



Regular Article

Analytical and clinical validation of a new point-of-care testing system for determination of D-Dimer in human blood

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ABSTRACT

D-Dimer testing is used for exclusion of deep venous thrombosis (DVT). AQT90 FLEX D-Dimer (AQT D-Dimer) is a novel time-resolved fluorescence based point-of-care test for quantification of D-Dimer in whole blood or plasma. Presently we have determined the analytical and clinical performance of AQT D-Dimer and compared it with four routine D-Dimer assays.

The within-run CV of AQT D-Dimer was 3.8–7.2% and the between-run CV was 5.7–9.7%. Excellent agreement was found between the D-Dimer concentrations recorded in citrate-, heparin- and EDTA stabilised blood. The plasma concentration of D-Dimer was determined with AQT D-Dimer, AxSYM, Biopool Auto-Dimer, STA-Liatest and Vidas New in 170 consecutive patients suspected for DVT. Phlebograms were positive in 64 patients (22 distal, 42 proximal). ROC-curves (ROC), the negative and positive predictive values (NPV, PPV), the sensitivity and specificity of the tests were compared. The area under ROC was comparable for all tests. NPV for all DVT was 87–88%, the sensitivity was 88–92% and the PPV was 45–55%. For proximal DVT the NPV and sensitivity were 100% for all tests, whereas the PPV was 37–48%. For distal DVT we obtained a NPV of 87–88%. The sensitivity was 64–77%, the PPV was 19–24% whereas a specificity of 32–58% was observed.

The AQT D-Dimer demonstrates excellent analytical and diagnostic performance. The test is rapidly performed and the measuring range of the assay is wide. The NPV, PPV, specificity, sensitivity and AUC of AQT D-Dimer for both proximal and distal DVT are comparable to routine D-Dimer assays.

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Introduction

The AQT90 FLEX is an integrated point-of-care (POC) test system aimed for determination of markers of congestive heart disease, acute myocardial infarction, infection and pregnancy using a single whole blood sample. Recently, the determination of D-Dimer was included in the analytical panel. The present study is aimed to establish the analytical and clinical performance of the AQT90 FLEX D-Dimer test (AQT D-Dimer) and to compare it with D-Dimer assays performed at central laboratory analyzers.

The value of D-Dimer testing in the exclusion of deep venous thrombosis (DVT) is related to the rapidity, turnaround time and the analytical and clinical performance of the test. The introduction of POC devices capable of measuring D-Dimer [1] has improved the turnaround time of D-Dimer testing compared to the classical enzyme linked immunosorbent assays (ELISA) and several POC devices for determination of D-Dimer are presently available [2–6]. A major

drawback, however, of the POC-based D-Dimer assays, is a higher imprecision than that of routine D-Dimer assays [2,5].

The clinical performance of POC-based D-Dimer assays varies [6] and the performance of some of the tests is reportedly inferior to the quality of D-Dimer assays performed at central laboratory analyzers [5,7,8]. The clinical performance, however, depends not only on the equipment used for testing but also on a number of other factors [9–11] such as the study design, the analytical principle of the D-Dimer method used and the choice of the D-Dimer cut-off threshold. Of particular importance, in this respect, is the validity of the performed radiological examination and the location of the thrombosis [12,13]. Phlebography is accepted as the gold standard in detecting DVT. Ultrasonography is the most frequently used diagnostic procedure for ruling out DVT, but the sensitivity of ultrasonography is lower when compared with phlebography, and in particular the presence of distal DVT can be difficult to visualise when ultrasonography is used as diagnostic tool [14,15]. This can lead to a false increase in the negative predictive value (NPV) of the D-dimer test employed [16].

The present study is the first to investigate the analytical and diagnostic performance of the AQT D-Dimer test, which is a novel POC-based time-resolved fluorescence (TRF) immunoassay for quantification of D-Dimer in whole blood or plasma. We evaluated the analytical imprecision of the test, and in order to establish the clinical performance

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of AQT D-Dimer with respect to both distal and proximal DVT we used phlebography as the radiological tool for diagnosis of DVT. We compared the results obtained by the AQT D-Dimer with results obtained with validated central laboratory equipment, i.e. two ELISA-based D-Dimer assays and two enzyme linked fluorescence -based D-Dimer assays (ELFA) applied on 170 consecutive patients referred to hospital with a tentative diagnosis of DVT.

Materials and methods

Study populations

Reference interval

The reference population consisted of 272 apparently healthy individuals (78 women and 54 men <50 years of age, 66 women and 74 men >50 years of age). Reference intervals were defined as concentrations below the 95th percentile range of the D-Dimer concentrations obtained in the citrate-stabilised whole blood and plasma samples.

Effect of plasma vs whole blood and choice of anticoagulant

The population used for evaluation of the effect of anticoagulant consisted of 60 apparently healthy individuals. The potential constant (systematic and consistent) and proportional (progressive with concentration) bias induced by the effect of anticoagulant was evaluated by comparison of the D-Dimer concentrations obtained in citrate-stabilised whole blood with the concentrations obtained in citrate-stabilised plasma, lithium heparin stabilised whole blood and EDTA-stabilised whole blood.

