

# Comparing D-dimer assays

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When a new D-dimer method is to be implemented either as a substitute for a currently used D-dimer assay or as a supplement, maybe as a point-of-care assay, it is necessary to do a clinical comparison of the “old” assay versus the “new” assay. The major pitfalls in D-dimer comparisons are addressed in this paper.

Comparing two D-dimer assays based on publications of different studies should be done with great caution and the following should always be kept in mind:

- Including many patients with elevated D-dimer concentrations due to other causes than VTE may give a false increase in sensitivity and negative predictive value
- The prevalence of VTE has an influence on the predictive values – higher prevalence gives lower NPV
- A high ratio of proximal to distal DVT will increase both the sensitivity and the negative predictive value
- A study focused on PE will have a higher sensitivity and negative predictive value compared to a study including DVT without PE

- Changing cut-off will change clinical sensitivity and specificity
- When ultrasound is used as the “gold standard”/ confirmatory method, the sensitivity and negative predictive value are falsely increased compared to when venography/angiography is used
- When all the persons in the tested population are not examined by a confirmatory method, falsely increased clinical sensitivity and negative predictive value may be seen

The ideal D-dimer method comparison is based on results obtained with both/all assays on the same samples and when all test persons are examined with a confirmatory method.

Less ideal is a comparison based on results obtained with both/all assays on the same samples where only all the test persons with discrepant results (below cut-off with one assay, above cut-off with the other assay) are examined with a confirmatory method.

## Glossary and abbreviations

Abbreviation / term Meaning	
Discrepant	Result below cut-off with one method, above cut-off with the other method
Distal	Located away from the centre of the body
DVT	Deep venous thrombosis
NPV	Negative predictive value, fraction of test-negatives who do not have disease
PE	Pulmonary embolism
PPV	Positive predictive value, fraction of test-positives who do have disease
Proximal	Located near the centre of the body
Sensitivity	Fraction of persons with disease characterized as sick with the test in question
Specificity	Fraction of persons without disease characterized as healthy with the test in question
Spectrum of disease	The range of disease states found in the patient population upon which the test is to be used
VTE	Venous thromboembolism (includes DVT and PE)

## Introduction

When a new D-dimer method is to be implemented either as a substitute for a currently used D-dimer assay or as a supplement, maybe as a point-of-care assay, it is necessary to do a clinical comparison of the "old" assay versus the "new" assay.

Comparing D-dimer assays is challenging. First of all a comparison should be primarily a clinical comparison because the specificities for D-dimer assays can be in the range from 30 to above 90 % [1, 2]. As the number of false-positives is equal to 100 % minus specificity, it can be seen that a relatively high number of false-positives seems to be accepted. The main reason for this is that a D-dimer test is used for ruling out VTE in low-risk patients and that patients with a positive test are examined with a confirmatory method (imaging) [3].

The challenges come from the fact that D-dimer is not a standardized method [4]. There exists no reference method and there are no international standards available. Furthermore, the D-dimer molecules belong to a heterogeneous group of molecules. This means

that it should be known which method was used to interpret a result, and you cannot directly compare two results obtained with different methods.

A clinical comparison can be based on:

- Data from several studies described in the literature, thus comparing separate studies
- A comparison study with the same samples measured by both/all the assays to be compared

Several issues have to be taken into consideration to make a comparison scientifically reliable.

## Comparisons of D-dimer methods

### Comparison based on several literature studies

Caution should be exercised when comparing two or more D-dimer methods between studies based on data in literature. In this context literature includes articles in peer-reviewed journals and the information given in

510(k) summaries/decision summaries from the FDA and in manufacturer's inserts.

The following has a major impact on the results of a D-dimer study and may vary from study to study [2, 5]:

- Patient population
- Prevalence of VTE
- Spectrum of disease/mix of VTE according to position
  - Distal – located in the calf vein
  - Proximal – located in the thigh
  - Pulmonary embolism
- Assay performance – clinical sensitivity and specificity
- Gold standard used to establish diagnosis
  - Ultrasound
  - Angiography/venography
  - Is the chosen gold standard used in all patients?

