

Performance of the AxSYM[®] D-dimer Assay* as a Rule-Out Test in Outpatients with Suspected Deep Vein Thrombosis (DVT)

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Abstract (revised)

With an annual incidence of 1/1000, venous thromboembolism is a common disorder. Clinical manifestations are thrombosis in the leg (DVT) and its major complication, pulmonary embolism (PE). Approximately 20-30% of outpatients with suspected DVT prove to have thrombosis after Doppler Ultrasound. Therefore, simplified and more cost-effective strategies have been explored. The D-dimer test is increasingly used as a first line test in patients with suspected DVT to accurately exclude DVT and the need for costly imaging. The aim of this study was to determine the negative predictive value (NPV) of the new AxSYM D-dimer assay as a rule-out test.

The AxSYM D-dimer assay is a fully automated microparticle enzyme immunoassay (MEIA) for measuring D-dimer in citrated plasma. Time to first result of the AxSYM assay is 15 minutes with throughput of 57 tests per hour. The reportable range is up to 9000 ng/mL FEU with no dilutions. In a 20 day study, the assay demonstrated total imprecision of <7% at 520 and 4346 ng/mL FEU, respectively (CLSI Protocol EP5-A2).

In a prospective study, plasma samples were taken from 278 individuals presenting at Aberdeen Royal Infirmary with suspected DVT following a pre-specified clinical investigation protocol which included the Vidas[®] D-dimer test. These samples were also tested in the AxSYM D-dimer assay to determine the D-dimer concentration. Diagnosis of DVT was confirmed by a positive ultrasound.

The negative predictive value (NPV) of the AxSYM D-dimer assay in this population was >98%, allowing use of the assay as a rule-out test.

A method comparison to the VIDAS D-dimer assay on these samples gave the following relationship: AxSYM=0.95 VIDAS-2.7ng/mL FEU and r=0.96.

In conclusion, the AxSYM D-dimer test provides a rapid, cost-effective method for exclusion of DVT.

Introduction

D-dimer is a specific marker of the breakdown of a fibrin clot and thus an indirect marker of clot formation. Elevated levels of D-dimer are present in a wide variety of disorders known to be associated with activation of coagulation.

Recent data suggests that up to 75% of outpatients who present with signs and symptoms suggestive of deep vein thrombosis (DVT) do not have the disease.¹ Objective diagnosis of DVT requires costly and time-consuming imaging by compression ultrasound or ascending venography. Alternative approaches to diagnosis and decision making in those presenting with symptoms suggestive of DVT have therefore been developed. Often clinical risk scoring to stratify patients into low, moderate or high risk of DVT combined with D-dimer testing are applied.

Here we present data from development and evaluation of an automated D-dimer immunoassay which reacts specifically with true D-dimer as well as higher molecular weight complexes. The clinical performance of the assay has been evaluated by retrospectively testing samples collected from consecutively presenting patients at a DVT outpatient clinical to determine the negative predictive value (NPV) of the assay. The AxSYM results were not included in the routine diagnosis at the clinic where the nurses collecting samples are blind to this data.

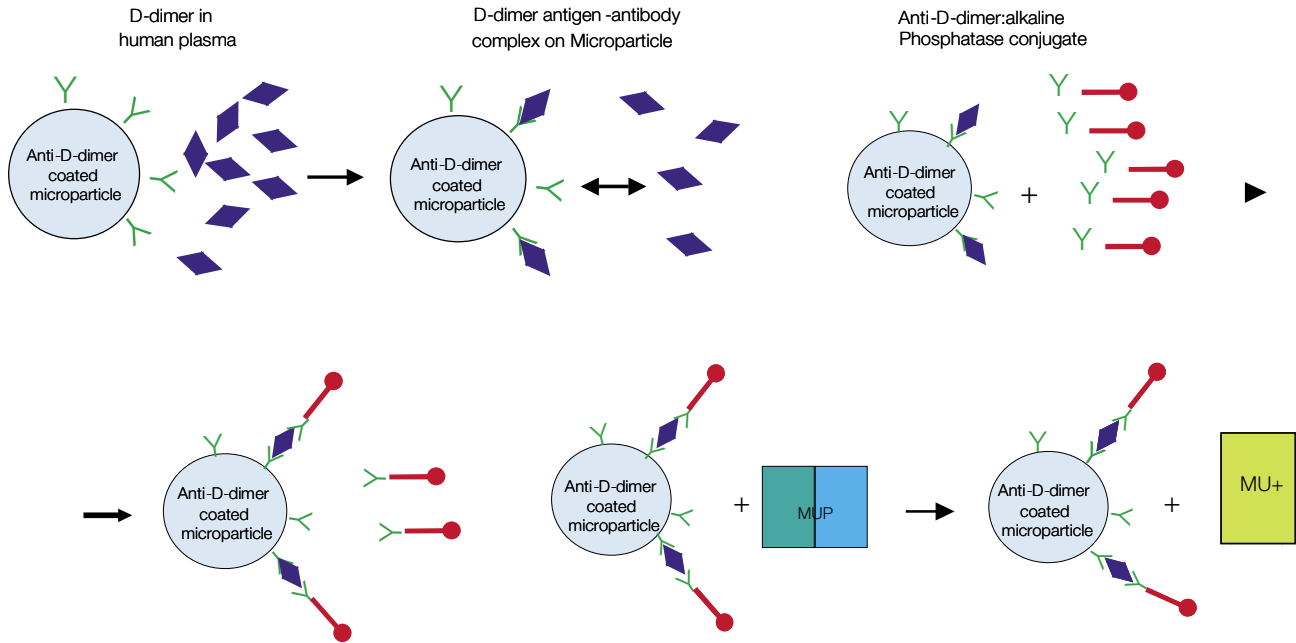
* Currently available outside the U.S.