DVT study population

The DVT study population has been described previously [17–19]. In brief, 201 consecutive outpatients, all more than 18 years old and referred to the acute ward of the Hospital of South West Denmark with clinically suspected DVT of the lower limbs, were included in the study. All patients were referred to hospital from general practitioners and they were enrolled in the study 24 h/d and 7 d/week. The patients should be eligible for phlebography, and should not receive anticoagulant therapy. Twenty patients were excluded from the study due to pregnancy ($n=3$), malignancy ($n=15$), and systemic lupus erythematosus ($n=2$). Informed written consent could not be obtained from 11 patients, leaving 170 patients eligible for examination. The study was approved by the local Ethical Committee and the Helsinki II declaration was observed.

Blood collection

Citrate-stabilised blood samples aimed for imprecision studies, establishment of reference interval and evaluation of clinical performance were collected in evacuated tubes containing 0.11 mol/L sodium

citrate (Venoject VT053SBC07). Blood aimed for evaluation of the effect of anticoagulant was additionally collected in evacuated tubes containing 45 USP lithium heparin (VenoSafe VF-053SHL) and 5.9 mg K_2EDTA (VenoSafe VF-053SDK). All tubes were obtained from Terumo Europe, Leuven, Belgium. Whole blood samples were kept at room temperature and analysed within two hours after collection. Plasma was collected after centrifugation of the tubes for 20 min at 2000 g and stored in a bio bank at -65°C in tightly capped cryotubes specifically aimed for long time storage at low temperature. The temperature of the freezer storing the samples was continuously logged and the freezer was equipped with internal and external alarm units. Studies have demonstrated that the antigenic properties of D-Dimer are long-term stable when stored at low temperature [20]. Before analysis the samples were thawed for 5 min at 37°C , kept at room temperature, and analysed within one hour.

Imprecision studies

Two pools of citrate-stabilised plasma with mean plasma concentrations of D-Dimer of 0.70 mg/L and 62.3 mg/L were prepared by mixing plasmas from patients with very high concentrations of D-Dimer and plasmas from healthy individuals. The pools were kept at -65°C . Before analysis the pools were thawed for 5 min at 37°C , kept at room temperature, and analysed within one hour. Within-run and between-run imprecision were determined by analyzing the two pools of plasma with the AQT D-Dimer test over 20 days with two runs per day and four replicates per run. The imprecision of the other tests used in the study was evaluated using the control samples supplied by the manufacturer.

Diagnosis of deep vein thrombosis

All patients were subjected to phlebography performed according to Rabinov [21] without compression during injection of at least 100 ml iodine 240 mg/mL (Ultravist, Schering AG, Albertslund, Denmark). The leg being examined was not weight-bearing. The radiological examination included a description of the localisation of the thrombus. Distal DVT was defined as thrombus in the calf veins, while proximal DVT was defined as thrombus in the popliteal vein or above.

Determination of D-Dimer

The plasma concentration of D-Dimer was determined with five different methods. The AQT D-Dimer (Radiometer, Copenhagen, Denmark) is a novel TRF based test for quantification of D-Dimer in whole blood or plasma. The assay performs on the AQT90 FLEX POC test system from Radiometer. The AQT D-Dimer assay is based on a concept in which all the specific reagents are provided in a dry stable form within an assay cup (Fig. 1). Biotinylated monoclonal anti-D-Dimer antibodies have been pre-immobilised to the streptavidin surface of the

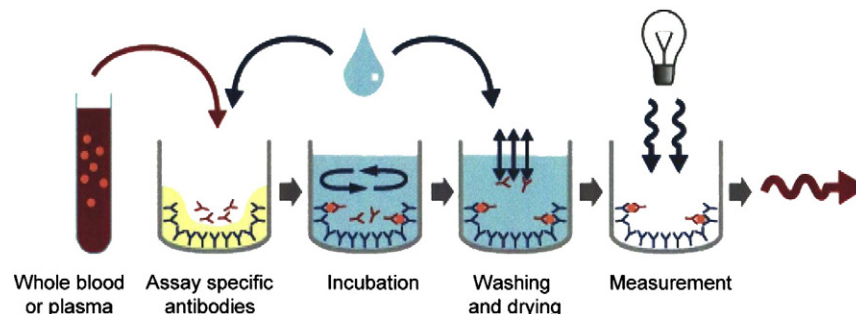


Fig. 1. The analytical principle of the AQT D-Dimer test.

Table 1

The within-run and between-run imprecision of AQT D-Dimer, Auto-Dimer, AxSYM D-Dimer, LIA-test D-Di and Vidas D-Dimer at two concentrations of D-Dimer.

