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D-dimer: preanalytical, analytical, postanalytical variables, and clinical applications

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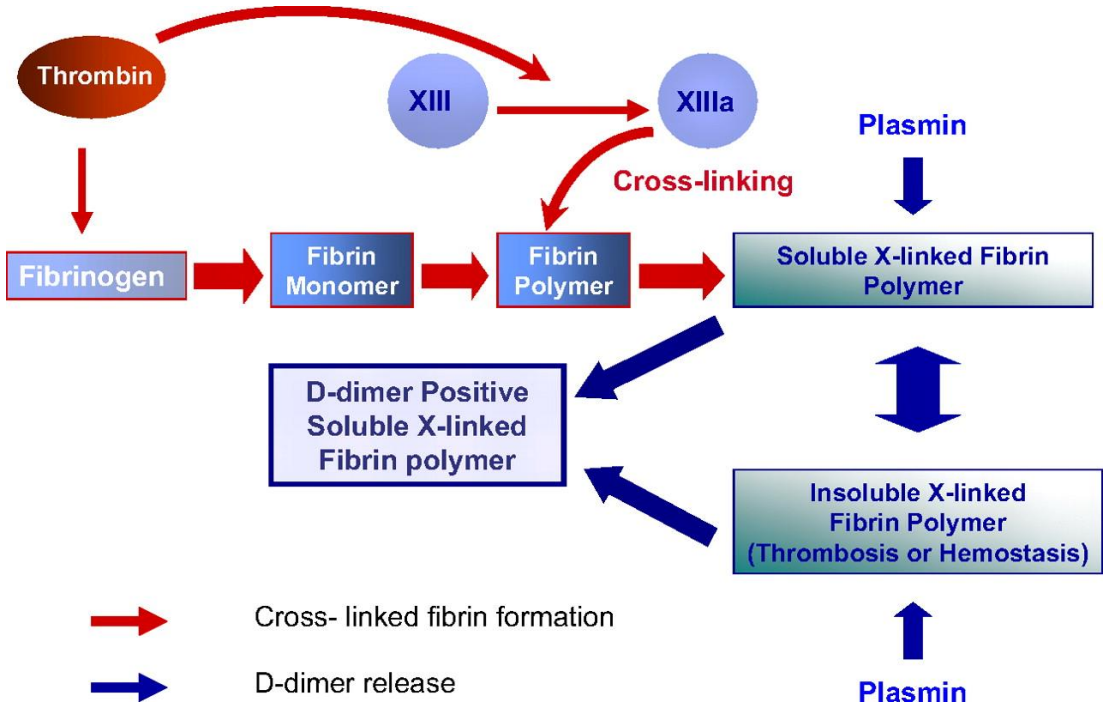
Summary

1. What is D-dimer
2. Preanalytical variables
3. Analytical variables
4. Postanalytical variables
5. Clinical applications

1. What is D-dimer

What is D-dimer

- The D-Dimer antigen is a marker of fibrin degradation that is formed by the sequential action of 3 enzymes: (1) thrombin, (2) factor XIIIa, and (3) plasmin
- Their presence reflects concomitant activation of both coagulation and fibrinolysis



Adam et al. Blood 2009 Mar 26;113(13):2878-87

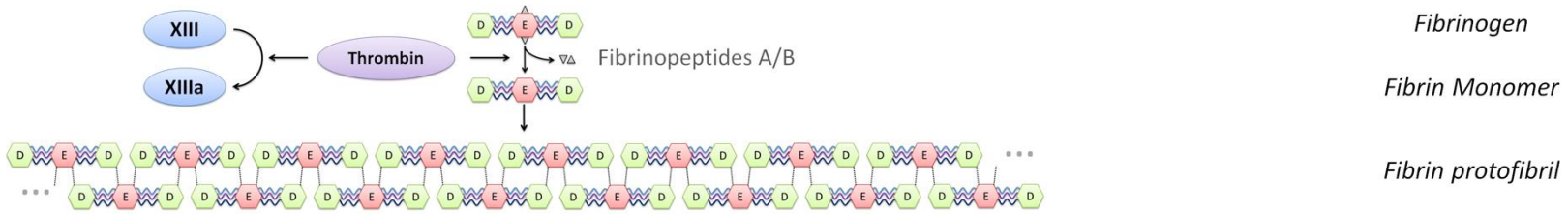
Mechanism of D-dimer production

Fibrinogen

- **Fibrinogen = plasma glycoprotein composed of three different pairs of polypeptide chains (Aα-, Bβ-, and γ-) connecting two-outer D-domains to the central E-domain**

Thachil et al. Methods Mol Biol. 2017;1646:91-104

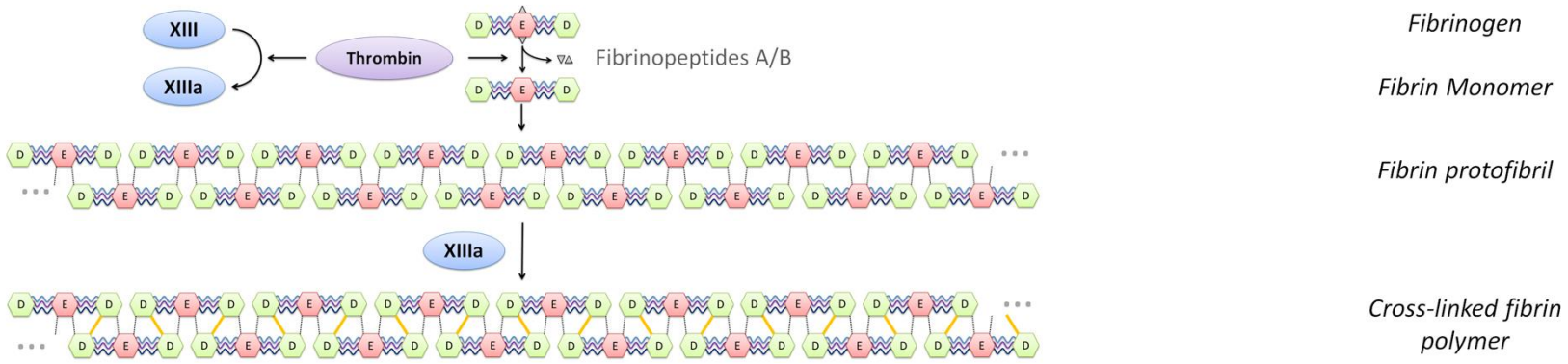
Mechanism of D-dimer production



- **Thrombin enzymatically cleaves two cryptic polymerization sites located on the E-domain, thus leading to generation of both highly self-adhesive fibrin monomers and fibrinopeptides A and B**
- **Fibrin monomers will then bind one another, to form a soluble network**

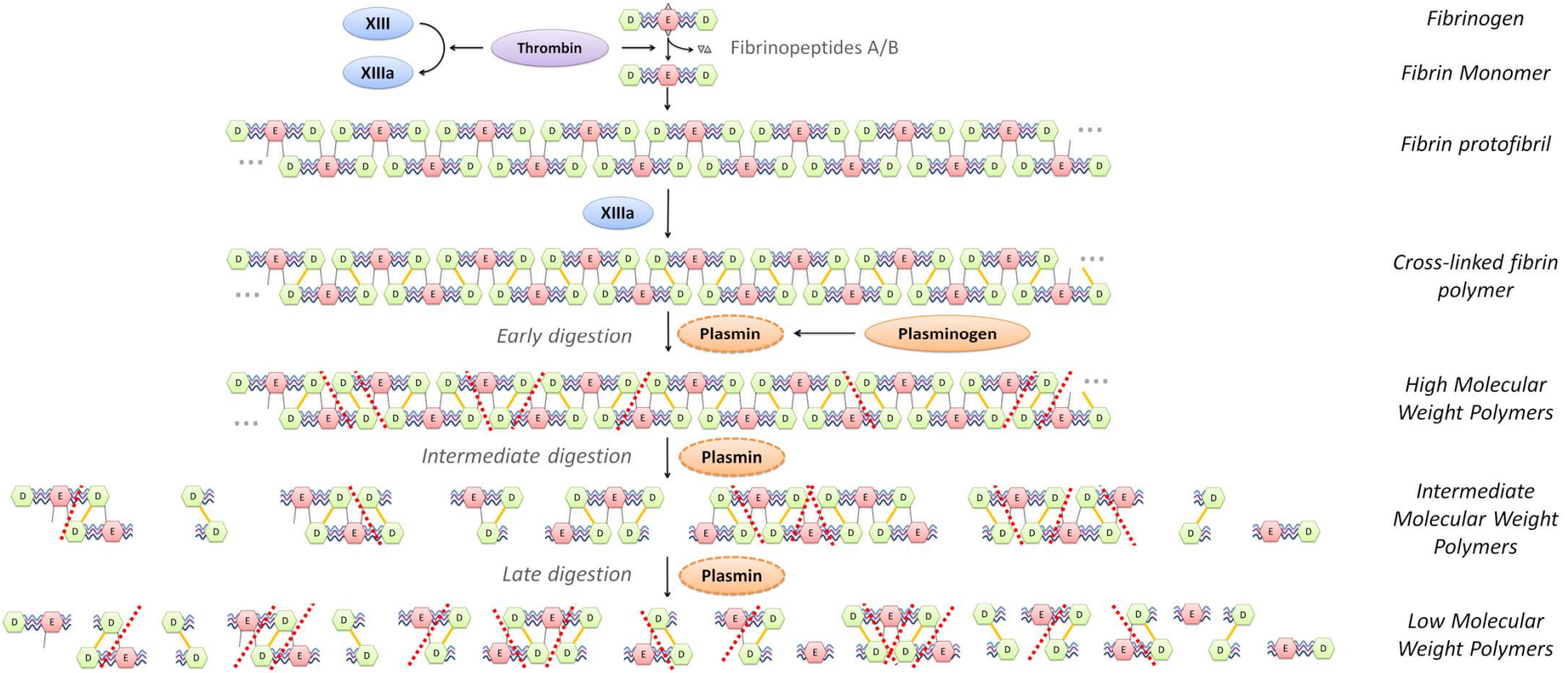
Favresse et al. Crit Rev Clin Lab Sci. 2018, minor revision

Mechanism of D-dimer production



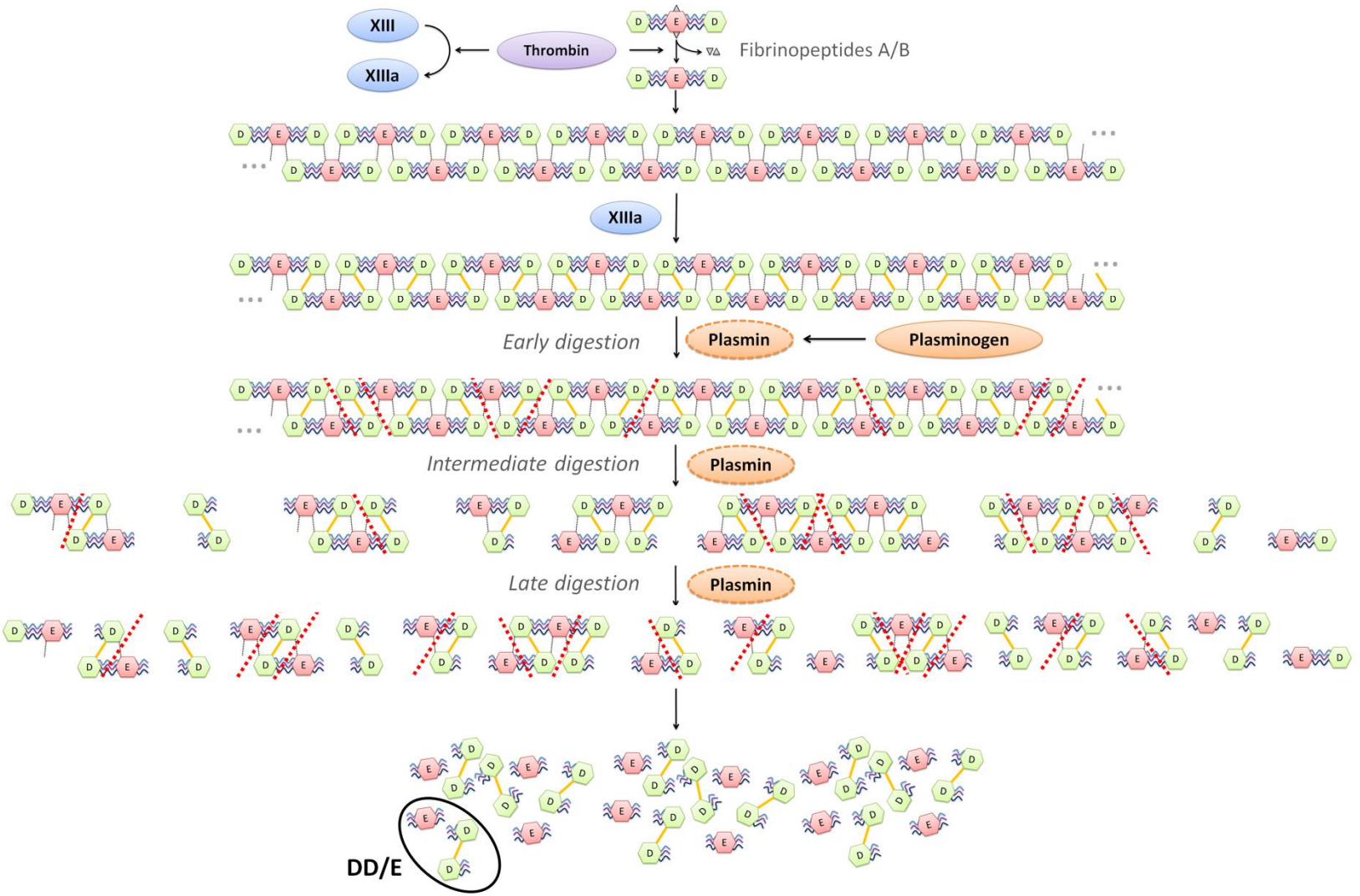
- **Simultaneously, the complex between soluble fibrin polymers, thrombin, and plasma factor XIII promotes the formation of factor XIIIa, which catalyzes covalent cross-linking of fibrin polymer via intermolecular bonds formed between lysine and glutamine residues, thus enabling the generation of stable and insoluble clots**

Mechanism of D-dimer production



- **The further fibrinolytic pathway leads to degradation of stabilized clot through plasmin activation**

