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D-Dimer: Quality of laboratory testing and its implications in clinical practice

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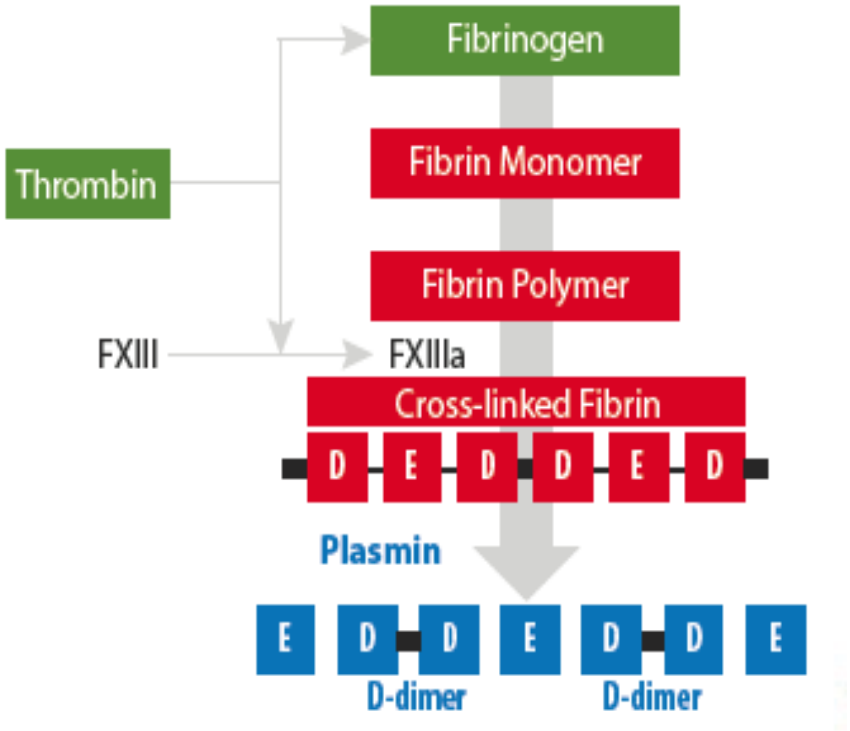
Disclosures for Rita Selby

I have no financial or other conflicts of interest to declare

What we will discuss

- A brief background on D-dimer
- D-dimer assay heterogeneity and establishment of “cut-offs”
- Variability in D-dimer assay results: Focus on recent EQA data
- Impact on clinical decision making
- How do we bridge this Knowledge-to-Action Gap?

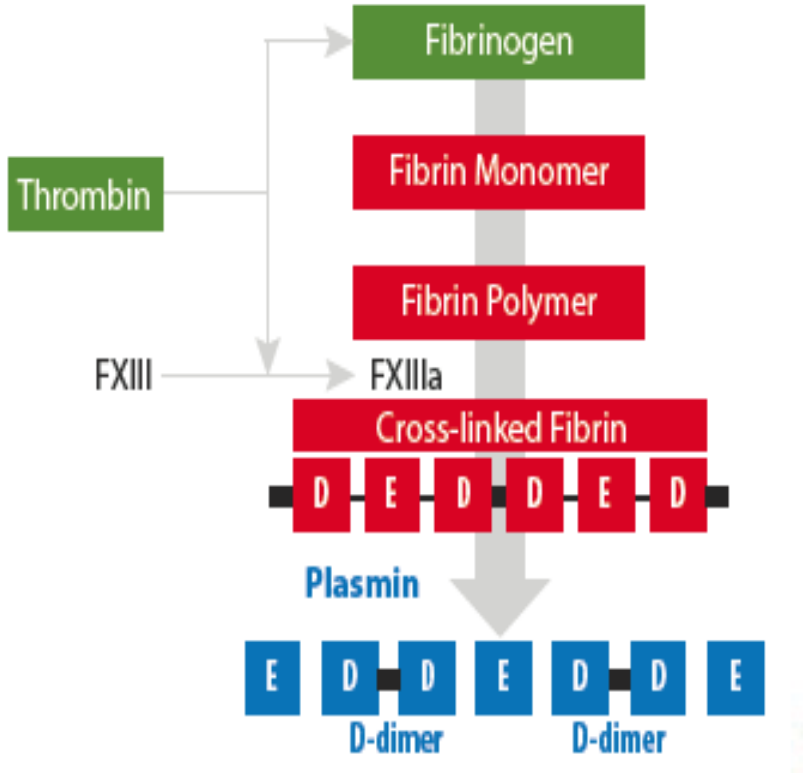
What is D-dimer?