	D-Dimer (mg/L)	Within-run CV%	Between-run CV%
AQT D-Dimer	0.70	4.6	6.6
	62.3	3.8	5.7
Auto-Dimer	0.30	2.1	3.3
	1.8	1.8	9.1
AxSYM D-Dimer	0.50	3.0	9.8
	4.1	4.2	12.8
LIA-test D-Di	0.26	10.3	12.5
	2.4	3.5	5.3
Vidas D-Dimer	0.51	5.9	8.8
	1.0	5.4	7.9

cup, and a separating layer and tracer antibodies have been added on top of the capture antibodies. The separating layer prevents the direct contact of the capture and tracer antibodies in storage. Prior to sampling, the sample is automatically diluted 1:50 with an assay solution. In the assay process, the diluted sample and assay solution are automatically added to the cup containing the assay-specific reagents. During the 15 min incubation period, the tracer and capture antibodies form a complex with D-Dimer present in the sample. After the incubation, the assay cup is washed with the assay solution and dried, after which the signal from the tracer antibody labelled with europium is measured by means of TRF directly from the dry surface of the assay cup. The concentration of D-Dimer is directly proportional to the measured europium signal. The measured signal is converted to a concentration using the calibration curve stored in the memory of the instrument. The analyzer performs sampling from a manually loaded capped primary sample tube, adjusts for the blood volume in the sample tube and converts the concentration of the D-Dimer in the sample to the concentration obtained in the corresponding citrate stabilised plasma. Time from sample collection to the first D-Dimer result is 20 min and subsequent results are obtained every 4th min thereafter. The AQT D-Dimer assay is calibrated against purified human D-Dimer obtained from HyTest Ltd, Turku, Finland and the content of D-Dimer is traced according to Lowry with a protein kit from Sigma-Aldrich, St. Louis, MO, USA. The measuring range of the assay is 0.08 – 100 mg/L and the detection limit is 0.035 mg/L of D-Dimer.

The Auto-Dimer is a latex-based immunoassay from Biopool, Umeå, Sweden. The assay was performed on the Dade-Behring Blood Coagulation System from Siemens, Marburg, Germany. The detection limit of the assay is 0.098 mg/L of D-Dimer, and concentrations below this limit are recorded as 0.098 mg/L. The AxSYM D-Dimer [22], a latex-based assay using the ELFA technique, was obtained from Axis-Shield, Dundee, UK. The kit performed on the AxSYM System from Abbott Laboratories, Gentofte, Denmark. The detection limit of the assay is 50 ng/mL. The LIA-test D-Di is a latex-based immunoassay obtained from Stago Diagnostica, Asnières-sur-Seine, France. The assay was performed using the STA-R equipment from Stago. The detection limit of this assay is 0.22 mg/L of D-Dimer and concentrations below this limit are recorded as 0.22 mg/L. The Vidas D-Dimer assay is a rapid ELISA based on the ELFA technique. The kit and the Mini Vidas equipment used for analysis were obtained from Biomérieux, Marcy-l'Etoile, France. All D-Dimer assays were performed by two experienced laboratory technicians. All D-Dimer kits used in the study were generous gifts from the companies.

Statistical analysis

The D-Dimer concentrations determined with the AQT D-Dimer were compared with the results obtained with the four other kits using the 170 citrate stabilised plasma samples from the patients suspected for DVT. Spearman's rank correlation analysis was used for the comparison. The D-Dimer concentrations obtained in citrate stabilised

