



## Quantification of D-dimer using a new fully automated assay: its application for the diagnosis of deep vein thrombosis

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### ABSTRACT

**Background and Objectives.** A D-dimer assay can be helpful to rule out thromboembolism provided it is sensitive, reliable, fast and easy to perform. Tests based on the ELISA methodology have a high diagnostic sensitivity, and are therefore adequate for excluding deep venous thrombosis (DVT). The drawbacks are their long assay times, unsuitability to be run on single samples and cost. New methods have been developed, based either on the same principle, by immunofiltration or by microlatex immunoturbidimetric assays which seem to reach the high sensitivity and negative predictive value (NPV) required, but allowing fast and quantitative single sample analysis. The aim of this work was to evaluate one rapid test, a fully automated quantitative assay (IL Test™ D-dimer, run on an ACL™ 7000 coagulation analyzer, Instrumentation Laboratory).

**Design and Methods.** We compared the diagnostic value of IL Test™ for DVT with that of an ELISA (Dimertest® Gold EIA Agen Biomedical Limited, Aca-cia Ridge, Australia). Eighty-six patients (43 men, 43 women, mean age: 61 years) showing DVT symptoms formed the population for this non-randomized controlled trial in a referral center. The diagnosis of DVT based on the clinical history, was confirmed by serial compression ultrasonography (CUS) with Doppler flow in 62 patients.

**Results.** The IL Test™ D-dimer proved to be rapid, automated and well suited for individual tests with a good reproducibility in three control plasmas with different concentrations of D-dimer (coefficient of variation range 0.54-3.87%). Its performance was comparable to that of the Dimertest® Gold EIA, as indicated by the areas under the receiver operating characteristic curves (Dimertest® Gold EIA 0.748; IL Test™ D-dimer 0.70). On the basis of kappa coefficients, there was a good concordance between the Dimertest® Gold EIA and IL Test™ D-dimer when the receiver operating characteristic (ROC) curves suggested cut-offs were used. The sensitivity (98.3%) and NPV (88.9%) shown by IL Test™ D-dimer are comparable or even better than those obtained for EIA (95%, 80%, respectively).

**Interpretation and Conclusions.** This study shows that the new method can be included in prospective clinical trials to test the utility of D-dimer measurement in combination with other non-invasive diagnostic procedures in the management of DVT diagnosis.

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Key words: D-Dimer, automated assay, deep vein thrombosis

Deep venous thrombosis (DVT) affects approximately 84 individuals per 100,000 every year.<sup>1</sup> Objective testing for DVT is crucial because clinical assessment alone is unreliable,<sup>2</sup> undiagnosed DVT can cause fatal pulmonary embolism<sup>3</sup> and DVT treatment is effective.<sup>4</sup> However, it is expensive and it is associated with side effects. Its inappropriate use should be avoided.

Contrast venography is the gold standard for DVT diagnosis<sup>5</sup> but it is not ideal because of its invasive nature and the risks associated with contrast media. Many non-invasive tests have therefore been developed for the diagnosis of these patients and their efficacy, at least for proximal DVT, is well established.<sup>6</sup>

Recent reviews have been published suggesting improved strategies for the diagnosis of suspected DVT.<sup>6,7</sup> The authors considered that a diagnosis strategy combining clinical assessment and D-dimer blood tests as adjuncts to compression ultrasonography (CUS) gives a non-invasive diagnosis in the vast majority of patients with suspected DVT, and appears to be safe.<sup>6,7</sup> However if the results of this testing are non-diagnostic or are discordant with the clinical assessment, venography should be considered.<sup>6,7</sup>

It is now generally accepted that the D-dimer blood test is a valuable tool for exclusion of venous thromboembolism in symptomatic patients. However, the test performance must reach high sensitivity and negative predictive values (NPV) with figures close to 100%. Several D-dimer assays are currently available but their clinical efficiency differs markedly. Although all of them have a low positive predictive value (PPV) for DVT, only some have high sensitivity and NPV.<sup>8,9</sup> Methods based on ELISA technology fulfil these requirements, but they are not suitable for emergency or individual determinations.<sup>9,10</sup> However, new meth-

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ods have been developed, automated ELISA,<sup>11,12</sup> an immunofiltration system,<sup>13,14</sup> and microlatex immunoturbidimetric assays<sup>15-17</sup> seem to reach the required high sensitivity and NPV, allowing fast and quantitative single sample analysis.