Methods

Materials

The AxSYM D-dimer assay is a Microparticle Enzyme Immunoassay (MEIA) for the quantitative determination of D-dimer in citrated human plasma. Time to first result is 15 minutes with throughput of 57 tests per hour. The assay format is shown below,

Two Step Sandwich Assay



Alkaline phosphatase enzyme cleaves the substrate MUP, which releases the fluorescent product MU+ and phosphate. Rate of MU+ production is proportional to D-dimer concentration.

Results

Precision (Tables 1 and 2)

Assay imprecision was assessed using two human plasma controls. Each control was run in replicates of two at two separate times of the day for 20 days (n=80). Testing was performed on two AxSYM systems using a single calibration on each instrument and one reagent lot. The total precision observed is shown in the tables below:

Table 1: LOW PANEL

Instrument	Mean (ng/mL FEU)	Total	
		SD	(%CV)
1	599.7	27.92	4.6
2	604.5	33.46	5.6

Table 2: HIGH PANEL

Instrument	Mean (ng/mL FEU)	Total	
		SD	(%CV)
1	4451.6	269.34	6.1
2	4379.9	320.30	7.3

Method Comparison (Table 3 and Figure 1)

A comparison of the AxSYM D-dimer assay and the BioMerieux Vidas assay was carried out on 271 citrated plasma samples (figure 2). Results from the Passing-Bablok linear regression analysis are summarized in the following table chart:

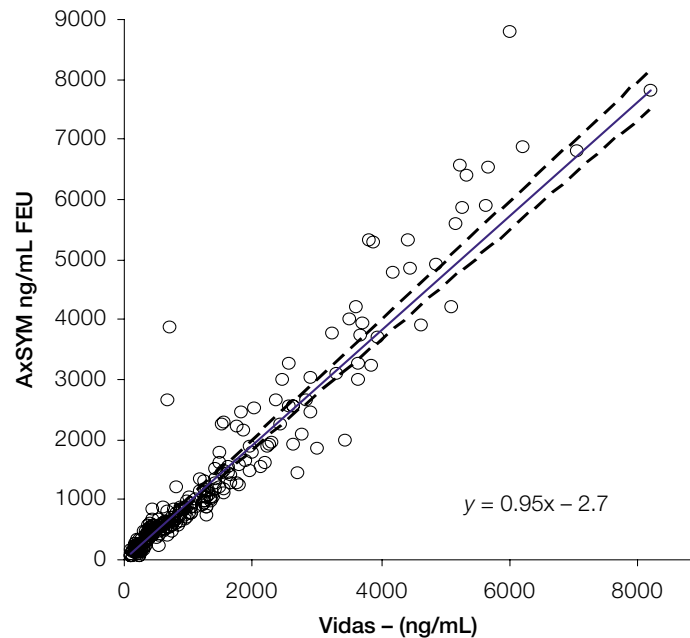
Table 3 AxSYM D-dimer vs. Comparison Assay

Specimen Type	n	r (95% CI)	Intercept (95% CI)	Slope (95% CI)
Plasma	271	0.96 (0.95 to 0.97)	-2.7 (-30.8 to -14.2)	0.95 (0.92 to 0.99)

Results (continued)

Method Comparison continued

Figure 1: AxSYM D-dimer vs Vidas Ddimer



Clinical Performance in The Evaluation OF DVT

A total of 278 patients were referred to the DVT outpatient clinic during the course of this prospective study carried out at Aberdeen Royal Infirmary. Four specimens collected were omitted from analysis; two due to clerical errors and two that had previous DVT (an exclusion criteria). Of the remaining 274 specimens, 49 (18%) were diagnosed with DVT by color flow duplex ultrasound.

Patients were classified as having a high, moderate or low pre-test probability (PTP) of DVT using a clinical risk score based on the original Wells model.² The AxSYM D-dimer results were analyzed using a clinical cut-off of 500ng/mL FEU, whereby a result of ≥ 500 ng/mL FEU was considered positive. The NPV of AxSYM D-dimer testing alone was 98% (95% CI 95 to 101). The incidence of DVT in each of the clinical risk categories was 7.9%, 14.3% and 37.0% for low-, moderate-, and high-risk groups respectively.

Applying Bayes' rule to the PTP categories, the probability of having a DVT if the D-dimer test was negative was 0% and 1.5% in the low- and moderate-risk groups respectively.

Calculated NPV, Sensitivity and Specificity of the AxSYM D-dimer assay in each of the clinical risk score categories are shown in the table below. With NPV of $>98\%$ in the moderate and low PTP groups the AxSYM assay may be recommended for use in conjunction with PTP scoring to safely rule-out DVT in patients presenting with symptoms suggestive of DVT.

PTP	ALL n=274, DVT=49	Low PTP n= 38, DVT=3	Moderate PTP n=182, DVT=26	High PTP n=54, DVT=20
NPV (95% CI)	98 (95 to 101)	100 (100 to 100)	99 (96 to 101)	92 (78 to 107)
Sensitivity (95% CI)	96 (90 to 101)	100 (100 to 100)	96 (89 to 104)	95 (85 to 105)
Specificity (95% CI)	43 (37 to 50)	46 (29 to 62)	44 (36 to 52)	35 (19 to 51)

Conclusions

In conclusion, this automated D-dimer assay for the AxSYM analyzer with a short test time and high throughput is both sensitive and precise. When used in conjunction with pre-test probability scoring this assay is an effective aid in the rule-out of DVT.