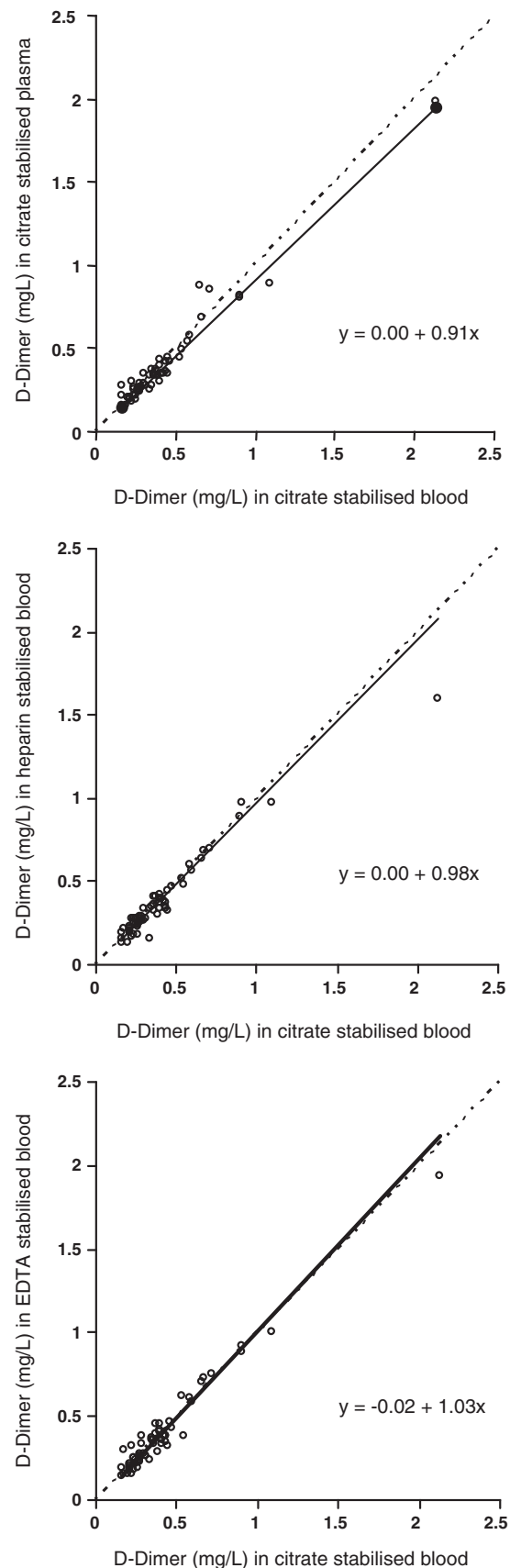


Fig. 2. Comparison between the D-Dimer concentrations obtained in citrate stabilised blood and citrate stabilised plasma (upper panel), lithium heparin stabilised blood (central panel), and EDTA stabilised blood (lower panel). The comparisons were performed according to Passing & Bablok. The solid lines and the equations represent the Passing & Bablok fit. The identity line is given as dots.

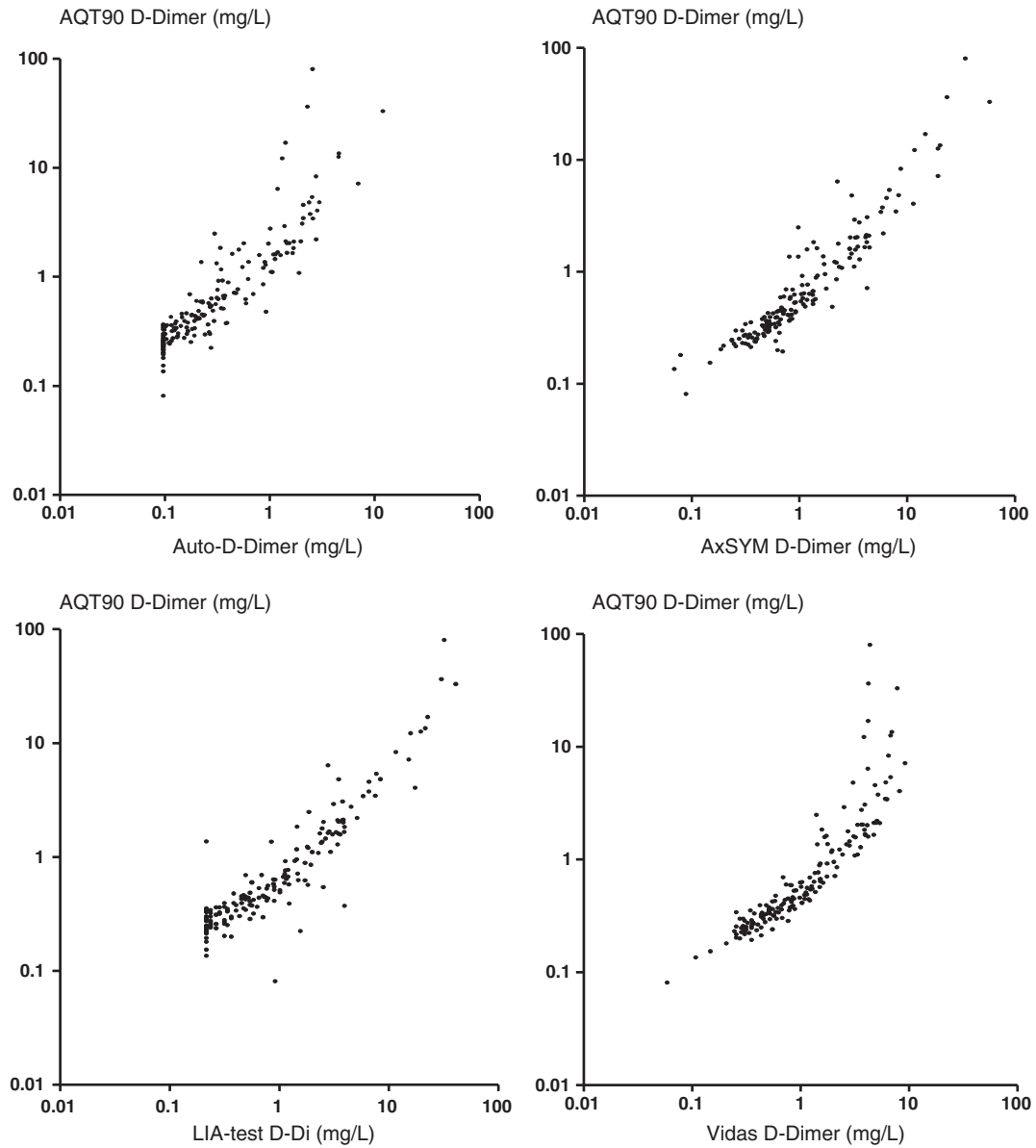


Fig. 3. Correlations between the AQT D-Dimer test and Auto D-Dimer, AxSYM D-Dimer, Liatest D-Di and Vidas D-Dimer. Citrate stabilised plasma samples from 170 consecutive patients suspected for DVT were used for comparison.