Mechanism of D-dimer production



Fibrinogen

Fibrin Monomer

Fibrin protofibril

Cross-linked fibrin polymer

High Molecular Weight Polymers

Intermediate Molecular Weight Polymers

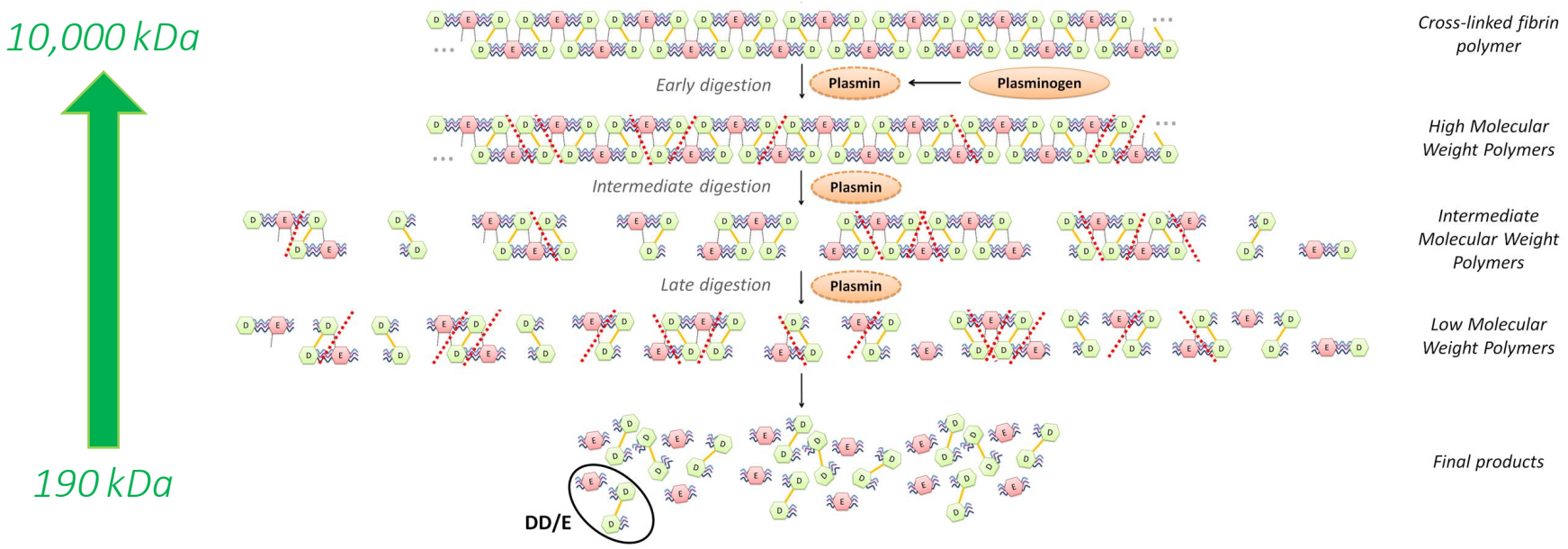
Low Molecular Weight Polymers

Final products

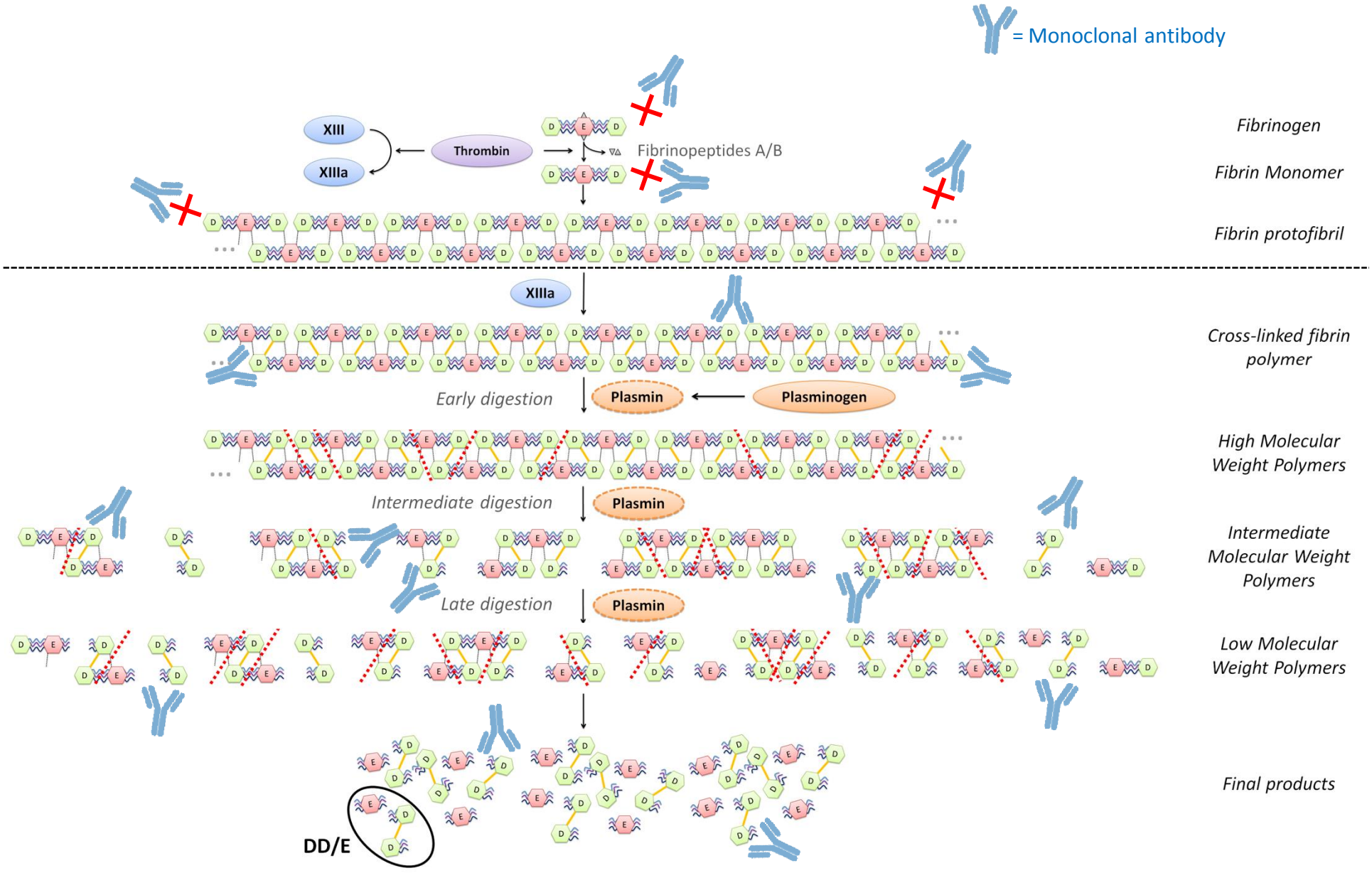
By Nicolas Bailly

What is D-dimer

- "Fragment D-dimer" initially used to describe the **final plasmin digestion products** (resistant to further plasmin breakdown) of factor XIIIa–cross-linked fibrin clot (fragment D-dimer/fragment E complex)
- However, the actual D-dimer **antigen** (which can be detected by current immunoassays) is not necessarily the DD/E complex. In fact, the term D-dimer comprises a **broad mixture of degradation products of cross-linked fibrin**



What is D-dimer



Realized by Nicolas Bailly

Fibrinogen degradation

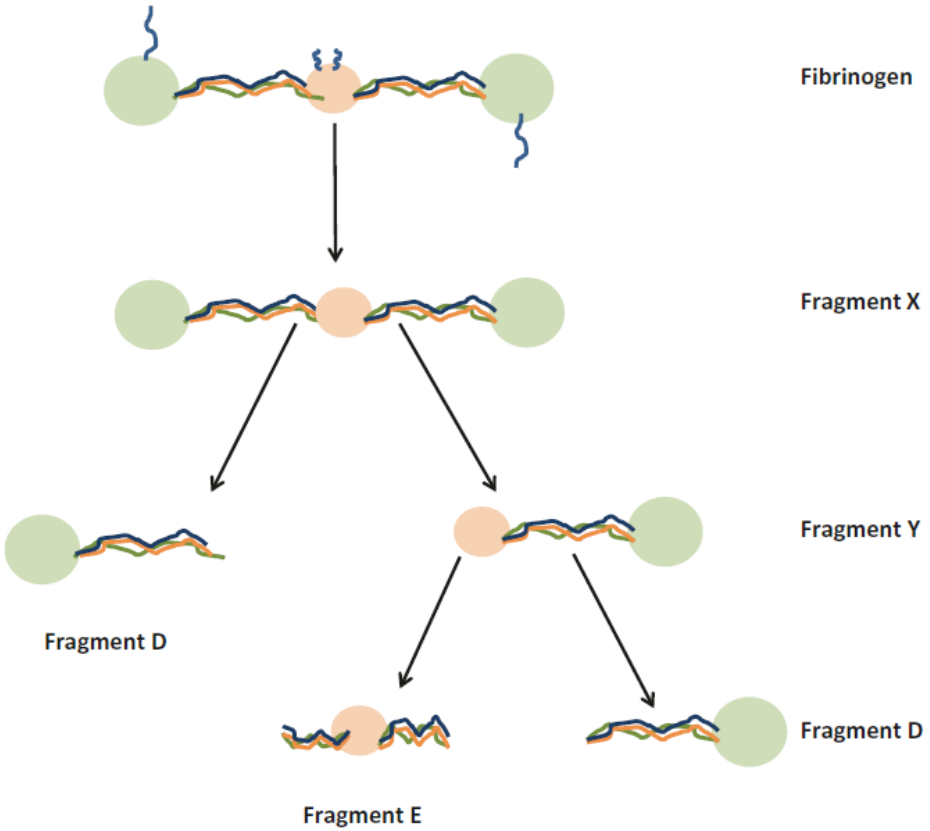


Fig. 1 (**a**) Schematic representation of human fibrinogen. The Aα-, Bβ-, and γ-chains are shown as *fibrils*. The central nodules contain all the six chains and are referred to as the "E" regions, and are flanked by the two distal "D" nodules. (**b**) The process of fibinogenolysis by plasmin. During the conversion of fibrinogen to fibrin by thrombin, fibrinopeptides A and B are removed. This will create fragment X which is further broken down to fragment D and fragment Y and then into a second fragment D and a fragment E

Thachil et al. Methods Mol Biol. 2017;1646:91-104

What is D-dimer

- **Mechanism of D-dimer production**

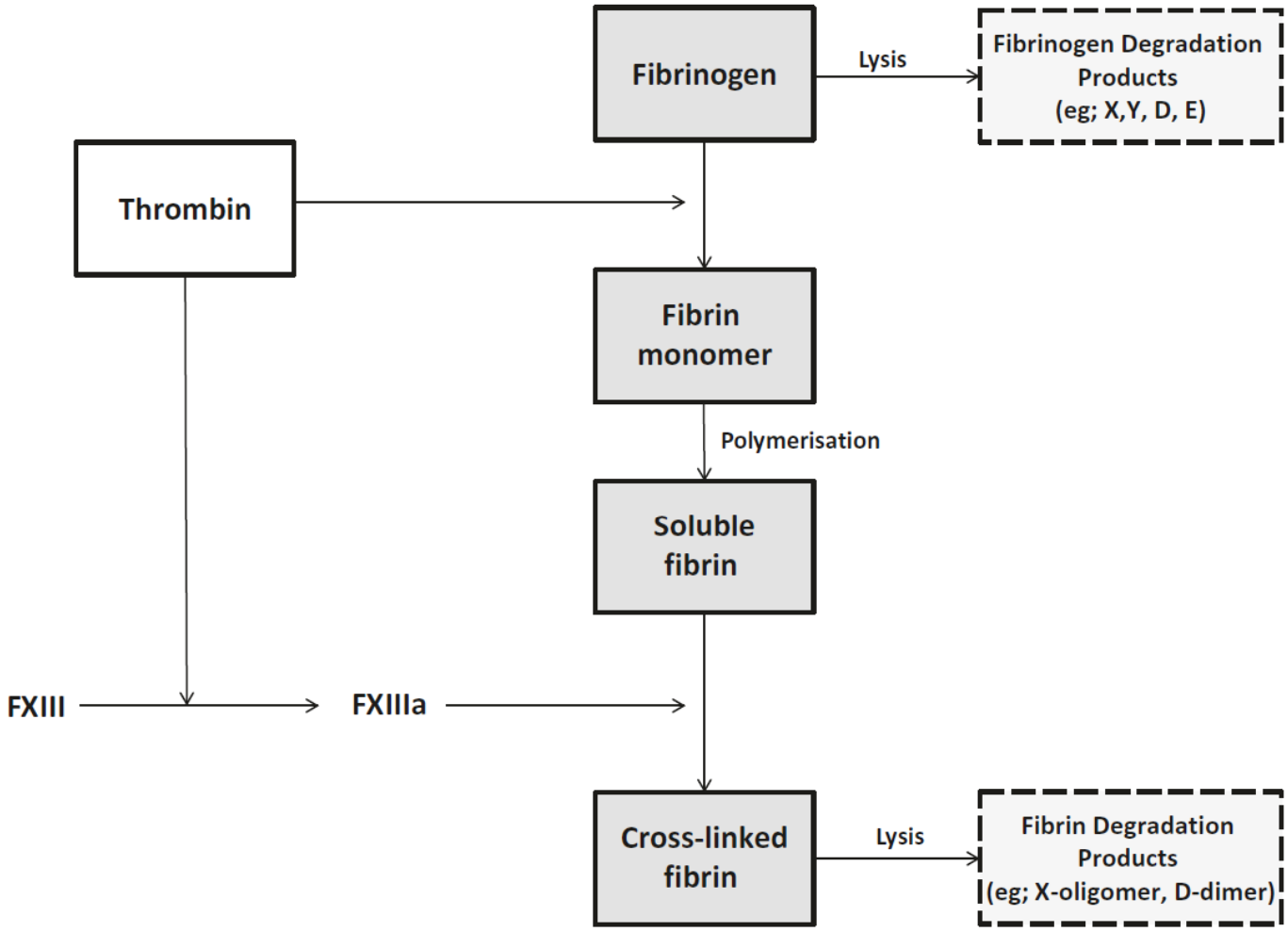


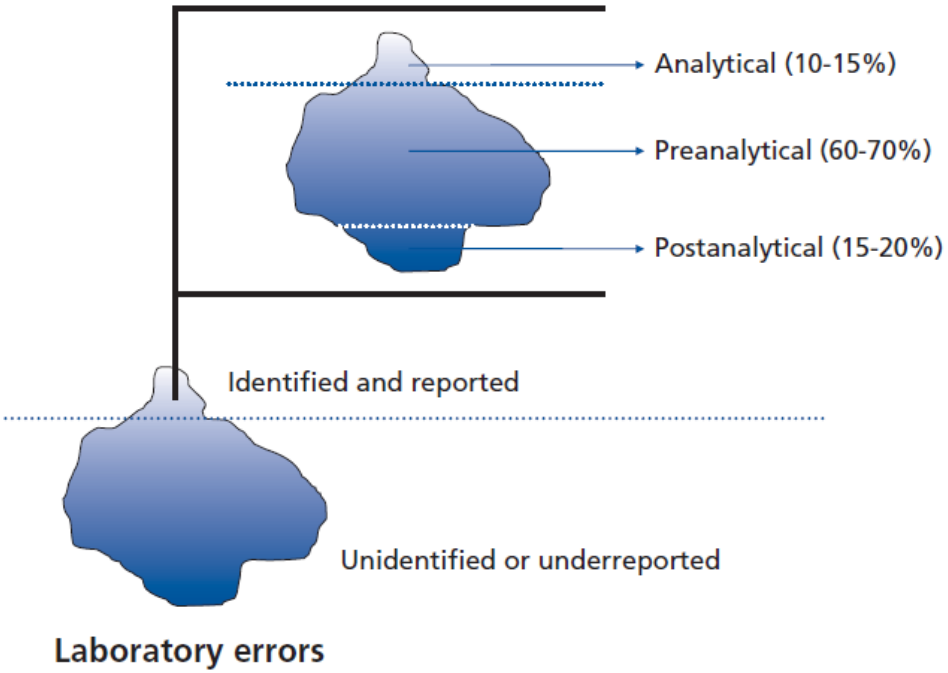
Fig. 2 Fibrinogen to fibrin conversion by thrombin. Once fibrinopeptides are removed, the resulting fibrin monomers are acted upon by activated coagulation factor XIII to form a stable cross-linked fibrin

Thachil et al. Methods Mol Biol. 2017;1646:91-104

2. Preanalytical variables

Preanalytical phase in medical laboratories

- Preanalytical errors have a frequency of **60-70%**, thus much higher than those occurring in the analytical phase (i.e., **10-15%**) and in the postanalytical phase (i.e., **15-20%**). Preanalytical errors are mainly related to intensive manual activities.