The aim of this study was to evaluate the IL Test™ D-Dimer, a fully automated rapid quantitative assay for the measurement of D-dimer in patients with symptomatic clinically suspected DVT.

## Design and Methods

### Patients

Between January 1998 and April 1999, 86 out-patients (43 women and 43 men) with a moderate or high clinical suspicion of DVT were entered into a non-randomized, controlled trial in a referral center. The exclusion criteria were the following: previous recent episode of DVT, stable symptoms lasting more than 20 days, anticoagulant or fibrinolytic therapy already underway at presentation. Informed consent was obtained from all patients. The geometric mean age  $\pm$  SD of the patients was  $61 \pm 16$  years. Patients were classified as having comorbid conditions when the following circumstances or disorders were present: a) 19 patients (15 with DVT diagnosis) subjected to immobilization due to a medical, traumatic or surgical condition; b) 23 patients (18 with DVT diagnosis) with cancer or metastatic cancer; c) 21 patients (13 with DVT diagnosis) with severe venous insufficiency. The remaining 23 patients (16 with DVT diagnosis) showed no comorbid conditions. Hypertension, dyslipidemia, obesity and smoking habit were taken into account in the clinical questionnaire and treatments were carefully recorded.

The delay between the first clinical symptoms giving rise to the suspicion of DVT and the CUS Doppler examination was also recorded: 69 patients were examined in the first days (*recent* events) and 17 at least 10 days (*old* events) after symptom occurrence.

The pre-test clinical probability (PCP) for DVT was assessed by means of a standard questionnaire.<sup>18</sup>

### Diagnostics

All patients were subjected to objective tests to confirm the diagnosis within 48 h of admission. Diagnosis of DVT was established by CUS with Doppler flow. Ultrasonography studies also assessed the distal veins of the lower limbs. It was repeated after 7 days in those patients with a negative CUS. DVT diagnosis was ruled out when CUS remained negative and no symptoms were observed. Contrast venography was only performed in one patient who had negative CUS results but a clinical suspicion of DVT which led to proximal DVT being diagnosed. All patients with a diagnosis of DVT as well as those in whom this entity had been ruled out were followed-up in the out-patient clinic.

### Blood sampling and D-dimer determination

Blood samples were collected into vacuum tubes that contained 0.129 M trisodium citrate. Samples were centrifuged at  $2,000 \times g$  for 15 min to obtain platelet poor plasma, which was stored at  $-70^\circ\text{C}$  until tested. A new automated immunoturbidimetric assay, IL Test™ D-dimer, was used and was com-

pared to the ELISA Dimertest® Gold EIA.

The IL Test™ D-dimer is a quantitative latex micro-particle enhanced turbidimetric immunoassay. The assay is performed fully automated on IL Coagulation Analysers. The latex reagent consists of polystyrene latex particles of uniform size coated with a monoclonal antibody highly specific for the D-dimer domain contained in the fibrin soluble derivatives. The degree of agglutination is directly proportional to the concentration of D-dimer in the sample, and is determined by measuring the decrease of transmitted light at 405 nm caused by the aggregates. The test was automatically processed on the ACL™ 7000. Calibration is performed by the instrument using the D-dimer calibrator included in the kit. Recalibration is only required monthly or when a new lot of reagents is used. Low and high D-dimer controls were used to check the lower and upper measurement ranges, respectively, and were included in each working session. The concentration of D-dimer was calculated automatically by the analyzer. The measurement range is from 200 to 1,050 ng/mL. The samples above the measurement range were re-tested using a 1/5 dilution. The upper limit of the normal range declared by the manufacturer is 255 ng/mL.