### Patient population

An elevated D-dimer level may be due to [5, 6, 7]:

- Hospitalization
- Old age
- Pregnancy
- Surgery
- Trauma
- Disseminated intravascular coagulation (DIC)
- Coronary disease
- Thrombolytic treatment
- Cancer
- Liver disease
- Infection/inflammation
- Hematoma

The mixture of these conditions is most often not known or disclosed in published studies.

Many elevated samples due to other causes than DVT or PE will have a strong negative influence on the specificity of an assay. Furthermore, testing on groups with a high incidence of elevated D-dimer due to other causes may also have an influence on sensitivity. In a patient with a D-dimer elevation above the cut-off due to reasons other than DVT or PE, a small DVT may be detected due to the elevation, which was present even before the DVT.

This gives a false increase in the measured clinical sensitivity.

### Prevalence of VTE

Prevalence has an impact on the predictive values of a test. This can be seen by simple theoretical calculations.

		Disease (the "the truth")		Predictive values
		+	-	
Test	+	True positives (TP)	False positives (FP)	PPV=TP/(TP+FP)
	-	False negatives (FN)	True negatives (TN)	NPV=TN/(TN+FN)
		All with disease	All without disease	

TABLE I: Comparing a method with the "truth"

If we take a method with the following performance characteristics:

Sensitivity = 90 %

Specificity = 45 %

and we test four different populations, all with 100,000 individuals but with different prevalence of disease, we get differences in predictive values.

Prevalence		Disease (the "the truth")		Predictive values
		+	-	
0.1 %	+	90	54945	PPV = 0.16 %
	-	10	44955	NPV = 99.98 %
3 %				
Test	+	2700	53350	PPV = 4.8 %
	-	300	43650	NPV = 99.3 %
17 %				
Test	+	15300	45650	PPV = 25.1 %
	-	1700	37350	NPV = 95.6 %
75 %				
Test	+	67500	13750	PPV = 83.1 %
	-	7500	11250	NPV = 60 %

TABLE II: Test used in populations with different prevalence

As the prevalence increases, so does the positive predictive value, while the negative predictive value decreases.

Hospitals may group patients into low-pretest-probability, medium-pretest-probability and high-pretest-probability patients. The pretest probabilities of DVT for these groups are often given as 3 %, 17 % and 75 % [8].

However, at one hospital the true prevalence of DVT in the low-pretest-probability group may be 7 %, at another hospital it may be 1 %. This means that the calculated NPV at one site would be several percentage points lower than at the other site due solely to the differences in prevalence (all other factors being equal).

### Spectrum of disease/mix of VTE according to position

Ideally, the ability of a test to correctly identify patients with and without a specific disease should not vary between patients. However, the spectrum of disease found among patients in the population upon which the test is to be used will often have an influence on the clinical performance of the test.

Therefore performance measures apply only to populations having a similar spectrum of disease to that used to estimate the performance measures [9].

The spectrum of disease for VTE patients is primarily determined by the site of the DVT [10], distal (calf veins) or proximal (thigh veins) and whether PE is present or not.

A DVT in the calf veins is usually smaller and gives rise to lower D-dimer concentrations than a DVT in the thigh veins [10]. For this reason the risk of getting a false negative result is larger when the DVT is distal compared to the risk when the DVT is proximal.

Most D-dimer studies are performed in hospitals on patient populations with a predominance of proximal DVT. In an outpatient study including 393 patients with clinically suspected symptomatic DVT of the lower extremities [10] a total of 137 of 393 patients had a proven DVT and the majority (59 %) presented with distal DVT.

Twenty-eight out of 81 patients with distal DVT had a normal D-dimer, compared with two of 56 patients with

proximal DVT. The sensitivity for distal DVT was only 65 % compared with 96 % for proximal DVT; the negative predictive values were 84 % and 99 %, respectively.

PE is usually associated with a higher D-dimer concentration than a DVT without PE [11]. This means that clinical sensitivities, specificities and predictive values cannot be compared between studies focusing on PE and DVT, respectively.