Lippi and Favaloro. Quality in Laboratory Hemostasis and Thrombosis. Causes of Errors in Medical Laboratories. 2013: p. 22-31
Magnette et al. Thromb J. 2016;14:49

Sample collection

1. Butterfly devices and needle bore size

Recommendations	Specific data regarding D-dimer
19 to 22 G	19 to 25 G
Butterfly deviced discouraged	Tolerated*

*A discard tube is mandatory for removing air contained within the tubing, which may be associated with collection of an inadequate volume of blood

Adcock. Quality in Laboratory Hemostasis and Thrombosis. Sample Integrity and Preanalytiocal Variables. 2013: p. 45-56
Magnette et al. Thromb J. 2016;14:49
Lippi et al. Clin Chem Lab Med 2006;44(8):1009-14
Lippi et al. J Thromb Haemost. 2005 Feb;3(2):389-91

Sample collection

2. Anticoagulant type and tube material

Recommendations	Specific data regarding D-dimer
- 105–109 mmol/L sodium citrate, buffered anticoagulant - Serum, heparinized/EDTA plasma samples cannot be accepted	- EDTA or heparinized plasma sample tolerated* - Serum discouraged**
Respect the required ratio of sodium citrate to whole blood (1:9)	Underestimation
Non-activating material (silicone-coated glass or polypropylene plastic)	Glass or plastic

* Dilution factor has to be taken into account

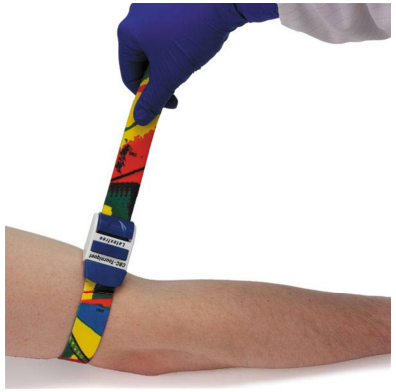
** False positive results were frequently encountered in patients under anticoagulant treatment, whilst false negative values were also seen when FDP were entrapped in the clot

Clinical and Laboratory Standards Institute (CLSI). H21-A5. 2008
Magnette et al. Thromb J. 2016;14:49
Adcock. Quality in Laboratory Hemostasis and Thrombosis. Sample Integrity and Preanalytiocal Variables. 2013: p. 45-56
Leroy-Matheron et al. Trhomb Res. 1994;74:399-407
Gosselin et al. Am J Clin Pathol. 2004;122:843-8
Yavas et al. Turk J Haematol. 2012;29:367-75

Sample collection

3. Tourniquet use

Recommendations	Specific data regarding D-dimer
Never remain in place for more than 1-2 min	↗ 13.4% after 3 min venous stasis



Adcock. Quality in Laboratory Hemostasis and Thrombosis. Sample Integrity and Preanalytiocal Variables. 2013: p. 45-56
Magnette et al. Thromb J. 2016;14:49
Lippi et al. J Thromb Haemost. 2005;3:289-91

Preanalytical phase in haemostasis laboratories

DE GRUYTER Clin Chem Lab Med 2018; aop

EFLM Paper

EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

COLABIOCLI

Ana-Maria Simundic*, Karin Bölenius, Janne Cadamuro, Stephen Church, Michael P. Cornes, Edmée C. van Dongen-Lases, Pinar Eker, Tanja Erdeljanovic, Kjell Grankvist, Joao Tiago Guimaraes, Roger Hoke, Mercedes Ibarz, Helene Ivanov, Svetlana Kovalevskaya, Gunn B.B. Kristensen, Gabriel Lima-Oliveira, Giuseppe Lippi, Alexander von Meyer, Mads Nybo, Barbara De la Salle, Christa Seipelt, Zorica Sumarac and Pieter Vermeersch, on behalf of the Working Group for Preanalytical Phase (WG-PRE), of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and Latin American Working Group for Preanalytical Phase (WG-PRE-LATAM) of the Latin America Confederation of Clinical Biochemistry (COLABIOCLI)

Joint EFLM-COLABIOCLI Recommendation for venous blood sampling

v 1.1, June 2018

https://doi.org/10.1515/cclm-2018-0602
Received June 9, 2018; accepted June 10, 2018

Simundic et al. Clin Chem Lab Med 2018, Epub ahead of print

Sample delivery to the laboratory

Recommendations	Specific data regarding D-dimer
At ambient temperature (15-22°C)	4°C or less possible
Vertical position	Pneumatic system tube tolerated*
Usually <1 hour, no more than 4 hours	Stable at various conditions

*It is advisable that each laboratory assesses its local PTS, since the systems are rather heterogeneous in terms of length, internal diameter, maximal acceleration force and speed

Adcock. Quality in Laboratory Hemostasis and Thrombosis. Sample Integrity and Preanalytiocal Variables. 2013: p. 45-56
Magnette et al. Thromb J. 2016;14:49
Schutgens et al. Clin Chem. 2002;48:1611-3
Wallin et al. Clin Chem Lab Med. 2008;46:1443-9
Le Quellec et al. Thromb Res. 2017;153:7-13

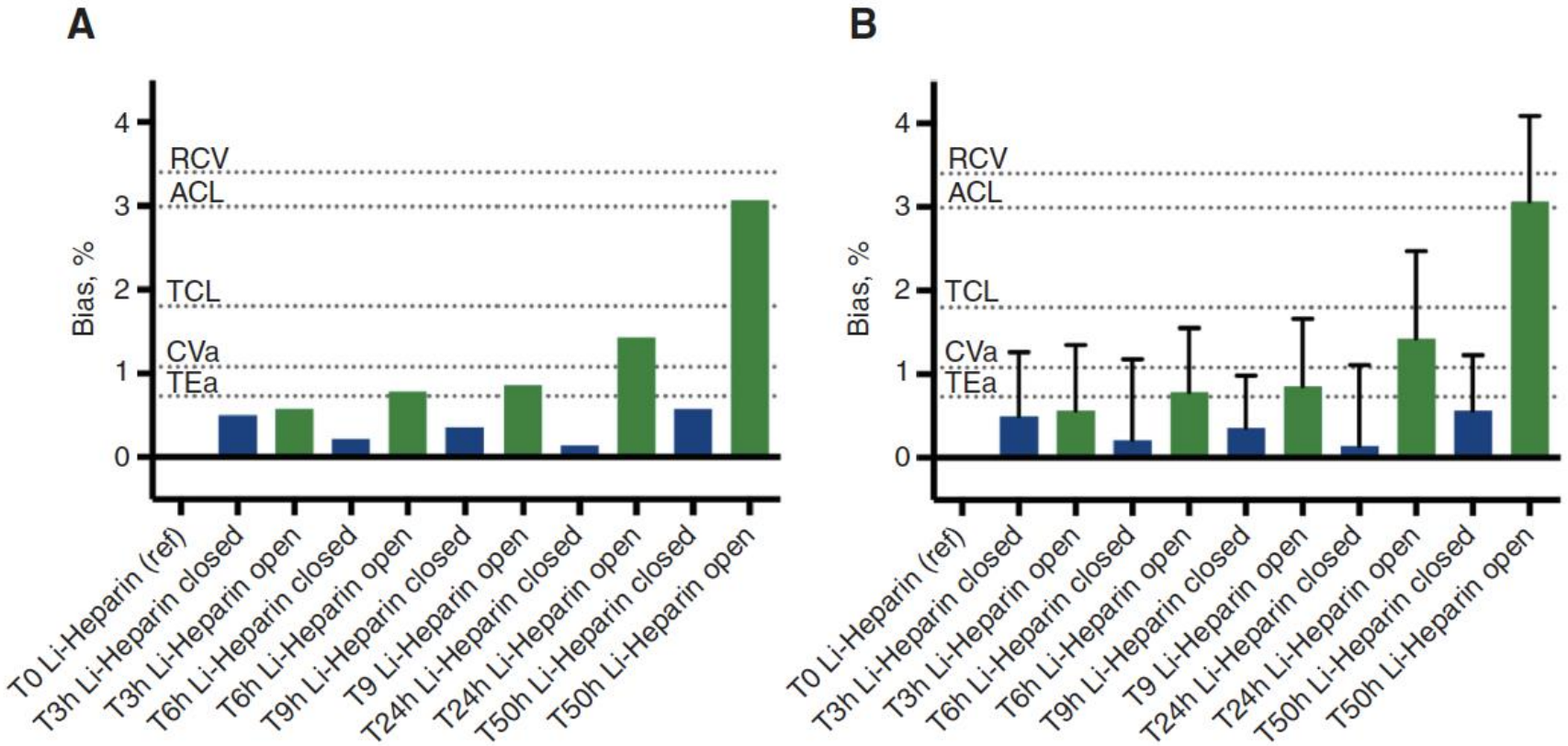
Stability of D-dimer

Stability	Conditions	Anticoagulant	Plasma/whole blood	D-dimer assay	Subjects	Stability criteria	Reference
24h	RT	Heparin	Plasma	Tina-quant® (Roche)	17 patients	Student t-test and regression equation	[39]
24h	RT	Citrate	Plasma	Tina-quant® (Roche)	15 patients	Student t-test and regression equation	[39]
6h	RT	Citrate	Plasma	Innovance® (Siemens)	40 patients	10% deviation from baseline, regression equation and discordance at the cutoff level of 0.5 mg/L FEU	[69]
24h	RT	Citrate	Plasma	Innovance® (Siemens)*	80 patients	10% deviation from baseline, analysis of variance, regression equation and Pearson correlation coefficient	[78]
24h	RT	Citrate	Whole blood	Vidas® (bioMérieux)	117 patients	Spearman correlation coefficient, regression equation and discordance at the cutoff level of 500 ng/mL FEU	[79]
24h	RT	Citrate	Whole blood	Innovance® (Siemens)	44 patients	10-20% deviation from baseline, Student t-test and regression equation	[76]
24h	RT	Citrate	Whole blood	ACL-TOP® (Werfen)	26 patients	Wilcoxon's paired t-test, regression equation and bias plot	[46]
52h	RT	Citrate	Whole blood	Asserachrom® (Stago)	59 patients	Analysis of variance, 10% deviation from baseline	[75]
8h	RT	Citrate	Whole blood	ACL-TOP® (Werfen)	144 patients	Analysis of variance, Student t-test or Wilcoxon signed rank test, Bland-Altman plot and discordance at the cutoff level of 0.5 ng/mL FEU	[47]
24h	2-8°C	Citrate	Plasma	Innovance® (Siemens)	40 patients	10% deviation from baseline, regression equation and discordance at the cutoff level of 0.5 mg/L FEU	[69]
24h	4°C	Citrate	Plasma	Innovance® (Siemens)*	80 patients	10% deviation from baseline, analysis of variance, regression equation and Pearson correlation coefficient	[78]
24h	4°C	Citrate	Plasma	Vidas® (bioMérieux)	20 patients	Wilcoxon's paired t-test, 10% deviation from baseline	[77]
24h	4°C	Citrate	Whole blood	ACL-TOP® (Werfen)	26 patients	Wilcoxon's paired t-test, regression equation and bias plot	[46]
24 months	-24 and -75°C	Citrate	Plasma	STA-Liatest® (Stago)	Plasma pool (6 patients)	Statistical change**, 5-10% deviation from baseline	[72]
2 weeks	-20°C	Citrate	Plasma	STA-Liatest® (Stago)	23 HV and 18 patients	Paired t-test, 10% deviation from baseline	[71]
36 months	-60°C (or less)	Citrate	Plasma	Innovance® (Siemens)	40 patients	10% deviation from baseline, regression equation and discordance at the cutoff level of 0.5 mg/L FEU	[69]
9 years	-80°C	Citrate	Plasma	STA-Liatest® (Stago)	60 patients	Wilcoxon's paired t-test	[70]

RT = room temperature, FEU = fibrinogen equivalent units, HV = healthy volunteers, *using the Sysmex® CA 7000 platform, **specific test not mentioned.

Favresse et al. Crit Rev Clin Lab Sci. 2018, minor revision

Assessment of *in vitro* stability



Bastin, Favresse et al. Clin Chem Lab Med. 2018 Apr 25;56(5):e121-e124

Sample processing

1. Centrifugation

Recommendations	Specific data regarding D-dimer
1,500 x g for at least 15 minutes at RT	- 4,500 x g for 2 minutes at RT - 4°C or 12° also possible

Clinical and Laboratory Standards Institute (CLSI). H21-A5. 2008
Bernard et al. Clin Chem Lab Med. 2002;40 (Suppl. S9):S350
Lippi et al. Clin Chem. 2006 Mar;52(3):537-8

Sample processing

2. Interfering susbtances

Recommendations	Specific data regarding D-dimer
Not analyse if visible hemolysis*,**	Cell-free hemoglobin (i.e., <3 g/L)
Icterius	Less widely discussed in the literature
Lipemia	
Paraproteinemia	

* *In vitro* hemolysis still represents one of the most frequent causes of preanalytical problems in clinical laboratories, with a prevalence ranging between 30-70% of all unsuitable specimens

** The majority of hemolyzed samples (±95%) from clinical laboratories are only mildly hemolytic (cell-free hemoglobin **0.3-0.6** g/L)

Clinical and Laboratory Standards Institute (CLSI). H21-A5. 2008
Adcock. Quality in Laboratory Hemostasis and Thrombosis. Sample Integrity and Preanalytiocal Variables. 2013: p. 45-56
Magnette et al. Thromb J. 2016;14:49

Specific preanalytical data regarding D-dimer testing

Pre-analytical variables	General recommendations in hemostasis laboratories	Specific data regarding D-dimer
Sample collection		
- Needle bore size	19-22 G	23-25 G also tolerated
- Butterfly devices	Discouraged	Tolerated
- Tube material	Non-activating material (silicone-coated glass or polypropylene plastic)	Glass or plastic
- Anticoagulant sample	Sodium citrate 3,2% (105-109 mmol/L)	Heparin and EDTA tolerated*
- Tourniquet use	Removed as soon as the needle is in the vein (max 1-2 minutes)	Longer tourniquet use (i.e., 3 min) not tolerated
Sample delivery to the laboratory	At RT (15-22°C), in vertical position, usually <1 hours	PTS tolerated
Sample processing		
- Centrifugation	At RT, 1,500 x g for at least 15 min	Faster protocol allowed (at RT, 4,500 x g for 2 min)
- Interfering substances	Do not analyze samples with hemolysis	Cell-free hemoglobin i.e., <3 g/L tolerated
Stability, storage and F/T effects	At RT (15-22°C), no more than 4 hours	At least 24h at RT or at 2-8°C or years at -60 to -80°C No impact of F/T procedure

G = gauge, RT = room temperature, PTS = pneumatic tube system, F/T = freezing/thawing, * correction factor needed (dilution).