Patient plasma is a "soup" of both D-dimer fragment, and breakdown products of Soluble and Insoluble fibrin monomers and polymers

Lin, Selby. Bloody Easy: Coagulation Simplified. Second Edition. April 2019. www.transfusionontario.org

Assaying D-dimer



Patient plasma is a
"soup" of
both D-dimer fragment,
and breakdown products of
Soluble and Insoluble fibrin
monomers and polymers

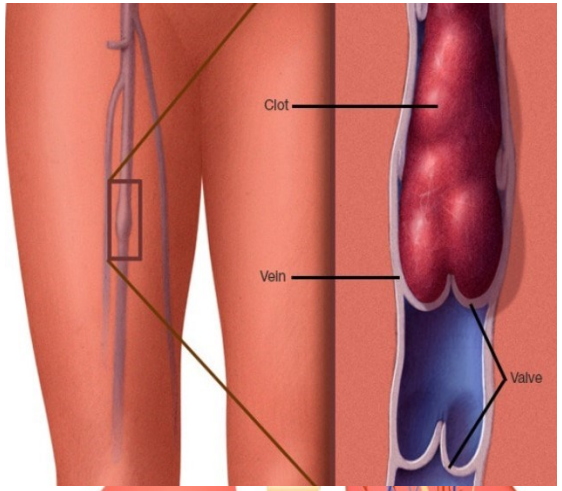
Monoclonal antibodies raised against antigen D-dimer, variably cross-react with other higher or lower molecular weight, cross-linked, fibrin(ogen) degradation products in the patient plasma

Lin, Selby. Bloody Easy: Coagulation Simplified. Second Edition. April 2019. www.transfusionontario.org

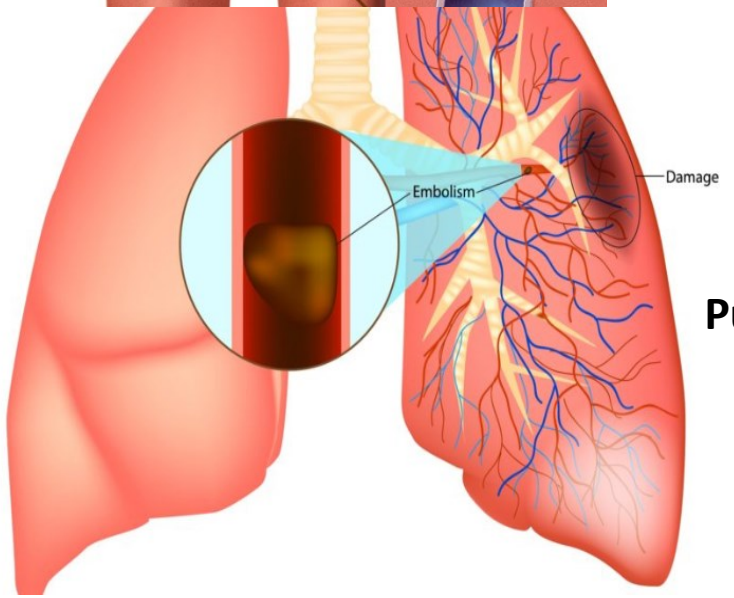
D-dimer - Clinical indications

- Diagnosis of DVT and PE in outpatients with symptoms
- Prediction of recurrence of DVT and PE after a first episode
- Scoring for DIC (ISTH DIC score)
- Recent: D-dimer to risk stratify COVID 19 illness severity