blood were compared with the concentrations determined in citrate-stabilised plasma, lithium heparin-stabilised blood and EDTA-stabilised blood. The comparisons were performed according to Passing & Bablok [23]. The analyses were performed by Analyse-it for Microsoft Excel ver. 2.12 from Analyse-it Software, Ltd., Leeds, UK, and this programme was also used for calculation of NPV, PPV, sensitivity, specificity and negative likelihood ratio (LR-neg). Receiver operating characteristic (ROC) curves were prepared by plotting the sensitivity versus 1- specificity and the area under the ROC-curves (AUC) and the 95% confidence interval of AUC for the D-Dimer test were calculated. The overall diagnostic performance of each D-Dimer test was evaluated by comparing the AUCs with a non-parametric approach [24].

Results

Imprecision

For the AQT D-Dimer the within-run CV was determined to be 3.8% at 62.3 mg/L of D-Dimer and 4.6% at 0.70 mg/L of D-Dimer, whereas the

between-run CV varied from 5.7% at 62.3 mg/L and 6.6% at 0.70 mg/L of D-Dimer. The within-run imprecision of the AQT D-Dimer was comparable with the imprecision of AxSYM D-Dimer and Vidas D-Dimer. The AQT D-Dimer demonstrated lower imprecision than the Liatest D-Di at low concentrations of D-Dimer whereas the within-run imprecision of the Auto-Dimer was better than the other tests employed (Table 1).

Reference intervals

The 95th percentile range for whole-blood was determined to be 0.61 mg/L of D-Dimer for subjects <50 years of age and 0.65 mg/L for subjects >50 years of age. The 95th percentile range for plasma was determined to be 0.55 mg/L of D-Dimer for subjects <50 years of age and 0.66 mg/L for subjects >50 years of age.

DVT study population

The study population consists of 170 consecutive patients suspected for DVT. We have previously published the characteristics

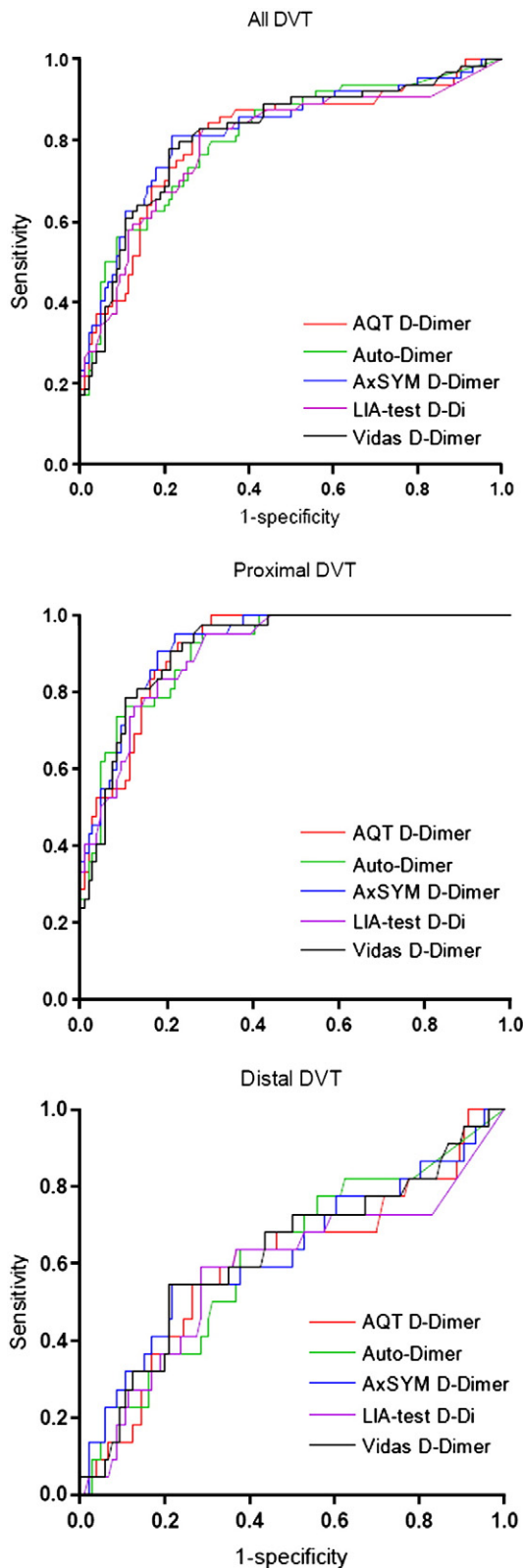


Fig. 4. Receiver operating characteristic (ROC) curves for the five D-Dimer tests investigated. The study population consists of 170 consecutive patients suspected for DVT. All patients (64 patients with DVT, 106 without DVT) are included in the upper panel. The central panel represents the 42 patients with proximal DVT and the 106 patients without DVT, whereas the ROC curves given in the lower panel correspond to the 22 patients with distal DVT and the 106 patients without DVT.