Favresse et al. Crit Rev Clin Lab Sci. 2018, minor revision

3. Analytical variables

Inter-laboratory variations

- **History of D-dimer assays**

1970s

- First-generation of D-dimer assays (qualitative assays: hemagglutinin inhibition, immunoelectrophoresis, immunodiffusion, …)
- Polycloncal antibodies (detection of both fibrinogen and FDP)
- Only in serum (loss of fibrinopeptide A during serum preparation)

1980s

- Development quantitative latex-enhanced immunoturbidimetric assays
- First monoclonal antibody targeting D-domains developed by Rylat *et al.* in 1983
- Utilization of plasma due to minimal fibrinogen (or FDP) cross reactivity

1990s

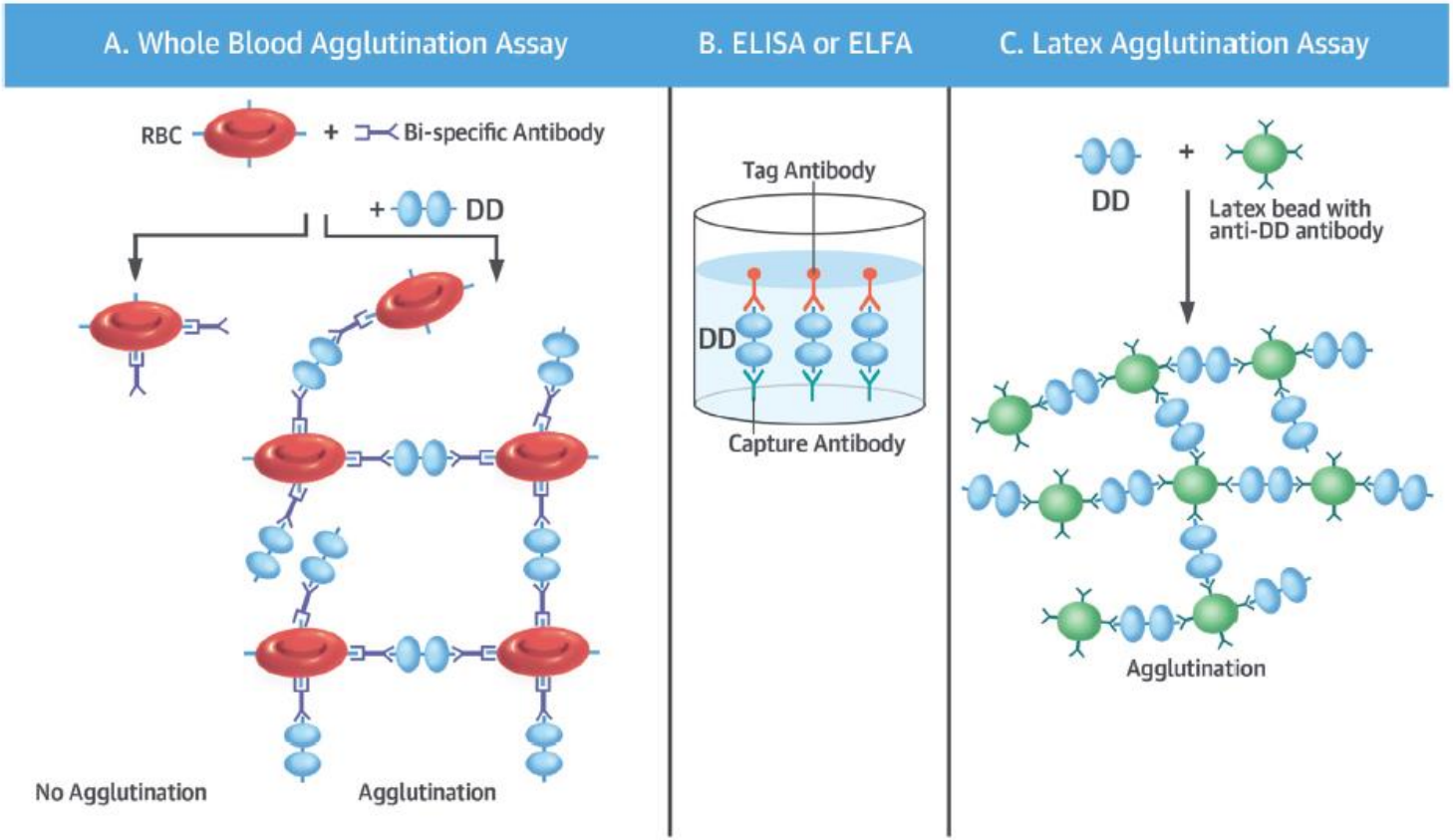
- Automated (more precise results in a shorter time)
- ELISA based-assay with fluorescence end-point detection (Se/Sp similar to microplate ELISAs)
- The Vidas® assays (bioMérieux) is still considered the reference commercial quantitative immunoassay. Most clinically validated D-dimer measurement technique

2000s

- Chemiluminescent enzyme immunometric assays
- Similiar Se compared to ELISAs and latex-enhanced immunoturbidimetric assays
- POC D-dimer assays

Inter-laboratory variations

- **D-dimer assays**



(A) Whole blood agglutination assays utilize a bi-specific antibody directed against an epitope on D-dimer (DD) and an epitope on red blood cells (RBC). In the presence of D-dimer, RBC agglutination is monitored by turbidity. **(B)** Enzyme-linked immunosorbent assays (ELISA) or enzyme-linked immunofluorescent assays (ELFA) involve capture of D-dimer with an immobilized antibody specific for D-dimer. A second antibody tagged with horseradish peroxidase or a fluorescent marker binds D-dimer and is used to generate a chromophore or fluorophore that is detected with a spectrophotometer or fluorimeter. **(C)** The latex agglutination assay uses latex beads coated with D-dimer specific antibodies. In the presence of D-dimer, latex bead agglutination is detected by turbidity.

Weitz et al. J Am Coll Cardiol. 2017 Nov 7;70(19):2411-2420

Characteristics of D-dimer assays

	ELISA	ELFA	Unenhanced Latex agglutination assay	CLIA	Latex-enhanced immunoturbidimetric assay	POC assay
Type	Quantitative	Quantitative	Qualitative/semi-quantitative	Quantitative	Quantitative	Qualitative/quantitative
TAT	2-4h	35-40min	Rapid	25-40min	15min	2-20min
Pros	Considered as the gold standard, Sensitivity, observed independent	Considered as reference method, most validated method, sensitivity, automation, wide linear range (0-1,000 µg/mL), automated, observed independent	Rapid, inexpensive	Sensitivity, rapid, automated, observed independent	Sensitivity, automated, rapid, observed independent	Readily available, fast, higher specificity, whole blood
Cons	Highly manual, technical skills, time-consuming, not optimal linear range, moderate specificity	Moderate specificity	Moderate sensitivity, manual, observer dependent	Lack clinical validation, moderate specificity	Moderate specificity	Sensitivity, not all FDA cleared, observer dependent, manual
Example	Asserachrome® (Stago), Enzygnost® (Dade Behring)	Vidas® (bioMérieux), AxSYM® (Abbott), Stratus CS® (Dade Behring)	Dimertest latex® (IL); Fibrinosticon® (bioMérieux) ; Dade Dimertest® (Siemens)	AcuStar® (Werfen), Immulite® (Siemens)	Tina-quant® (Roche), STA-Liatest® (Stago), HemosIL HS® (Werfen) Innovance® (Dade-Behring)	SimpliRed® (Agen), Clearview Simplify® (Agen)

ELISA = Enzyme-linked immunosorbent assay, ELFA = Enzyme-linked immunofluorescence assays, CLIA = Chemiluminescent enzyme immunometric Assay, POC = Point of care.

Favresse et al. Crit Rev Clin Lab Sci. 2018, minor revision

Inter-laboratory variations

- **High inter-laboratory variability**

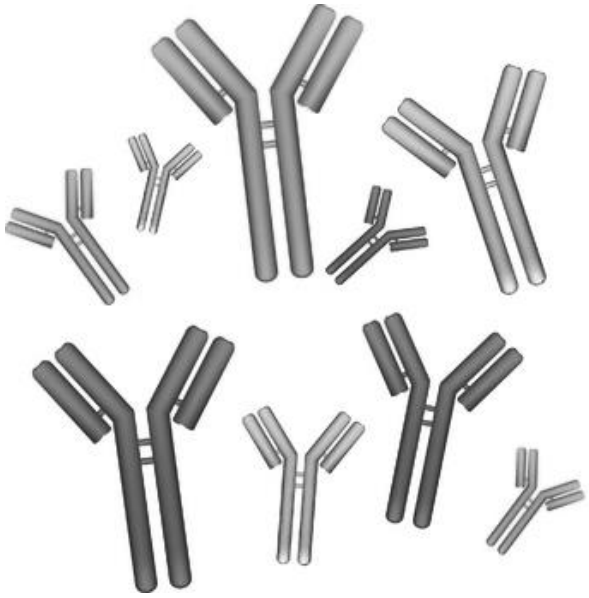
Reference	Number of assays	Differences	Number of labs
Dempfle et al. 2001	23	from 630 to 13,350 µg/L (21x)	12
Meijer et al. 2006	7	20x	357
Olson et al. 2013	13	CV high as 42%	3,800

Demfle et al. Thromb Haemost. 2001 Apr;85(4):671-8
Meijer et al. Thromb Haemost. 2006 Mar;95(3):567-72
Olson et al. Arch Pathol Lab Med. 2013 Aug;137(8):1030-8

Inter-laboratory variations

- **Leading sources of inter-laboratory variability**

1. Use of different monoclonal antibodies with different specificity towards D-dimer epitopes (>20 differents)



Inter-laboratory variations

- **Leading sources of inter-laboratory variability**

1. Use of different monoclonal antibodies with different specificity towards D-dimer epitopes (>20 differents)
2. Heterogeneity of fragments derived from plasmin digestion of cross-linked fibrin (from LMWF to HMWF)

Inter-laboratory variations

- **Heterogeneity of D-dimer containing fragments**

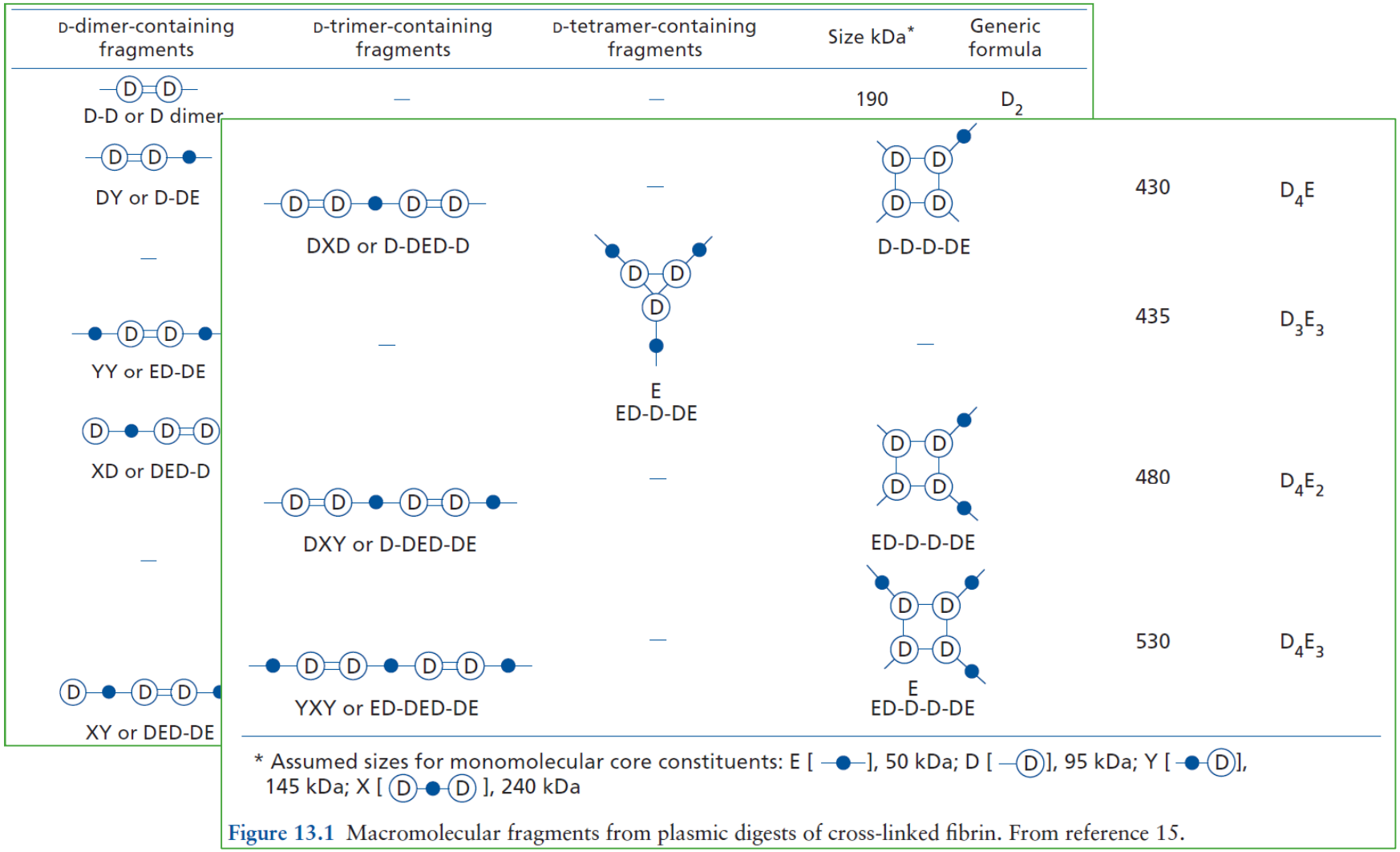
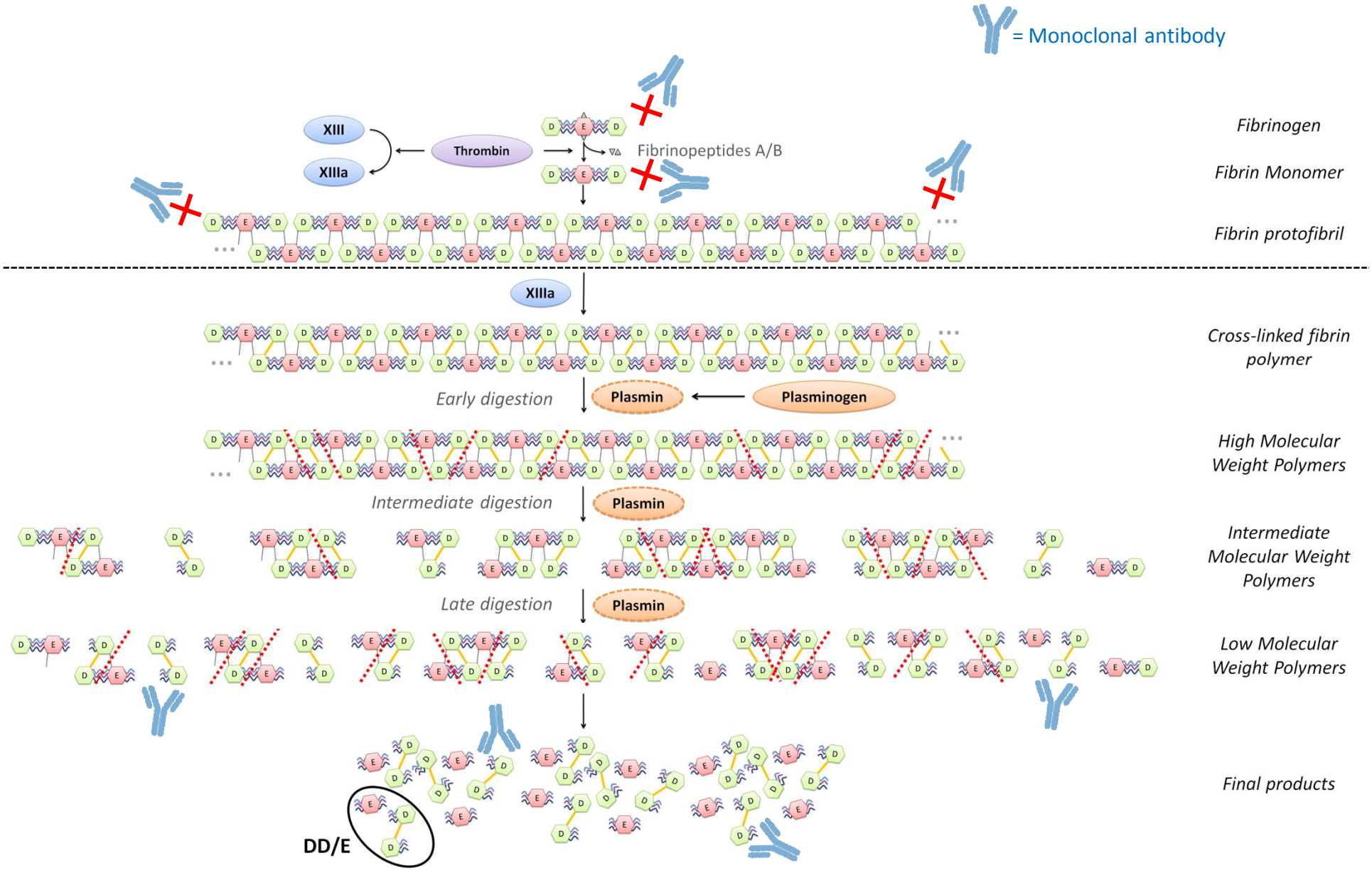


Figure 13.1 Macromolecular fragments from plasmic digests of cross-linked fibrin. From reference 15.