### Assay performance – clinical sensitivity and specificity

A comparison of either clinical sensitivities or specificities without the other parameter is an arbitrary one, since changing the cut-off can increase sensitivities or specificities at the expense of lowering the other parameter [9]. A joint comparison of clinical sensitivity/specificity pairs may still be meaningful if the sensitivity and specificity of one method are both higher than the sensitivity and specificity of the other method.

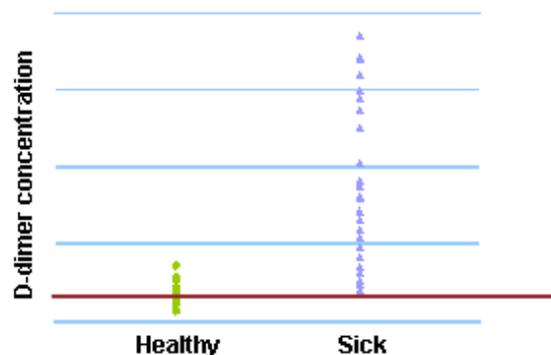


FIG. 1: Cut-off = 400 µg/L

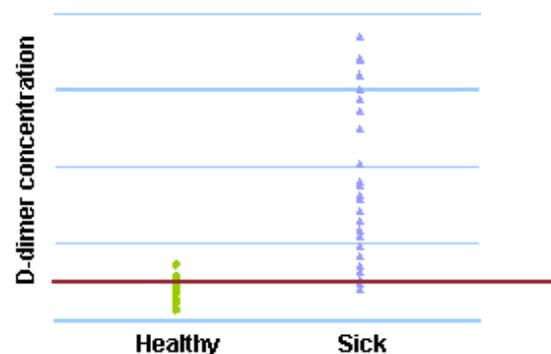


FIG. 2: Cut-off = 500 µg/L

The figures above are examples of the trade-off between clinical sensitivity and specificity. When 400 µg/L is chosen as cut-off, the sensitivity is 100 % and the specificity is 54 %. When the cut-off is increased to 500 µg/L, the sensitivity decreases to 92 % and the specificity increases to 79 %.

### Gold standard used to establish diagnosis

The gold standard used to establish a VTE diagnosis is usually based on either ultrasound or angiography/venography.

Ultrasound has a limited ability to image the calf veins where the thrombi are smaller [2]. This means that there is a risk of classifying patients with calf DVT as non-sick. This will give bias such as falsely increased sensitivity and negative predictive value because a negative D-dimer in combination with a false negative ultrasound DVT test will be registered (in error) as a true negative test.

A review of 26 studies [2] shows up to 9.2 % differences in NPV for each D-dimer test when evaluated versus a diagnostic test detecting all clots, and when evaluated versus a test detecting primarily the clots in the thigh.

It is also important to notice if all test persons were examined with a confirmatory method. In some studies only patients with a positive D-dimer test are examined with a confirmatory method. VTE is often asymptomatic, misdiagnosed, and unrecognized at death [12].

Some estimates of the likelihood of diagnosing DVT are as low as 10 %. Due to a lack of routine post-mortem examinations it is difficult to estimate the true incidence of VTE. We can only say that it is underestimated.

The fact that VTE is underestimated is also supported by a meta-analysis finding that ~70 % of the major (either fatal or contributing to death) pulmonary embolic events diagnosed at autopsy had been missed by the attending clinicians [13].

Falsely increased sensitivity and negative predictive value may be seen when a person has a negative D-dimer and a DVT that is not detected due to lack of examination.

### Comparison with the same samples measured by both/all the assays to be compared

The ideal D-dimer method comparison is based on results obtained with both/all assays on the same samples and when all tests persons are examined with a confirmatory method.

Less ideal is a comparison based on results obtained with both/all assays on the same samples where only all the test persons with discrepant results (below cut-off with one assay, above cut-off with the other assay) are examined with a confirmatory method.

It should be kept in mind that a comparison based on results obtained with both/all assays on the same samples can be directly used to compare the relative performances of the assays, but the relative performances may be different if the assays are tested on another population with a different prevalence, a different spectrum of disease, etc.

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