Table 2

The area under the ROC curve and the 95% confidence interval of the area (95%CI) for the AQT D-Dimer, Auto-Dimer, AxSYM D-Dimer, LIA-test D-Di and Vidas D-Dimer tests in relation to the location of deep venous thrombosis (DVT).

Test	DVT location	Area under ROC curve (95%CI)
AQT D-Dimer	All	0.81 (0.74 - 0.88)
	Distal	0.60 (0.46 - 0.74)
	Proximal	0.92 (0.87 - 0.96)
Auto-Dimer	All	0.81 (0.74 - 0.88)
	Distal	0.62 (0.48 - 0.75)
	Proximal	0.91 (0.87 - 0.96)
AxSYM D-Dimer	All	0.82 (0.75 - 0.89)
	Distal	0.63 (0.48 - 0.77)
	Proximal	0.93 (0.89 - 0.97)
LIA-test D-Di	All	0.80 (0.72 - 0.87)
	Distal	0.58 (0.44 - 0.73)
	Proximal	0.91 (0.86 - 0.95)
Vidas D-Dimer	All	0.82 (0.75 - 0.89)
	Distal	0.62 (0.49 - 0.76)
	Proximal	0.92 (0.87 - 0.96)

of the patients [17–19]. In brief, phlebograms were positive in 64 patients and negative in 106 patients. Stratification of the patients according to the localisation of the thrombus revealed that 22 patients had distal DVT, while 42 patients suffered from proximal DVT. Patients suffering from DVT and patients without DVT were comparable with respect to age, sex and use of oral contraceptives.

Effect of plasma vs whole blood and choice of anticoagulant

Comparison of the D-Dimer concentrations obtained in citrate-stabilised blood with the concentrations obtained in citrate-stabilised plasma demonstrated an insignificant constant bias of 0.00 (95% CI -0.02 - 0.03) mg/L of D-Dimer, whereas the proportional bias was 0.91 (95% CI 0.84 - 0.98). We calculated the bias corresponding to a D-Dimer concentration of 0.40 mg/L (our recommended cut-off value of the assay for exclusion of DVT as described below). At this D-Dimer level a bias of -0.03 (95% CI -0.048 to -0.002) mg/L was recorded. Comparison of the results obtained in citrate-stabilised blood with the results obtained in lithium heparin-stabilised blood showed a constant bias of 0.00 (95% CI -0.03 - 0.03) mg/L and a proportional bias of 0.98 (95% CI 0.90 - 1.06). When the D-Dimer concentrations recorded in citrate stabilised blood were compared to the results obtained in EDTA stabilised blood we observed a constant bias of -0.02 (95% CI -0.06 - 0.00) mg/L and a proportional bias of 1.03 (95% CI 0.93 - 1.12), Fig. 2.

Clinical performance

The results obtained with the AQT D-Dimer test showed good correlation with the results obtained with the other D-Dimer tests, $P < 0.0001$ (Fig. 3). The comparison between AQT D-Dimer and Auto D-Dimer, AxSYM D-Dimer, Liatest D-Di and Vidas D-Dimer revealed a Spearman's rho of 0.92 (95% CI 0.89-0.94), 0.94 (95% CI 0.92-0.96), 0.90 (95% CI 0.86-0.92) and 0.90 (95% CI 0.86-0.92), respectively.

We compared the AUC for the five D-Dimer assays in all patients, in patients with proximal DVT and in patients with distal DVT, Fig. 4. The AUC and the 95% confidence interval of the AUC for AQT D-Dimer, Auto-Dimer, AxSYM, Liatest D-Di and Vidas D-Dimer tests in relation to the location of DVT are presented in Table 2. The AUC for the D-Dimer assays in all patients varied from 0.80 to 0.82, in patients suffering from proximal DVT the AUC varied from 0.91 to 0.93 and in patients with distal DVT the AUC varied from 0.58 to 0.62. None of these differences were statistically different, $p > 0.05$.

Table 3

The relationship between the cut-off level for AQT D-Dimer and the negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity and the negative likelihood ratio (LR-neg) of the test.