The intra-assay reproducibility of the IL Test™ D-dimer assay was tested using three lyophilized control plasmas: D-dimer control plasma I (mean assigned value 1,055 ng/mL), D-dimer control plasma II (mean assigned value 525 ng/mL) and D-dimer control plasma III (mean assigned value 263 ng/mL). Eight replicates were performed on the same working day. The between-assay reproducibility was assayed by testing the same three samples on eight different working days.

The Dimertest® Gold stripwell EIA kit provides a quantitative measurement of cross-linked fibrin degradation products containing D-dimer. This kit utilizes the monoclonal DD-3B6 for antigen capture. The upper limit of normal range declared by the manufacturer is 120 ng/mL.

### Statistical management

ROC curves<sup>19</sup> were constructed by plotting the sensitivity (true positive fraction, among patients with thrombosis) versus specificity (false positive fraction, among patients without thrombosis). The area under the curves (AUC) was then calculated. ROC curves were used to determine the optimal cut-off levels for each test. The kappa coefficient (K)<sup>20</sup> which establishes the degree of agreement between two tests when classifying patients into positive and negative categories, was also calculated. Sensitivity, specificity, PPV, NPV, and 95% confidence intervals (CIs) were calculated according to standard methods for proportions.

## Results

Eighty-six patients showing DVT symptoms were enrolled for the study. The suspected diagnosis based on the clinical history was confirmed by compression ultrasonography Doppler or venography for 62 patients with proximal DVT (72%). The patients referred to our unit usually have a pre-test clinical probability rated from moderate to high. This is the reason for the high prevalence of DVT.

**Table 1. Intra-assay and inter-assay precision for the IL Test™ D-dimer.**

	Intra-assay	Inter-assay
D-dimer control plasma I, mean±SD	1038.5±11.61	1056±28.3
Mean assigned value, range	1025-1057	1016-1097
1,055 ng/mL, CV%	1.12	2.68
D-dimer control plasma II, mean±SD	443.2±2.38	481±18.64
Mean assigned value, range	440-446	446-501
525 ng/mL, CV%	0.54	3.87
D-dimer control plasma III, mean±SD	222±8.1	275.6±8.62
Mean assigned value, range	212-234	263-289
263 ng/mL, CV%	3.65	3.12

**Table 2. IL Test™ D-dimer on healthy individuals. Distribution by age.**

Age range (yrs)	14-20	24-40	43-59	63-91
Number of samples	10	17	19	17
Average age (yrs). Geometric mean	17.4	31.3	48.0	70.0
D-dimer (ng/mL). Geometric mean	179	178	185	203
SD	27	31	28	32