Reber and Moerloose. Quality in Laboratory Hemostasis and Thrombosis. Standardization of D-dimer Testing. 2013: p. 136-146

Inter-laboratory variations



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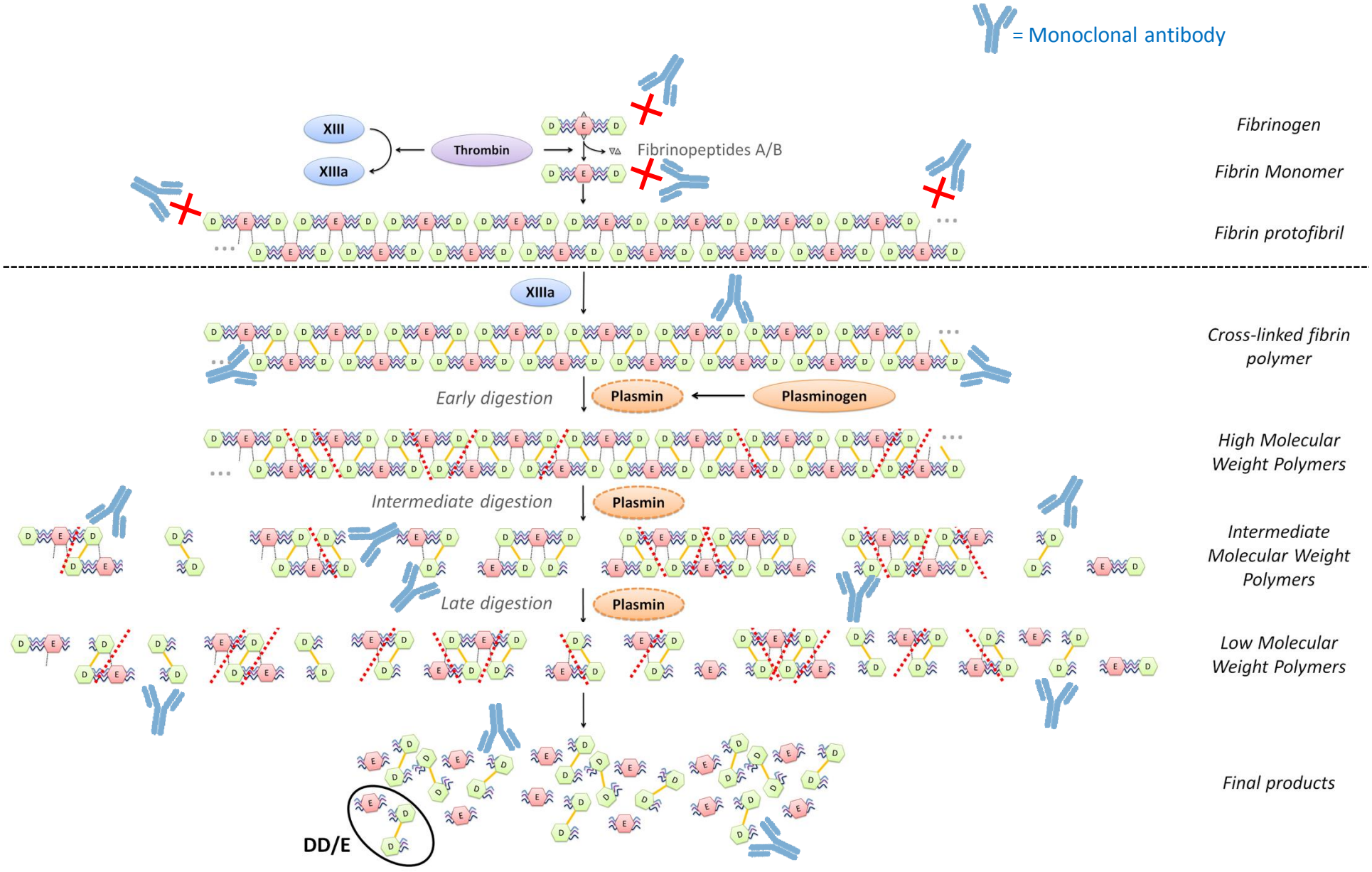
Inter-laboratory variations



Realized by Nicolas Bailly

Inter-laboratory variations

- **Leading sources of inter-laboratory variability**

1. Use of different monoclonal antibodies with different specificity towards D-dimer epitopes (>20 differents)
2. Heterogeneity of fragments derived from plasmin digestion of cross-linked fibrin (from LMWF to HMWF)
3. Lack of international certified internal control or calibrators
4. Use of different units or clinical cut-offs

➔ D-dimer assays standardization is a quite challenging, if not an impossible target

➔ Less stringent harmonization procedures have been proposed

Meijer et al. Thromb Haemost. 2006 Mar;95(3):567-72
Reber and Moerloose. Quality in Laboratory Hemostasis and Thrombosis. Standardization of D-dimer Testing. 2013: p. 136-146
Nieuwenhuizen. Thromb Haemost. 1997 May;77(5):1031-3
Dempfe et al. Thromb Haemost. 2001 Apr;85(4):671-8

Harmonization

- **First attempt in 1997**
- Pools of patients tested with 5 different D-dimer assays (one microlatex and four microplate ELISAs)
- « Mean pool consensus values » of each pool and assay were calculated
- Utilization of a conversion factor between assays (squared regression from 0.7 to 0.92)

Nieuwenhuizen. Thromb Haemost. 1997 May;77(5):1031-3

Harmonization

- **Second attempt in 2001**
- 39 individual samples with 23 D-dimer assays (including microlatex-enhanced, membrane-based and ELISA assays) were tested
- A conversion factor was calculated by using median values for each sample measured with all assays, and for each assay the median value obtained with all samples
- The multiplication of individual sample assay value with the corresponding conversion factor was found to be effective to improve the correlations among most assays

Dempfe et al. Thromb Haemost. 2001 Apr;85(4):671-8

Harmonization

- **Third attempt in 2006**
- A plasma pool of 50 patients diluted with normal plasma was used to prepare five different samples that were then distributed to 502 participants of an external quality control survey using seven different D-dimer assays
- For each D-dimer assay, the mean results of each sample were plotted against the amount of pool added (assay-specific regression line)

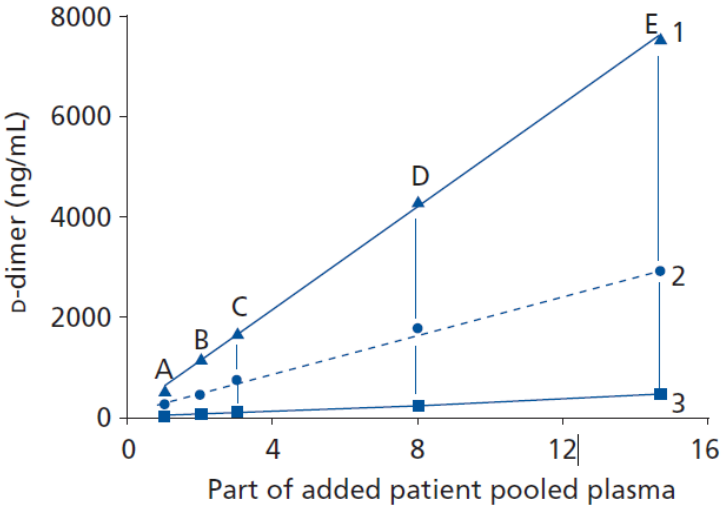


Table 13.5 Coefficients of variation for the method-specific consensus values of all methods included before and after harmonization for the five different plasma samples

Sample	Overall median value (ng/mL)	Before harmonization (%)	After harmonization (%)
A	252	91.0	18.2
B	425	92.3	7.4
C	736	86.8	6.1
D	1733	83.6	5.9
E	2816	82.3	1.5

Meijer et al. Thromb Haemost. 2006 Mar;95(3):567-72
Reber and Moerloose. Quality in Laboratory Hemostasis and Thrombosis. Standardization of D-dimer Testing. 2013: p. 136-146

Harmonization

- **Fourth attempt in 2007**
- Three calibrators and two test samples were delivered to more than 500 laboratories participating to the UKNEQAS external quality survey, using 9 different D-dimer techniques
- Individual laboratory results of calibrators were plotted against the median results obtained with all D-dimer immunoassays. The individual regression line was used to convert data generated on the two test samples into harmonized results.
- This approach was effective to improve the between-center agreement after calibration, with significant improvement of inter-laboratory variability (**from 25.9% to 11.6% and from 22.4% to 7.7% for FEU; from 55.3% to 21.6% and from 40.8% to 11.6% for data reported in DDU**)

Meijer et al. Thromb Haemost. 2006 Mar;95(3):567-72
Reber and Moerloose. Quality in Laboratory Hemostasis and Thrombosis. Standardization of D-dimer Testing. 2013: p. 136-146

3. Postanalytical variables

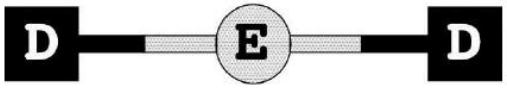
Different D-dimer units

- **Two different units**

	Definition	Preparation of calibrators	Molecular weight
FEU	Compare the mass of D-dimer of that of fibrinogen	Plasmin degradation of purified fibrinogen clotted in the presence of factor XIII	340 kD
DDU	The mass of the estimated weight of D-dimer	Composed of purified D-dimer	195 kD

Rem: different units according to the type of calibrators used

D-dimer Unit (DDU): 195 kDa



Fibrinogen Equivalent Units (FEU): 340 kDa

Olson et al. Arch Pathol Lab Med. 2013 Aug;137(8):1030-8
Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Different D-dimer units

- International survey on D-dimer reporting: a call for standardization
- 409 responses across the world

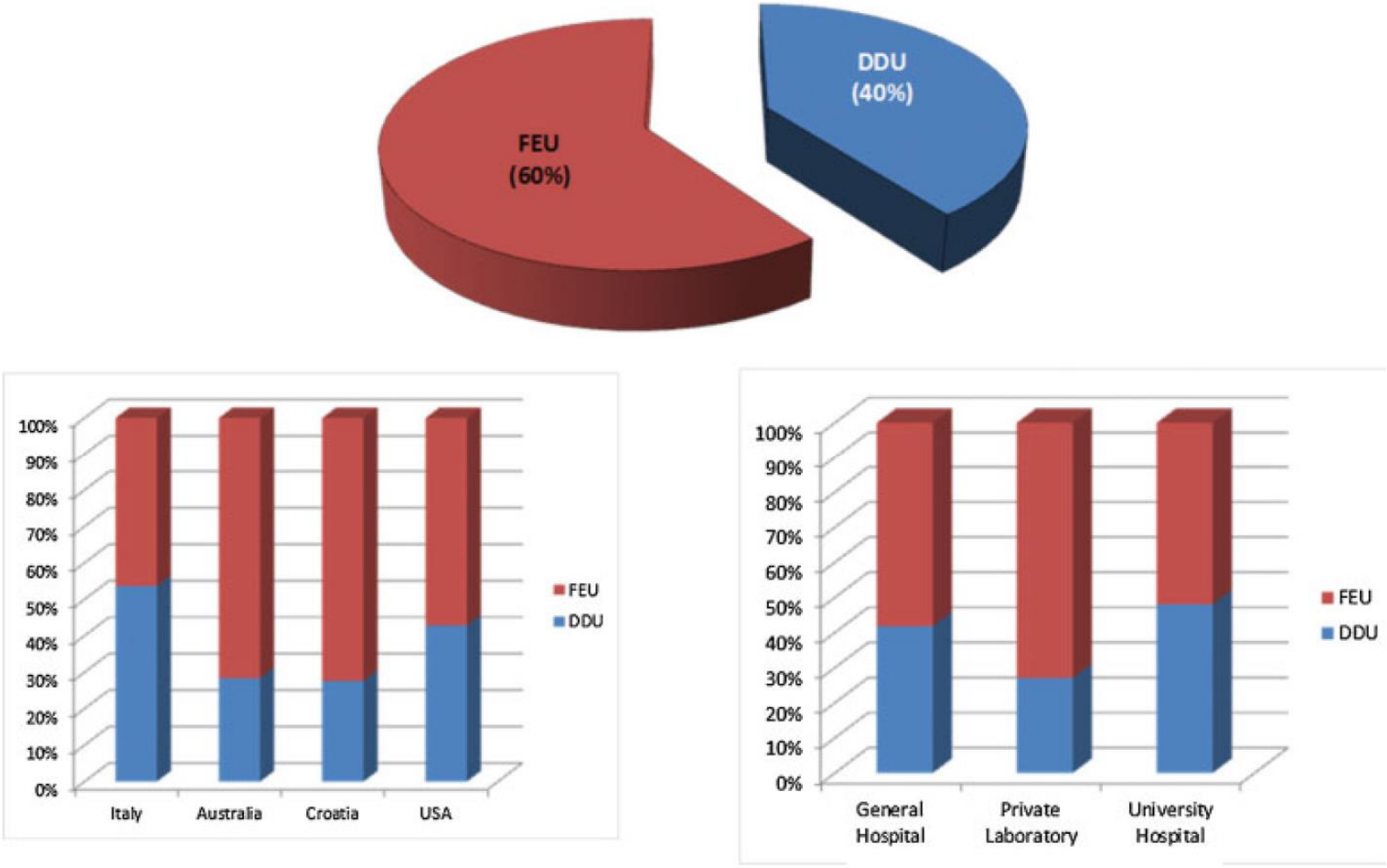
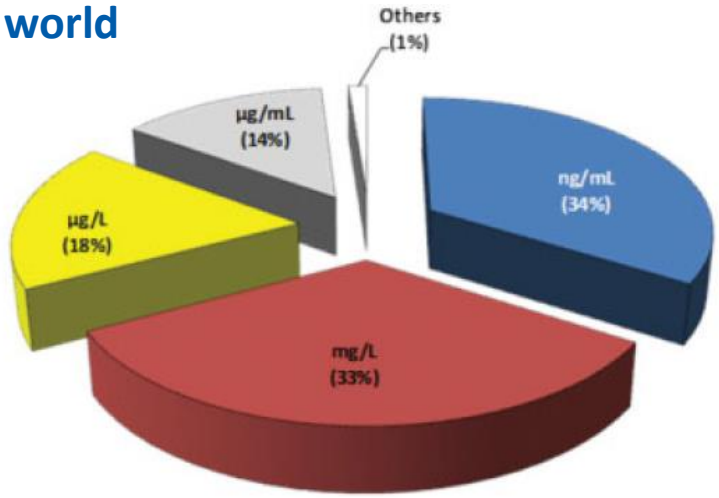


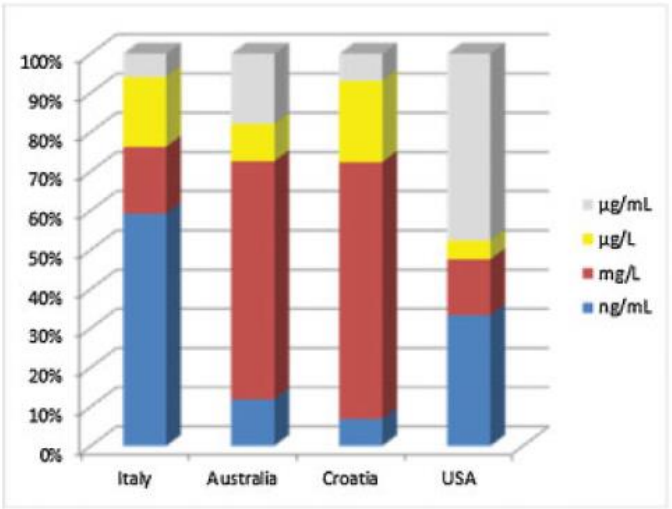
Fig. 3 Use of DDU or FEU for D-dimer reporting among respondents to the survey. DDU, D-dimer unit; FEU, fibrinogen-equivalent unit.

Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Different D-dimer units

- International survey on D-dimer reporting: a call for standardization
- 409 responses across the world





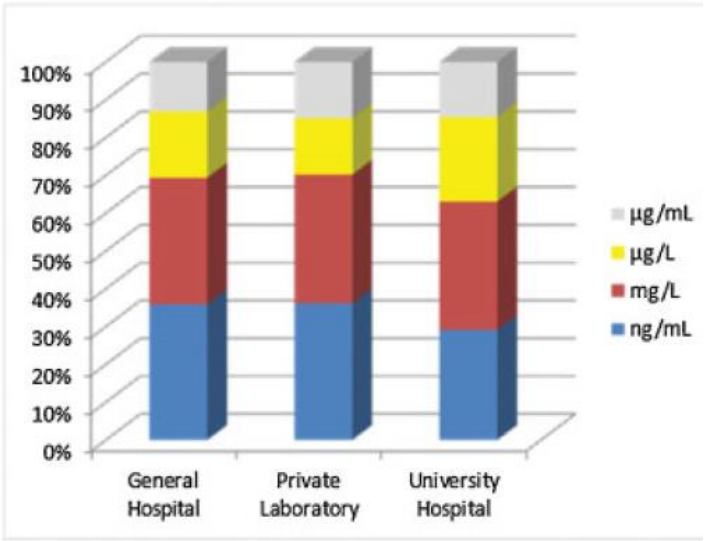


Fig. 4 Use of different measure units for D-dimer reporting among respondents to the survey.

Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Different D-dimer units

- **Survey performed by the College of American Pathologists (CAP)**

	ng/mL, No.	g/L, No.	g/mL, No.	mg/L, No.	Total
DDU	379	12	39	125	555
FEU	304	19	336	143	802
Total	683	31	375	268	1357

"*At least 14 combinations for D-dimer measurement coexist*"

"*Among the measure units that can be adopted, "**µg/L**" (or "**ng/mL**") is probably the unit that best approximates the **International System (IS)** and is also recommended by the Italian Consensus document*"

"*Some laboratories did not even acknowledge the type of measure unit they are using (8% of laboratories in the CAP survey)*"

Table 3 Interconversion of D-dimer values into SI units.

FEU=DDU×2
µg/L FEU=mg/L FEU×1000
µg/L FEU=µg/mL FEU×1000
µg/L FEU=ng/mL FEU

Olson et al. Arch Pathol Lab Med. 2013 Aug;137(8):1030-8
Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Turnaround time

- **Consensus document of AcEMC, CISMEL, SIBioC, and SIMeL**
  - A recommended overall **TAT <1h**
  - Impossible with manual ELISAs
  - Faster centrifugation process, PTS, reliable POC analyzers, wide range of linearity (up to 5,000 µg/L FEU), …
  - In a European study, **81%** of participants declared to measure D-dimer **24h per day**

Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93
Spannagl et al. Blood Coagul Fibrinolysis. 2005 Sep;16(6):439-43

4. Clinical applications

Clinical applications

1. Ruling out VTE (DVP/PE)
2. Prediction of recurrence of VTE
3. Diagnosis and monitoring of DIC
4. Excluding acute aortic dissection (AAD)
5. Predicting and managing thrombotic complications in patients with severe infections and sepsis
6. Prognostication of peripheral artery disease
7. Identification of vaso-occlusive crisis in sickle cell disease
8. Screening of intracardiac thrombus
9. Prediction of VTE in sleep apnea
10. Identifying patients with low probability of cerebral venous thrombosis (CVT)
11. Diagnosis of acute mesenteric ischemia (AMI)
12. …

Causes of D-dimer elevation

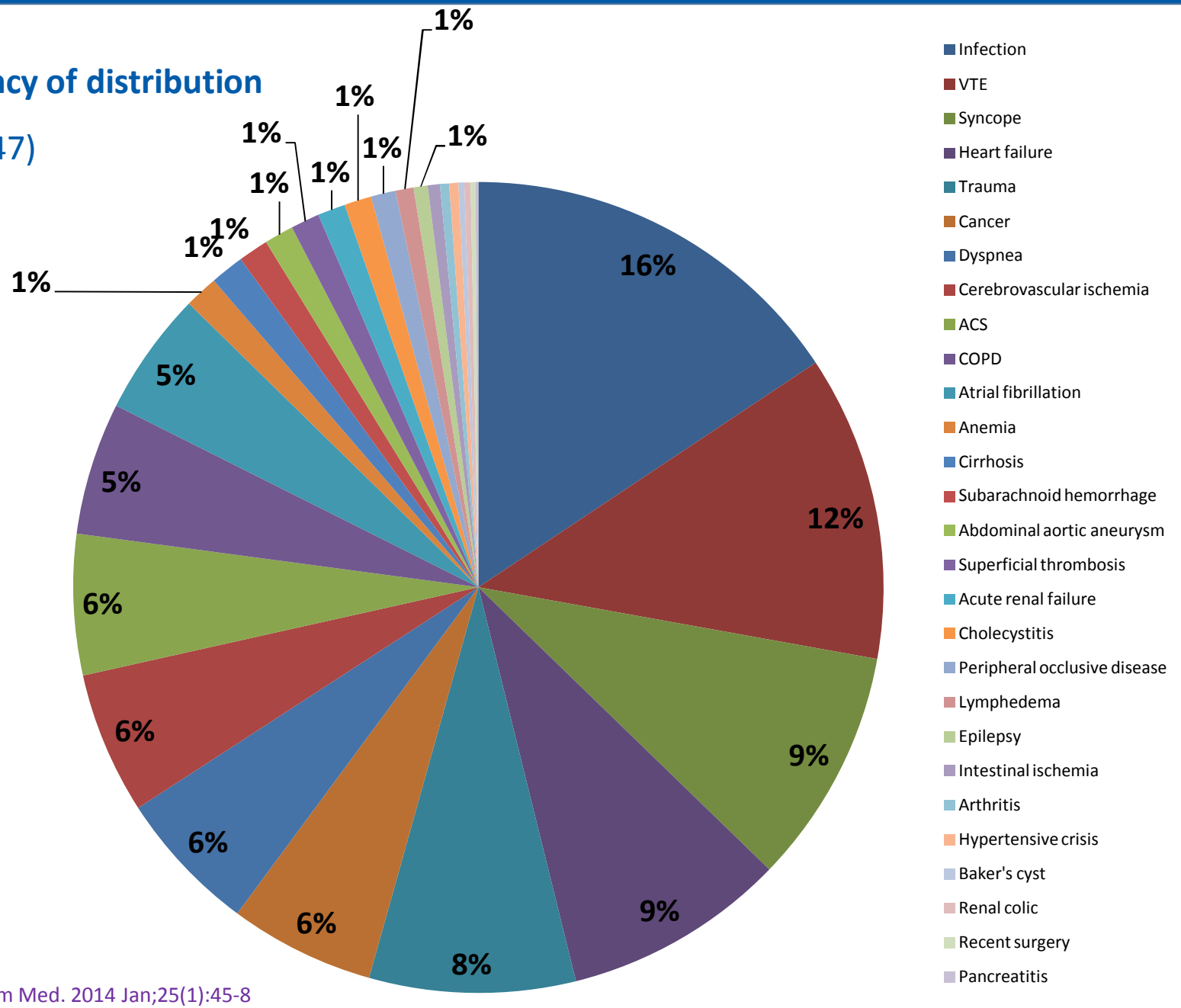
- **Lack of specificity**

Acute respiratory distress syndrome	Disseminated intravascular coagulation	Pancreatitis
Advance age	Heart failure	Post transplantation complications
Alzheimer	HELLP syndrome	Pregnancy or puerpenium
Aneurism	Hemolysis (falciform anemia)	Recent surgery
Aortic dissection	Hemorrhage	Renal disease
Arthritis	Hospitalization	Severe chronic urticaria
Atrial fibrillation	Inflammatory bowel disease	Thrombolytic therapy
Burns	Ischemic cardiopathy	Trauma
Cancer	Liver disease	Venous or arterial thrombosis
Chronic inflammation	Localized or systemic Infection	…
Disability	Neonatal period	

Causes of D-dimer elevation

- **Frequency of distribution**

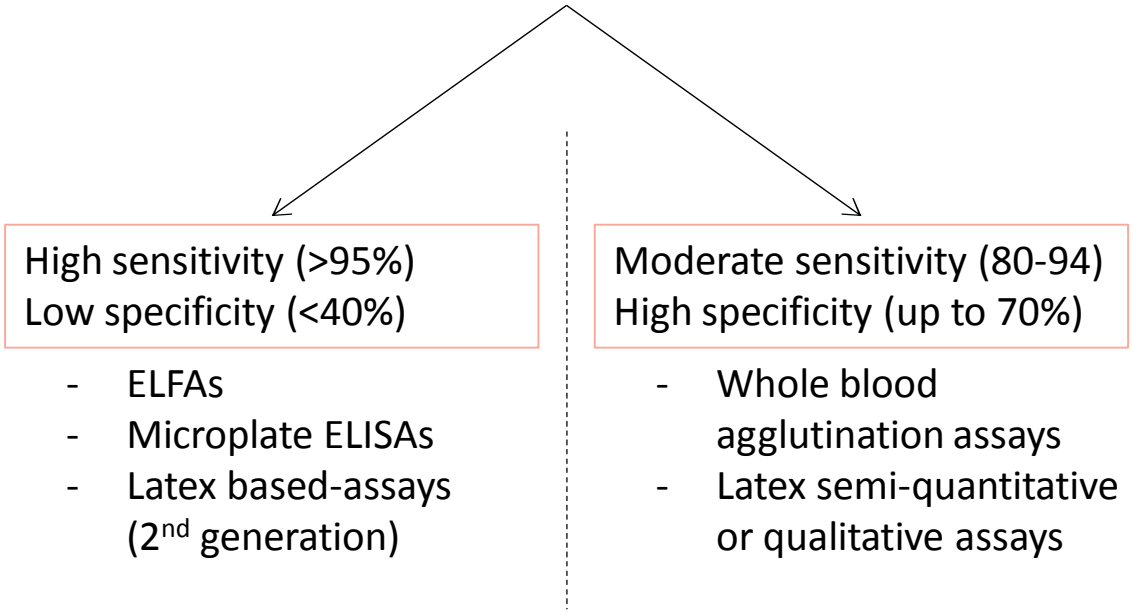
(n = 1,647)



Lippi et al. Eur J Interm Med. 2014 Jan;25(1):45-8

Recommended clinical performances for VTE exclusion

	FDA	CLSI
Sensitivity	≥95% (with lower limit of CI ≥90%)	≥97% (with lower limit of CI ≥90%)
Negative predictive value	≥97% (with lower limit of CI ≥95%)	≥98% (with lower limit of CI ≥95%)



Di Nisio et al. J Thromb Haemost. 2007 Feb;5(2):296-304
FDA and CLSI recommendations

Recommended cut-offs?

- Verification with a min. of 200 subjects (British Guidelines)
- Cut-offs validated in prospective studies (e.g., Vidas®, AxSYM®, STA-Liatest®)
- Otherwise, comparison with validated assays is encouraged
- Manufacturers should also stay abreast of the recent literature regarding the use of their immunoassays, in order to eventually adjust the cut-off

➔ The CAP survey showed that 488 laboratories out of 1,506 in USA were using cut-off values higher than those recommended by the literature or by the manufacturer

➔ A European survey also highlighted that 24% and 55% of participants used lower or higher cut-offs than those recommended, respectively

British Committee for standards in Haematology guidelines
Olson et al. Arch Pathol Lab Med. 2013 Aug;137(8):1030-8
Reber and Moerloose. Quality in Laboratory Hemostasis and Thrombosis. Standardization of D-dimer Testing. 2013: p. 136-146

Clinical Prediction Rules for VTE exclusion

- **False-negative D-dimer results**
  - Hypofibrinolytic state
  - Small thrombi (i.e. distal DVT or isolated subsegmental PE)
  - Anticoagulant therapy
  - D-dimer testing performed to early or late after the thrombosis
  - Severe infection, cancer
- **NPV is directly influenced by the prevalence of a disease**
  - Increase in the diagnostic specificity of D-dimer

Clinical Prediction Rules

Table 2 Most commonly used clinical prediction rules for suspected PE

Wells score [105]		Geneva score [106]		Revised Geneva score [107]	
Items	Score	Items	Score	Items	Score
Previous PE or DVT	1.5	Previous PE or DVT	2	Age > 65 years	1
Heart rate > 100	1.5	Heart rate >100	1	Previous DVT or PE	3
Recent surgery or immobilization	1.5	Recent surgery	3	Surgery or fracture within 1 month	2
Clinical signs of DVT	3	Age		Active malignancy	2
Alternative diagnosis less likely than PE	3	60–79	1	Unilateral lower limb pain	3
Hemoptysis	1	≥80	2	Hemoptysis	2
Cancer	1	Arterial blood gases		Heart rate	
		CO2 (kPa)		75–94	3
		<4.8	2	≥95	5
		4.8–5.19	1	Pain on lower limb deep vein palpation and unilateral edema	4
		O_2 (kPa)			
		<6.5	4		
		6.5–7.99	3		
		8–9.49	2		
		9.5–10.99	1		
		Chest X-ray			
		Atelectasia	1		
		Elevated hemidiaphragm	1		
Clinical probability		*Clinical probability*		*Clinical probability*	
Low	<2	Low	0–4	Low	0–3
Intermediate	2–6	Intermediate	5–8	Intermediate	4–10
High	>6	High	≥9	High	≥11
Dichotomized [71]					
PE unlikely	≤ 4				
PE likely	>4				

Righini et al. J Thromb Haemost. 2008 Jul;6(7):1059-71

Imaging tests

- **DVT**
  - Ultrasonography
  - Or Doppler flow studies
- **PE**
  - CT pulmonary angiogram (CTPA)
  - Or contrast-enhanced
  - Or not enhanced magnetic resonance imaging, especially when CTPA is unadvisable

Other recommendations

- **Quantitative assays**
- **Precision**
  - <10% close to diagnostic cut-off
- **Linearity**
  - Between 50 and 5,000 µg/L FEU

Clinical algorithms for VTE exclusion

- **Clinical algorithms**
  - **Low prevalence (<10%)**
  - **Moderate prevalence (±30%)**
  - **High prevalence (>50%)**