Cut-off level (mg/L)	NPV(%) (95%CI)	PPV (%) (95%CI)	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	LR-neg
0.30	83 (67-93)	44 (35-53)	89 (79-96)	31 (23-41)	0.36 (0.17-0.75)
0.35	87 (76-95)	50 (41-60)	89 (79-96)	46 (37-56)	0.24 (0.12-0.50)
0.40	88 (78-95)	55 (45-65)	88 (77-94)	57 (47-66)	0.22 (0.11-0.43)
0.45	88 (79-94)	61 (50-71)	84 (73-92)	67 (57-76)	0.23 (0.13-0.42)
0.50	87 (78-93)	63 (52-73)	83 (71-91)	71 (61-79)	0.24 (0.14-0.42)
0.55	85 (76-91)	64 (52-75)	78 (66-88)	74 (64-82)	0.30 (0.19-0.48)
0.60	83 (74-90)	66 (54-77)	73 (61-84)	77 (60-85)	0.34 (0.23-0.52)

Table 4

The negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity and negative likelihood ration (LR-neg) of the five different D-Dimer assays according to the location of DVT. The 95% confidence intervals are given in brackets. A D-Dimer concentration of 0.40 mg/L was chosen as cut-off value for the AQT D-Dimer assay. For the other assays the cut-off value was recommended by the manufacturer.

		NPV (%)	PPV (%)	Sensitivity (%)	Specificity (%)	LR-neg
AQT D-Dimer 0.40 mg/L	All	88 (78-95)	55 (45-65)	88 (77-94)	57 (47-66)	0.22 (0.11-0.43)
	Distal	88 (78-95)	23 (13-36)	64 (41-83)	57 (47-66)	0.64 (0.36-1.14)
	Proximal	100 (94-100)	48 (37-59)	100 (92-100)	57 (47-66)	0.00 (nd)
Auto-Dimer 0.20 mg/L	All	88 (78-95)	55 (45-65)	88 (77-94)	58 (48-67)	0.22 (0.11-0.42)
	Distal	88 (78-95)	24 (14-37)	64 (41-83)	58 (48-67)	0.63 (0.36-1.13)
	Proximal	100 (94-100)	48 (37-59)	100 (92-100)	58 (48-67)	0.00 (nd)
AxSYM D-Dimer 0.50 mg/L	All	87 (73-96)	45 (36-54)	92 (83-97)	32 (23-42)	0.24 (0.10-0.59)
	Distal	87 (73-96)	19 (12-29)	77 (55-92)	32 (23-42)	0.71 (0.31-1.61)
	Proximal	100 (90-100)	37 (28-46)	100 (92-100)	32 (23-42)	0.00 (nd)
LIA-test D-Di 0.50 mg/L	All	87 (76-94)	52 (42-62)	88 (77-94)	51 (41-61)	0.25 (0.13-0.48)
	Distal	87 (76-94)	21 (12-33)	64 (41-83)	51 (41-61)	0.71 (0.40-1.28)
	Proximal	100 (93-100)	45 (34-55)	100 (92-100)	51 (41-61)	0.00 (nd)
Vidas D-Dimer 0.50 mg/L	All	87 (73-95)	46 (37-56)	91 (81-97)	37 (28-47)	0.25 (0.11-0.57)
	Distal	87 (73-95)	19 (11-29)	73 (50-89)	37 (28-47)	0.74 (0.36-1.53)
	Proximal	100 (91-100)	39 (29-48)	100 (92-100)	37 (28-47)	0.00 (nd)

nd: not defined.

When we calculated the NPV, PPV, sensitivity, specificity and the LR-neg for the AQT D-Dimer at various concentrations used as cut-off for the test in relation to the location of the deep venous thrombosis we observed that the highest NPV and the lowest LR-neg were obtained with D-Dimer concentrations between 0.35 – 0.50 mg/L. The PPV of the AQT D-Dimer increased from 44% to 66% and the specificity increased from 31% to 77%, whereas the sensitivity decreased from 89% to 73% when the cut-off concentration of D-Dimer increased from 0.30 mg/L to 0.60 mg/L, Table 3. Based on these results a cut-off value of 0.40 mg/L for the AQT D-Dimer was used for comparison of the performance of the test with the other D-Dimer tests and the cut-off values recommended by the manufacturers of the four routine tests were used for comparison, Table 4. When all patients were included we observed that the NPV for all tests was 87-88% while the sensitivity was 88-92%. The PPV was 55% for the AQT D-Dimer and 45-55% for the other tests. The LR-neg varied between 0.22 and 0.25 and was lowest for AQT D-Dimer and Auto-Dimer. For proximal DVT the NPV and the sensitivity were 100% for all tests. The PPV of the AQT D-Dimer was 48% and 37-48% for the other tests, whereas the LR-neg was 0.00 for all tests. For distal DVT the NPV was 87-88%, the sensitivity was 64-77% and the PPV was 19-24% for all tests. The LR-neg varied between 0.63 and 0.74. The specificity of the D-Dimer assays was 32-58% and the best results were again obtained with the AQT D-Dimer and the Auto-Dimer assays.

