensitivity and 100% NPV. Given the VTE prevalence of 28.0%, the HemosIL AcuStar D-Dimer assay correctly classified 53.3% of the samples. Table 26 presents these data.

Table 26: HemosIL AcuStar D-Dimer in Clinical Study (n = 150)

Instrument	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	NPV (%) (95% CI)
HemosIL AcuStar D-Dimer	150	100.0 (91.6–100.0)	35.2 (26.2–45.0)	100.0 (90.7–100.0)

Assay Kit

HemosIL AcuStar D-Dimer contains a D-Dimer cartridge and two calibrators. The cartridge allows for approximately 100 tests. A separate set of controls is available for use with the kit.

- **D-Dimer Cartridge** for 100 determinations contains one vial of a magnetic particle suspension coated with a mouse monoclonal antibody (MA-8D3) directed against the D-Dimer domain, one vial of assay buffer, one vial of tracer consisting of an anti-XDP mouse monoclonal antibody labeled with isoluminol, and one vial of sample diluent used for the automatic dilution in the sample rerun. The phosphate buffer contains bovine serum albumin, mouse monoclonal IgG, stabilizers, and preservative.
- **D-Dimer Calibrator 1** is a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers, and preservative in a barcoded plastic tube.
- **D-Dimer Calibrator 2** is a lyophilized solution of D-Dimer partially purified from human fibrin digested with human plasmin containing bovine serum albumin, buffer, stabilizers, and preservative in a barcoded plastic tube.
- **Controls Kit** is available separately. It contains three different controls: low control, high control, and very high control.

Table 27: HemosIL AcuStar D-Dimer Assay Kit

Product	Part Number	Kit Configuration
HemosIL AcuStar D-Dimer	0009802000	1 D-Dimer Cartridge (liq) 1 x 1 mL D-Dimer Calibrator 1 (lyo) 1 D-Dimer Calibrator 1 barcoded plastic tube 1 x 1 mL D-Dimer Calibrator 2 (lyo) 1 D-Dimer Calibrator 2 barcoded plastic tube
HemosIL AcuStar D-Dimer Controls	0009802100	Low D-D Control: 3 vials x 1 mL (lyo) High D-D Control: 3 vials x 1 mL (lyo) Very High D-D Control: 3 vials x 1 mL (lyo)

Conclusions

The selection of D-Dimer assays offered by the HemosIL Assay Panel satisfies the requirements of virtually any clinical or hospital laboratory.

- HemosIL D-Dimer is FDA-cleared for VTE exclusion at a cut-off value of 230 ng/mL D-DU when used in conjunction with PTP assessment. HemosIL D-Dimer offers proven, accurate, and reliable solutions for D-Dimer testing. HemosIL D-Dimer runs across many Instrumentation Laboratory platforms. The assay has proven to be a reliable solution for hospital and laboratory networks that utilize different instruments because the same reagent can be used across all instruments using the same cut-off value. The reagent contains a blocking agent against HAMA.
- HemosIL D-Dimer 500 is a European Union CE IVD Mark assay for the quantitative determination of D-Dimer with a cut-off value of 500 ng/mL FEU for use in conjunction with a clinical PTP assessment model or the exclusion of VTE in outpatients suspected of DVT and PE. HemosIL D-Dimer 500 offers proven, accurate, and reliable solutions for D-Dimer testing for laboratories reliant on the FEU units. It utilizes the same reagents as the familiar and proven HemosIL D-Dimer, but reports outcomes in FEUs.
- HemosIL D-Dimer HS is FDA-cleared for VTE exclusion at a cut-off value of 230 ng/mL D-DU when used in conjunction with PTP assessment. HemosIL D-Dimer HS offers proven, accurate, and reliable solutions for D-Dimer testing. The modified monoclonal antibody used in the HemosIL D-Dimer HS assay greatly reduces RF interference (up to 1,400 IU/mL); increases specificity and greatly reduces the incidence of false positive results; and provides enhanced patient care at a lower cost. The reagent contains a blocking agent against HAMA. HemosIL D-Dimer HS runs on the ACL TOP Family of instruments.
- HemosIL D-Dimer HS 500 is FDA-cleared for VTE exclusion at a cut-off value of 500 ng/mL FEU when used in conjunction with PTP assessment. HemosIL D-Dimer HS 500 assay, with its liquid, ready-to-use reagent, may be a better alternative for laboratories processing greater volumes or reliant on the FEU units. The ready-to-use liquid reagent reduces preparation time and eliminates the variability in outcomes introduced by water source and operator pipetting technique. HemosIL D-Dimer HS 500 assay has a wide linearity and a favorable interference profile (reduces RF interference up to 1,400 IU/mL). The reagent also contains a blocking agent against HAMA. HemosIL D-Dimer HS 500 runs on the ACL TOP Family instruments.
- HemosIL AcuStar D-Dimer is FDA-cleared as an aid in the diagnosis of VTE using a cut-off value of 500 ng/mL FEU. HemosIL AcuStar D-Dimer assay is able to successfully assay extremely low and extremely high concentrations of D-Dimer antigen in the same run. HemosIL AcuStar D-Dimer has ready-to-use reagent and the most dynamic linear range of any IL D-Dimer assay. The ready-to-use liquid reagent reduces preparation time and eliminates the variability in outcomes introduced by water source and operator pipetting technique. The chemiluminescent technology offers an alternative to laboratories that do not wish to utilize latex-based products. The Chemiluminescent technology offers enhanced analytical sensitivity, an extensive dynamic range, and a 2-month onboard stability. Given the pre-calibrated Master Curve of the assay, the operator needs only to scan the barcode on the assay cartridge and establish only a two-point curve before beginning sample runs. HemosIL AcuStar D-Dimer runs on the ACL AcuStar instrument.