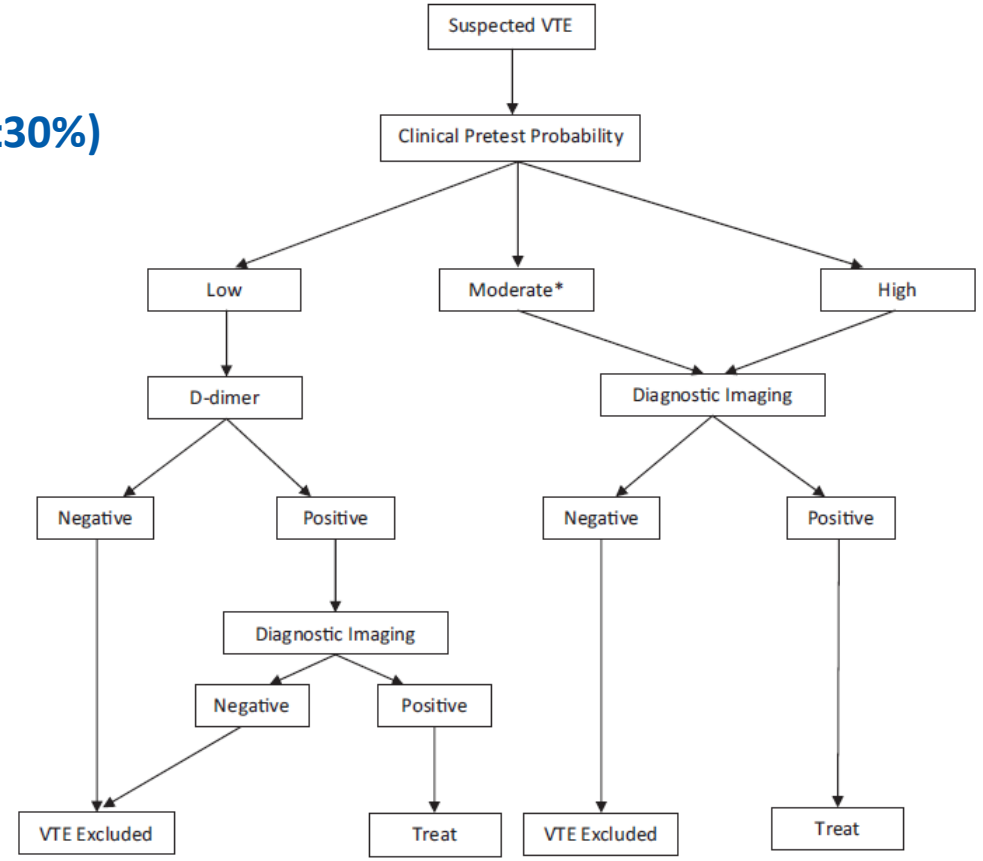
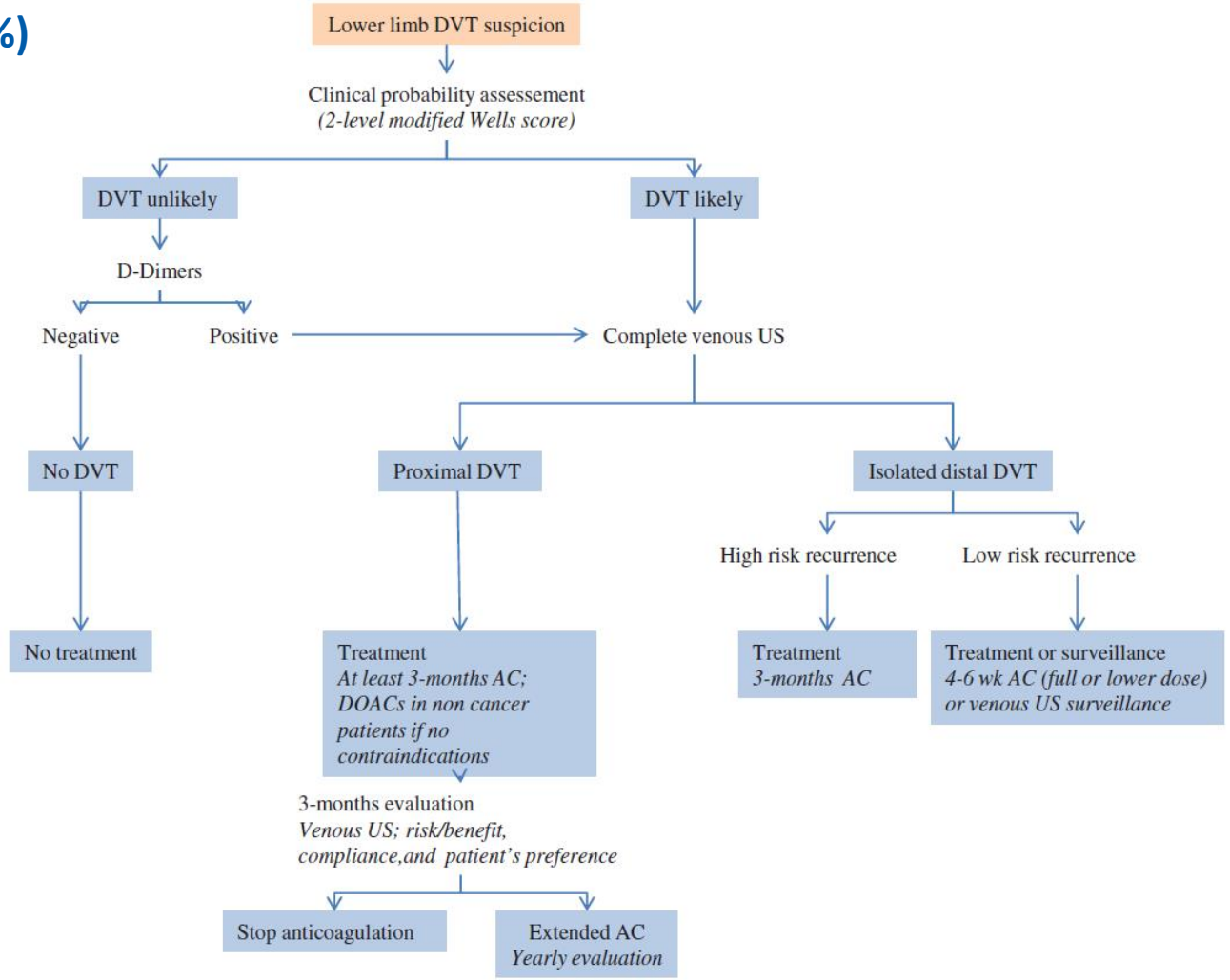


FIGURE 1 Diagnostic algorithm for venous thromboembolism. *D-dimer testing may also be performed to exclude VTE in this category depending on the assay used (Linkins et al.[21])

Linkins and Takach Lapner Int J Lab Hematol. 2017 May;39 Suppl 1:98-103

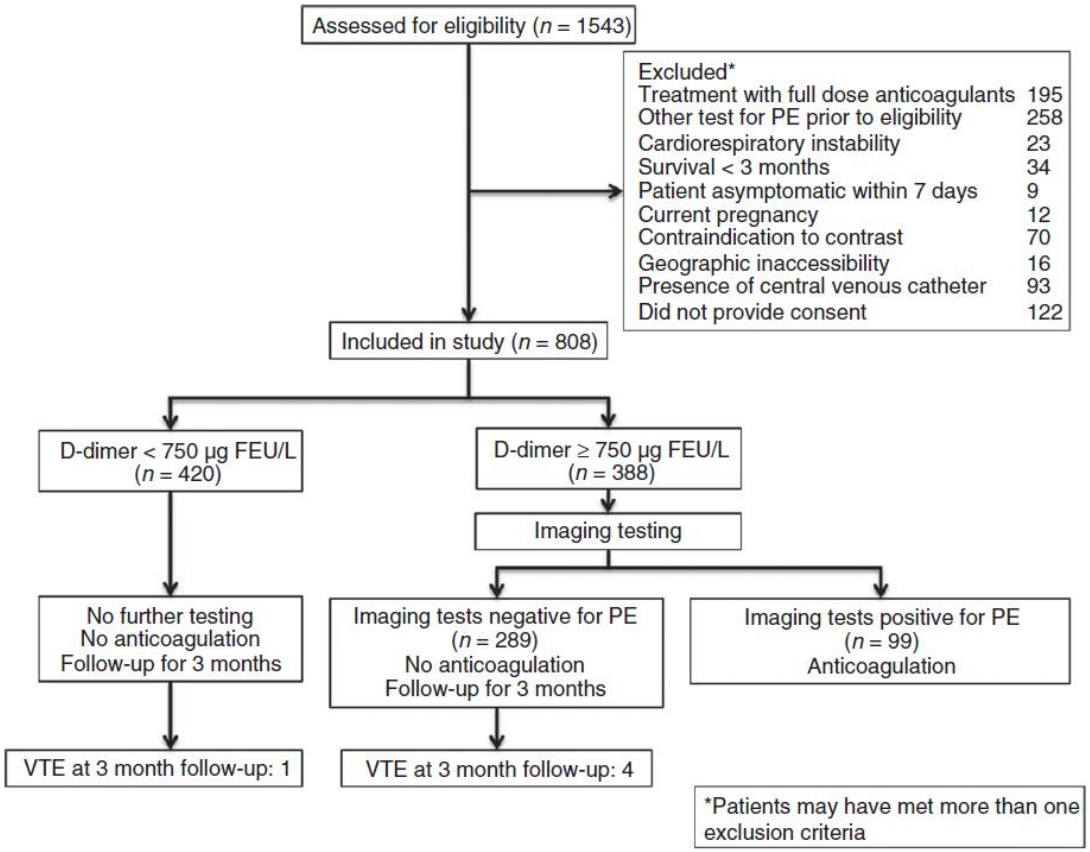
Clinical algorithms for VTE exclusion

- **Clinical algorithms**
  - **Unlikely (±10%)**
  - **Likely (±35%)**



Mazzolai et al. Eur Heart J. 2017 Feb 17. doi: 10.1093/eurheartj/ehx003

Clinical algorithms without CPR?



"Theoretical advantage of limiting the use of CPR is a decrease of the cost attributable to imaging techniques (e.g., CTPA and ventilation perfusion lung scanning) and prevention of radiation exposure"
"MDA D-dimer assay (quantitative latex agglutination assay, bioMérieux) no longer available"
"The number of patients with a high pretest probability was quite low in that study"

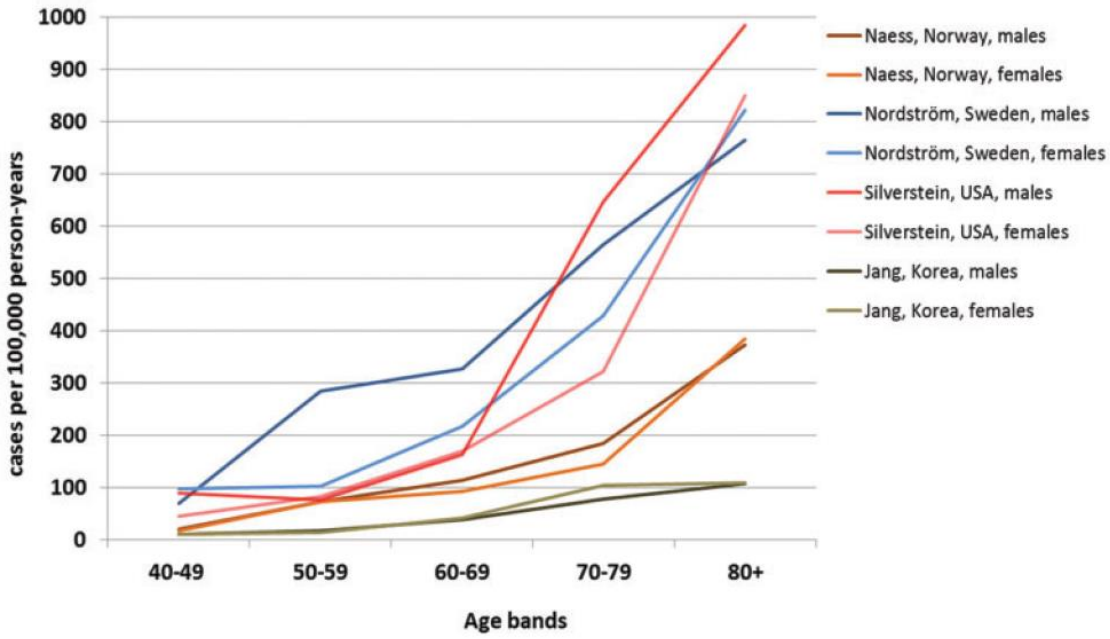
Bates et al. J Thromb Haemost. 2016 Mar;14(3):504-9

Clinical Prediction Rules: current practice

- **70.3%** of clinicians used pre-test probability scores
- **10%** could exclude or confirm DVT only based on D-dimer test results
- Moreover, a significant number of clinicians still order D-dimer testing in patients with high VTE probability, whilst others order imaging testing in case of low pre-test probability

Kristoffersen et al. Thromb Res. 2017 Nov;159:19-23
Kristoffersen et al. Thromb Res. 2016 Jun;142:1-7

Age specific cut-offs



- **The incidence of VTE is known to increase sharply with age**
- **D-dimer values tend to increase with ageing**
  - Å 60% of older patients have D-dimer values higher than classical cut-offs

➔ **A high rate of these patients with low clinical score would undergone unnecessary imaging testing!**

Mazzolai et al. Eur Heart J. 2017 Feb 17. doi: 10.1093/eurheartj/ehx003
Deitelzweig et al. Am J Hematol. 2011 Feb;86(2):217-20
Spyropoulos et al. J Manag Care Pharm. 2007 Jul-Aug;13(6):475-86

Age specific cut-offs

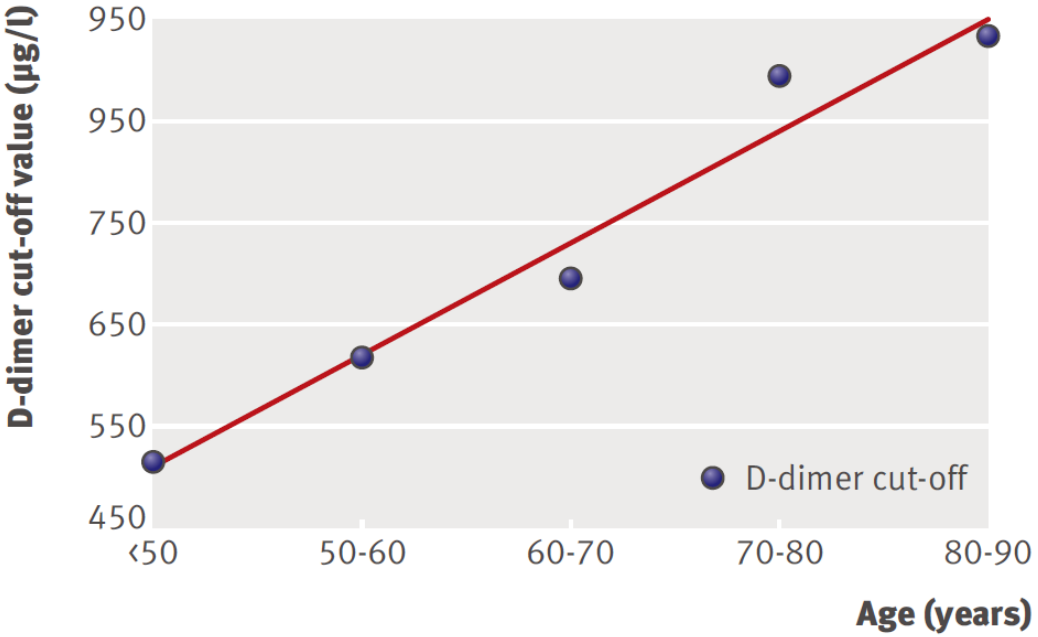


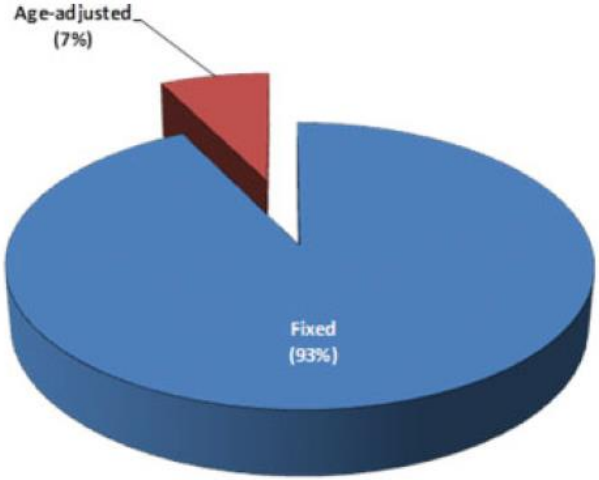
Fig 1 | Optimal cut-off values for D-dimer test for pulmonary embolism by age in patients with an unlikely clinical probability of pulmonary embolism (sensitivity set at 100%)