Discussion

The introduction of bedside analysis for determination of D-Dimer has facilitated rapid and reliable D-Dimer tests convenient for use in clinical situations where rapid sample turnaround time is

essential. D-Dimer tests are in particular used in the diagnostic work in patients referred to hospital suspected to suffer from venous thromboembolic disease.

The AQT FLEX platform is an integrated POC test system aimed for acute determination of markers of congestive heart disease, acute myocardial infarction, infection, coagulation and pregnancy using a single whole blood sample. The AQT D-Dimer test performs on the AQT FLEX platform and the present study is the first to establish the analytical and diagnostic performance of the test and to compare the performance of the assay with well-established D-Dimer assays. Our evaluation of the analytical performance revealed that the reproducibility of the AQT D-Dimer assay is comparable to methods performed using central laboratory analyzers. The D-Dimer concentrations obtained in citrate-stabilised blood are comparable to the concentrations recorded in lithium heparin-stabilised blood and EDTA-stabilised blood, i.e. the constant bias and the proportional bias between the various blood matrices are insignificant. We observe, however, a modest, but significant proportional bias between the results obtained in citrate stabilised blood and citrate stabilised plasma. Of particular notice we observe a small, but significant bias at the concentration we recommend as cut-off for exclusion of DVT (0.40 mg/L). As discussed below we demonstrate, however, that variation in the cut-off value between 0.35 and 0.50 mg/L of D-Dimer is without effect on the NPV of the AQT D-Dimer. Thus, the cut-off value established in citrate-stabilised plasma can also be used if citrate-stabilised blood is used for analysis.

Our correlation studies show good agreement between the results obtained with the AQT D-Dimer and the other tests investigated. It should be noticed, however, that the D-Dimer concentration in some of the samples is below the detection limit of the Auto-Dimer and the

Liatest D-Dimer. We recorded the D-Dimer concentration obtained in these samples as the concentration corresponding to the detection limit for the assay. This may influence the results we obtain when we compare AQT D-Dimer with Auto Dimer and Liatest D-Di.

The clinical performance of D-Dimer test in relation to diagnosis of DVT depends on the validity of the radiological examination performed [12,13] and on the location of the DVT. We observe that approximately one third of our patients with positive phlebogram suffers from distal DVT and this frequency is comparable with other clinical studies [25]. Notably, normal D-Dimer concentrations are regularly observed in patients suffering from distal DVT and the prevalence of distal DVT, and thereby the number of false negative patients, has great impact on the diagnostic performance of the D-Dimer test [9,13,25–27]. Our results are in complete agreement with this statement. High AUCs are observed for all five tests when ROC-curves are compared for patients suffering from proximal DVT, whereas the AUCs are lower for ROC-curves from patients with both proximal and distal DVT and much lower when only patients suffering from distal DVT are included demonstrating that the D-Dimer assays are not sufficiently sensitive to exclude distal DVT. In all cases, however, comparable AUCs are observed for all five tests. Thus, the overall clinical performance of the AQT D-Dimer is comparable with the performance of D-Dimer tests performed at central laboratory analyzers and the five tests show comparable NPV, whereas the sensitivity of the tests varies in particular when distal DVTs are examined. The specificity and PPV of the AQT D-Dimer, however, are higher than most of the other tests employed.

The cut-off for D-Dimer tests can be calculated based on the ROC-curves [28,29] of the tests applied, but the cut-off concentrations calculated on ROC-curves take into account the overall test performance [30]. D-Dimer tests are mainly used for exclusion of DVT and the D-Dimer concentration used as cut-off must ensure that the number of false negative patients is low, that is the NPV of the test must be high. Thus, the cut-off concentrations recommended in the present study and provided by the manufacturers of the D-Dimer kits favour high NPV more than a balanced approach between the sensitivity and the specificity of the tests.

We demonstrate that the AQT D-Dimer is a robust test because variation of cut-off values from 0.35 to 0.50 mg/L are virtually without effect on the NPV and LR-neg and in particular that the cut-off concentration may be increased to 0.50 mg/L without reduction of the clinical value of the test if only patients with proximal DVT are considered (data not shown). The clinical performance of D-Dimer assays, however, depends also on the prevalence of DVT in the study population and the pre-test clinical probability of DVT in particular the specificity of the test, being higher in patients with low clinical probability of DVT [15,31]. The prevalence of DVT in our study population is rather high, but comparable with other studies focusing on unselected patients [4,15]. Also, the pre-test clinical probability for DVT in our patients is presumably high as the study population is unselected. Thus, an improved clinical performance should be expected if the D-Dimer tests were applied on a population with low clinical probability and low prevalence of DVT.

We conclude that the AQT D-Dimer is a rapid POC test for determination of D-Dimer with analytical performance comparable with D-Dimer assays performed on central laboratory analyzers. The use of whole blood facilitates a rapid sample turnaround time, the reproducibility of the test is good and the clinical performance, in particular when dealing with proximal thrombosis, is excellent. These features indicate that the AQT D-Dimer can be a valuable tool for exclusion of DVT.

Conflict of interest statement

None.

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