In summary, the HemosIL panel of D-Dimer assays contains automated D-Dimer assays for coagulation analyzers that are FDA-cleared to exclude VTE in conjunction with PTP assessment. They exhibit excellent correlation and slope throughout the entire line of Instrumentation Laboratory instruments. In addition, the HemosIL AcuStar D-Dimer offers a new methodology for D-Dimer analysis that provides extremely wide working ranges for customers having special demands for D-Dimer measurement. Contact your local Instrumentation Laboratory representative for assistance in selecting the assay and instruments best suited to the particular needs of your laboratory and patient population.

Table 28: HemoIL Kit Components

HEMOSIL D-DIMER AND HEMOSIL D-DIMER 500 ASSAY KIT COMPONENTS		
D-Dimer	0020008500	4 x 3 mL Latex Reagent (lyo) 4 x 9 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer 500*	0020301000	4 x 3 mL Latex Reagent (lyo) 4 x 9 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer Controls	0020008610	5 x 1 mL Low D-Dimer Control (lyo) 5 x 1 mL High D-Dimer Control (lyo)
D-Dimer Controls *(liquid)**	0020013000	5 x 1 mL Level 1 D-Dimer Control (liq) 5 x 1 mL Level 2 D-Dimer Control (liq)
HEMOSIL D-DIMER HS ASSAY KIT COMPONENTS		
D-Dimer HS	0020007700	3 x 2 mL Latex Reagent (lyo) 3 x 8 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer Controls	0020008610	5 x 1 mL Low D-Dimer Control (lyo) 5 x 1 mL High D-Dimer Control (lyo)
D-Dimer Controls *(liquid)**	0020013000	5 x 1 mL Level 1 D-Dimer Control (liq) 5 x 1 mL Level 2 D-Dimer Control (liq)
HEMOSIL D-DIMER HS 500 ASSAY KIT COMPONENTS		
D-Dimer HS 500	0020500100	3 x 4 mL Latex Reagent (liq) 3 x 6 mL Reaction Buffer (liq) 2 x 1 mL Calibrator (lyo)
D-Dimer HS 500 Controls	0020500200	5 x 1 mL Low D-Dimer HS 500 Control (lyo) 5 x 1 mL High D-Dimer HS 500 Control (lyo)
D-Dimer HS 500 Controls *(liquid)**	0020500200	5 x 1 mL Level 1 D-Dimer HS 500 Control (liq) 5 x 1 mL Level 2 D-Dimer HS 500 Control (liq)
HEMOSIL ACUSTAR D-DIMER ASSAY KIT		
HemosIL AcuStar D-Dimer	0009802000	1 D-Dimer Cartridge (liq) 1 x 1 mL D-Dimer Calibrator 1 (lyo) 1 D-Dimer Calibrator 1 barcoded plastic tube 1 x 1 mL D-Dimer Calibrator 2 (lyo) 1 D-Dimer Calibrator 2 barcoded plastic tube
HemosIL AcuStar D-Dimer Controls	0009802100	Low D-D Control: 3 vials x 1 mL (lyo) High D-D Control: 3 vials x 1 mL (lyo) Very High D-D Control: 3 vials x 1 mL (lyo)

* Not currently 510(k) cleared.

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