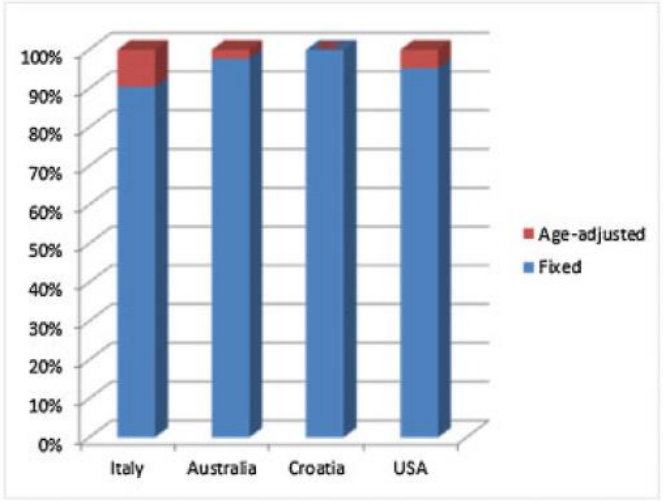
- **[age-adjusted cut-off, µg/L FEU] = [age, years]) x 10**
  - Å These cut-offs would enable to a substantially increase in the PPV without significantly impairing the NPV (and is cost-effective)

Douma et al. BMJ. 2010 Mar 30;340:c1475

Age specific cut-offs

- International survey on D-dimer reporting: a call for standardization
- 409 responses across the world





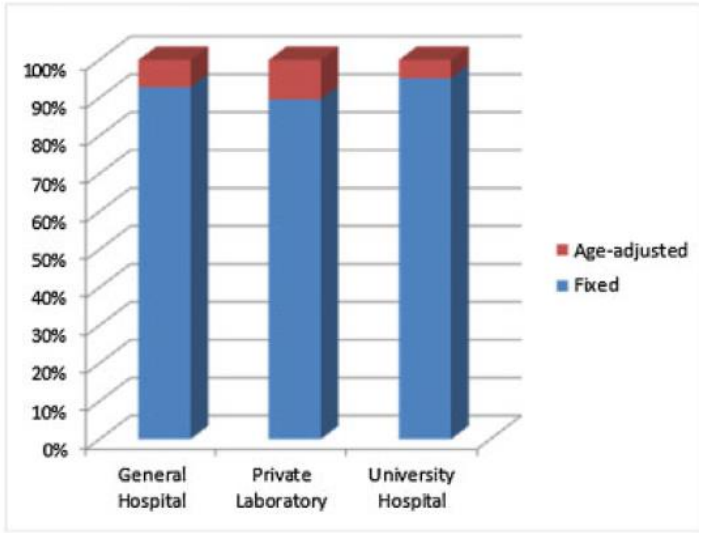
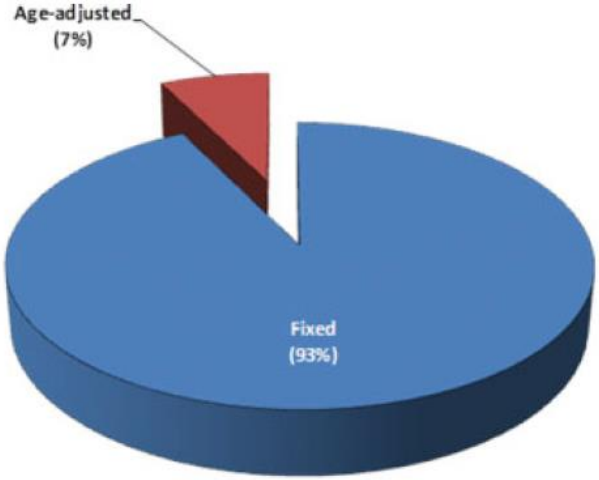


Fig. 5 Use of fixed or age-adjusted cutoff for D-dimer reporting among respondents to the survey.

Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Age specific cut-offs

- **International survey on D-dimer reporting: a call for standardization**
- **409 responses across the world**



"Along with the 14 different combinations of D-dimer units, the use of age-adjusted cut-off complicated further the clinical decision making due to the ***nearly 30 different possibilities for reporting D-dimer test results****"*

Lippi et al. Semin Thromb Hemost. 2015 Apr;41(3):287-93

Clinical probability-adjusted cut-offs

- Higher cut-off in patients with low clinical probability (**1,000 µg/L FEU**)
- Conventionnal cut-off in patients with moderate clinical probability (**500 µg/L FEU**)

Table 2: Accuracy of age-adjusted and clinical probability-adjusted D-dimer interpretation strategies for VTE.

Accuracy parameter	Age-adjusted Strategy	Clinical probability -adjusted Strategy	Difference p-value
Sensitivity			
n/N	106/109	106/109	1.0
% (95 % CI)	97.3 (92.2, 99.1)	97.3 (92.2, 99.1)	
Specificity			
n/N	837/1540	922/1540	<0.001
% (95 % CI)	54.4 (51.9, 56.8)	59.9 (57.4, 62.3)	
Negative predictive value			
n/N	837/840	922/925	0.095
% (95 % CI)	99.64 (99.11, 99.86)	99.68 (99.19, 99.87)	
Negative results			
n/N	840/1649	925/1649	<0.001
% (95 % CI)	50.9 (48.5, 53.4)	56.1 (53.7, 58.5)	

Takach Lapner et al. Thromb Haemost. 2017 Oct 5;117(10):1937-1943

Prediction of recurrence of VTE

- **1-year follow up after a first VTE episode**
  - Risk of recurrence in men = 9.5%
  - Risk of recurrence in women = 5.3%
- **3-year follow up after a first VTE episode**
  - Risk of recurrence in men = 19.7%
  - Risk of recurrence in women = 9.1%
- D-dimer value is a significant predictor of VTE
  - Risk x2 if > diagnostic cut-off after 3-months of anticoagulant therapy

➔ **D-dimer testing should be performed in all patients with clinical suspicion of recurrent VTE**

ISth™
International Society on Thrombosis and Haemostasis

Douketis et al. BMJ 2011 Feb 24;342:d813

Prediction of recurrence of VTE: scores

Table 5 Predictors included in final model

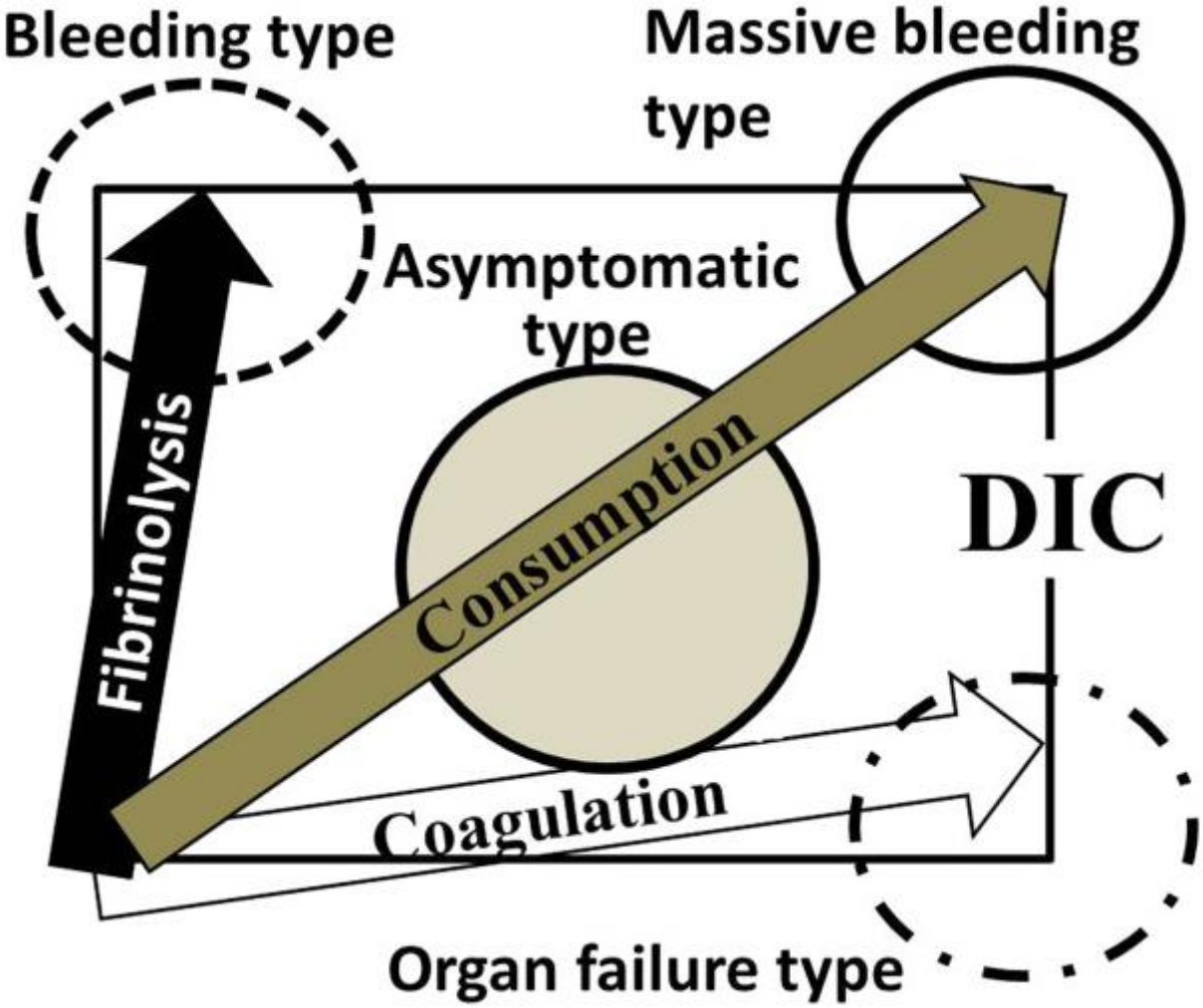
Model	HERDOO2	Vienna	DASH
Predictors included			
D-dimer	X	X	X
Age	X	–	X
Sex	–	X	X
BMI	X	–	–
Post-thrombotic signs	X	–	–
Site of index event	–	X	–
Hormone therapy	–	–	X

BMI, body mass index.

- « ***Should the anticoagulant treatment be discontinued or resumed after the usual 3-month period???*** »
  - Lack validation in interventional studies
  - Other D-dimer assays? (Vidas®, Liatest®). Appropriate timing of D-dimer monitoring?

Ensor et al. BMJ Open. 2016 May 6;6(5):e011190

Disseminate intravascular coagulation (DIC)



Wada et al. J Intensive Care 2014 ;2(1):15

DIC scoring system

Table The International Society of Thrombosis and Hemostasis Disseminated Intravascular Coagulation (DIC) Score

Laboratory Results	Points
Platelet count	
> 100,000/μL	0
< 100,000/μL	1
< 50,000/μL	2
Fibrinogen level	
> 100 mg/dL	0
< 100 mg/dL	1
Prothrombin time	
Prolonged < 3 sec	0
Prolonged 3 to 5 sec	1
Prolonged ≥ 6 sec	2
D-dimer or fibrin degradation products	
No increase	0
Moderate increase	2
Strong increase	3

According to this scoring system, the laboratory diagnosis of DIC can be made with a total of ≥ 5 points—*but only in patients with an underlying disorder known to be associated with DIC.* If the score is < 5, the tests should be repeated in 1–2 days. In laboratories where the prothrombin time is not reported in seconds, use of the prothrombin index is recommended (0 points for > 70%, 1 point for 40%–70%, and 2 points for < 40%).

ISTH DIC score

DIC scoring system

Table II. ISTH Diagnostic Scoring System for DIC.

Scoring system for overt DIC

Risk assessment: Does the patient have an underlying disorder known to be associated with overt DIC?

If yes: proceed

If no: do not use this algorithm

Order global coagulation tests (PT, platelet count, fibrinogen, fibrin related marker)

Score the test results

- Platelet count (>100 × 10^9/l = 0, <100 × 10^9/l = 1, <50 × 10^9/l = 2)
- Elevated fibrin marker (e.g. D-dimer, fibrin degradation products) (no increase = 0, moderate increase = 2, strong increase = 3)
- Prolonged PT (<3 s = 0, >3 but <6 s = 1, >6 s = 2)
- Fibrinogen level (>1 g/l = 0, <1 g/l = 1)

Calculate score:

≥5 compatible with overt DIC: repeat score daily

<5 suggestive for non-overt DIC: repeat next 1–2 d

ISTH DIC score

DIC scoring system

- **There is no unique test which is sufficient to make or exclude the diagnosis of DIC symptoms + association of lab tests**
  - **PLT count**
  - **PT**
  - **Fibrinogen**
  - **FDP**
    - D-dimer ≥2 the URL
    - Soluble fibrin may be more specific as suggested by some authors

Wada et al. J Intensive Care 2014 ;2(1):15

Pregnancy

- D-dimer levels increased physiologically along the pregnancy and postpartum period. In a study including 1,343 pregnant women with D-dimer measurement using turbidimetry method (STALiatest), the rate of pregnant healthy women with a D-dimer test below the usual cut-off (500 µg/L) was 85%, 29% and 4.1% during the first, the second and the third trimester, respectively [203]
- In postpartum, D-dimer returns to normal level around the 6th week
- In case of PE suspicion, since imaging tests may expose the mother and the fetus to radiation, the ability to rule-out PE on non-radiologic test is crucial [107].

Cancer

- The prevalence of VTE is increased (up to 20% of cancer patients develop VTE) and the NPV is therefore reduced [107, 208]. A large meta-analysis of 10,002 patients showed that the prevalence of both a low Wells score and a negative D-dimer value among patients with cancer was only 9% [209]
- It has also been shown that 88 to 94% of patients with malignancy will require additional tests to rule out VTE [210]
- Score do exist

Conclusion

- **Biomarker of activation of coagulation and fibrinolysis**
- **Mainly employed for the exclusion of VTE**
  - High sensitivity and NPV (≥95% and ≥97%, respectively)
  - Clinical Prediction Rules
  - Age-adjusted cut-offs (increased PPV)
  - Still challenging in specific populations (i.e., pregancy, cancer, renal failure)
  - ➔ **Major efforts for a larger implementation of these recommendations**
- **Major drawback high inter-variability between immunoassays**
  - Different units
  - Different monoclonal antibodies
  - Broad mixture of degradation products of cross-linked fibrin
  - Lack of international certified internal control or calibrator

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