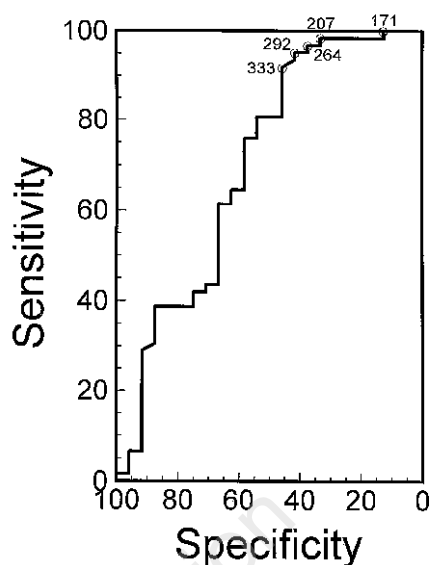
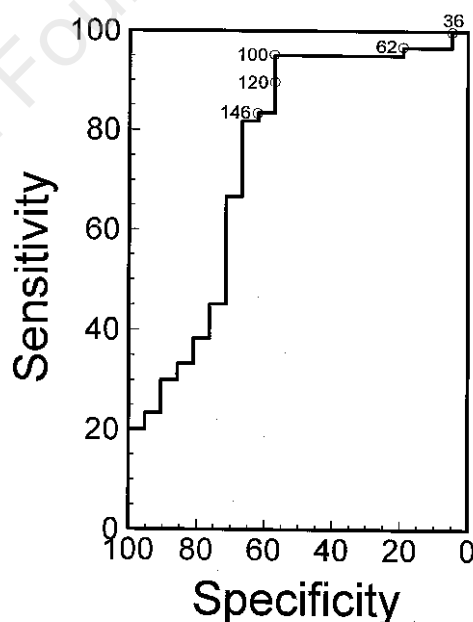
As reported in Table 1, the intra- and inter-assay reproducibility of the IL Test™ D-dimer for the three control plasmas with different concentrations of D-Dimer was good, with the coefficients of variation ranging from 0.54 to 3.65 for intra-assay and from 2.68 to 3.87 for inter-assay.

The upper limit of the normal range was assessed by analyzing 63 healthy individuals (geometric mean age 41.1 years, range 14-91), and was found to be 249.2 ng/mL (CI 236.1-262.4 ng/mL), very close to that declared by the manufacturer (255 ng/mL). Table 2 shows the D-dimer concentration for these patients classified into 4 age groups. A slight increase of the mean was observed as age increased.

ROC curves were plotted for both quantitative assays (Figures 1 and 2). The areas under the ROC curves were not significantly different between Dimertest® Gold EIA (0.748; CI 0.615-0.882) and IL Test™ D-dimer (0.700; CI 0.564-0.835).

The values of K between the two tests evaluated in this study were calculated for two different cut-off values: the upper limit of the normal range declared by the manufacturers and the cut-off suggested from the ROC curves. The results are shown in Table 3. The K coefficient expresses the concordance between the ability of the assays to classify a given patient as having a normal or an abnormal result. On the basis of the interpretation of K values by Fermanian,<sup>20</sup> a good concordance was found between the Dimertest® Gold EIA and IL Test™ D-dimer.

Table 4 summarizes the sensitivity, specificity, PPV and NPV of the two methods studied. Using the upper

**Figure 1. ROC curve for the IL Test™ D-dimer. Some cut-off values are shown on the curve.****Figure 2. ROC curve for the Dimertest® Gold EIA. Some cut-off values are shown on the curve.**

limit of normal range declared by the manufacturer, IL Test™ D-dimer showed the highest sensitivity and NPV. The ELISA Dimertest® Gold EIA improved its clinical efficiency very markedly (sensitivity rising from 90.0 to 95.0% and NPV rising from 66.7 to 80.0%) when the ROC determined cut-off was used instead of the upper limit of normal range declared by the manufacturer. Only one out of the 62 patients with proxi-

**Table 3. Kappa (K) coefficients. Dimertest® Gold EIA was taken as the reference test.**

Ref. test cut-off	Assay test	Cut-off	Kappa
120 ng/mL*	IL Test™ D-dimer	255 ng/mL*	0.61
100 ng/mL°	IL Test™ D-dimer	255 ng/mL*	0.65
100 ng/mL°	IL Test™ D-dimer	292 ng/mL°	0.73

\*The upper limit of normal range suggested by the manufacturer; °ROC determined cut-off.

**Table 4. Sensitivity, specificity, NPV and PPV of the investigated D-dimer assays.**

Test	Cut-off	Sensitivity	Specificity	NPV	PPV
IL Test™ D-dimer	<255 ng/mL*	98.4	33.3	88.9	79.2
Dimertest® Gold EIA	<120 ng/mL*	90.0	57.1	66.7	85.7
IL Test™ D-dimer	<292 ng/mL°	95.2	41.7	76.9	80.8
Dimertest® Gold EIA	<100 ng/mL°	95.0	57.1	80.0	90.5

\*The upper limit of normal range suggested by the manufacturer; °ROC determined cut-off.

mal DVT yielded a false negative value with IL Test™ D-dimer (using the upper limit of the normal range declared by the manufacturer), while Dimertest® Gold EIA yielded three (ROC cut-off: 100 ng/mL). Of these three EIA false negative results, which were classified as recent events, one corresponded to the false negative found with IL Test™ D-dimer and another reported just above its cut-off, suggesting patients with a poor fibrinolytic activity.

## Discussion

It is now firmly established that D-dimer determination is a valuable diagnostic tool for exclusion of venous thromboembolism in symptomatic patients with suspected DVT as an addition to venous ultrasonography. Enzyme-linked immunosorbent assay (ELISA) techniques have shown a very high sensitivity and NPV in the diagnosis of DVT, but they are not suitable for emergency or individual determinations.<sup>9,10</sup> To be able to cope with these situations, several alternatives to a well-established ELISA have been proposed. There are D-dimer tests with sensitivity and NPV similar to that of ELISA, based either on the same principle,<sup>11,12</sup> immunofiltration<sup>13,14</sup> or on microlatex immunoturbidimetric<sup>15-17</sup> assays.

We evaluated the clinical performances of the new IL Test™ D-dimer, a fully automated seven-minute assay performed on IL Coagulation analyzers ACL™, and compared the results with a well established ELISA (Dimertest® Gold EIA).

The lower specificity found in our study for both quantitative assays (IL Test™ D-dimer and Dimertest® Gold EIA) could be due to the high percentage of patients showing comorbid conditions in the group studied (16 for IL and 9 patients for EIA were classified as false positives), in whom the high D-dimer levels could have been due to situations other than DVT.

The higher sensitivities and NPV shown by IL Test™ D-dimer versus the EIA was caused by 2 samples from patients with DVT whose values were above the IL cut-off but below 70 ng/mL with the EIA.

The high sensitivity (98.4 and 95.0%) and NPV (88.9 and 80.0%) shown by the two quantitative methods (IL and EIA, respectively), support the idea that the use of D-dimer together with the prior clinical probability and a CUS Doppler examination, is the best strategy to exclude DVT, minimizing the situations in which venography is required.

When comparing the clinical efficiency of the D-dimer assays reported in our study with the results of other studies, it is important to take into account that the prevalence of DVT is usually significantly lower in these latter studies, being around 20%<sup>16,17</sup> while in ours the prevalence of DVT was close to 70%. However, the percentage of positive ultrasonography reported for patients with low, moderate and high PCP is 3, 17 and 75% respectively,<sup>17</sup> which is consistent with the fact that patients in our study were rated with moderate and high PCP and yielded 3 out of 4 positive ultrasonographies.

## Conclusions

The sensitivity and NPV values found in our study for IL Test™ D-dimer were comparable to or even higher than those for the EIA, and demonstrated that D-dimer determination by this method can be of great help as an exclusion tool for patients admitted with a suspicion of DVT. Tandem PCP, D-dimer and CUS Doppler allows management of the great majority of patients.

A possible limitation of this test is the recommendation to use the instruments for which it has been prepared: the fully automated series IL Coagulation Analysers (ACL™ and Futura™).

Advantages of IL Test™ D-dimer are its rapidness (7 minutes), and automation and the possibility to run single assays, which makes it very suitable in emergency laboratories. New studies with larger number of patients would be necessary to confirm our findings.

## Contributions and Acknowledgments

PV, FF, JS, HF and JA were responsible for the design, data handling, statistical analyses and writing of the paper. The order of the authors is related to their contribution. YM and AV took care of the patients.

## Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

## Manuscript processing

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## Potential implications for clinical practice

- ◆ This new fully automated assay of D-dimer may be useful for diagnosis of deep vein thrombosis.<sup>21,22</sup>

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