D-dimer is an accepted diagnostic tool for excluding DVT or PE



Deep vein thrombosis (DVT)



Pulmonary embolism (PE)

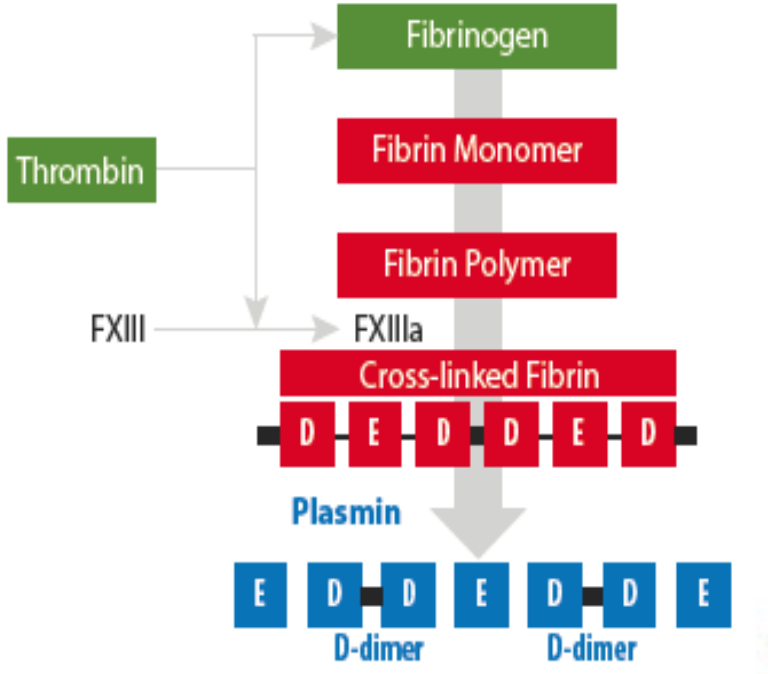
DVT + PE = Venous thromboembolism (VTE)

- Outpatients presenting with suggestive leg or respiratory symptoms
  - Apply a standardized clinical pre-test probability (PTP) assessment (several have been validated)
  - Assess D-dimer level
- **Low to moderate PTP + "Negative" D-dimer rules out DVT/PE**
- **Negative D-dimer = Below a "validated" VTE exclusion threshold (assay specific)**
- D-dimer is **sensitive,** but not **specific** for VTE
- Advantage – Avoid imaging, reduce wait times, resulting efficiency and cost savings

D-dimer: Sensitive, not Specific

Non specific increase in many physiological and pathological states

- Age
- Pregnancy
- Acute illness
- Post-operative
- Trauma
- Cancer
- Infections / Sepsis



Strategies to increase D-dimer "specificity"

- **Age adjusted D-dimer exclusion threshold instead of conventional "universal" threshold of 500 µg/L FEU**
  - Age X 10 in patients > 50 years
  - 85 year old patient – D-dimer exclusion threshold will be 850 µg/L instead of 500 µg/L to rule out VTE

- **Adjusting D-dimer cut-off depending on clinical probability of VTE**
  - <1000 µg/L in low PTP vs. < 500 µg/L (conventional cut-off) if moderate PTP
  - Further modifications with higher D-dimer cut-offs in recent studies

Righini et al. JAMA 2014 – ***ADJUST PE***
Van der Hulle Lancet 2017 - ***YEARS***
Kearon NEJM 2019 - ***PEGeD***
Kearon BMJ Open 2022 – ***4D***

Strategies to increase D-dimer “specificity”

GOAL

SAFELY RULE OUT VTE WITHOUT ANTICOAGULATION

(false negative rates of < 1%)

&

FURTHER REDUCE IMAGING FOR VTE

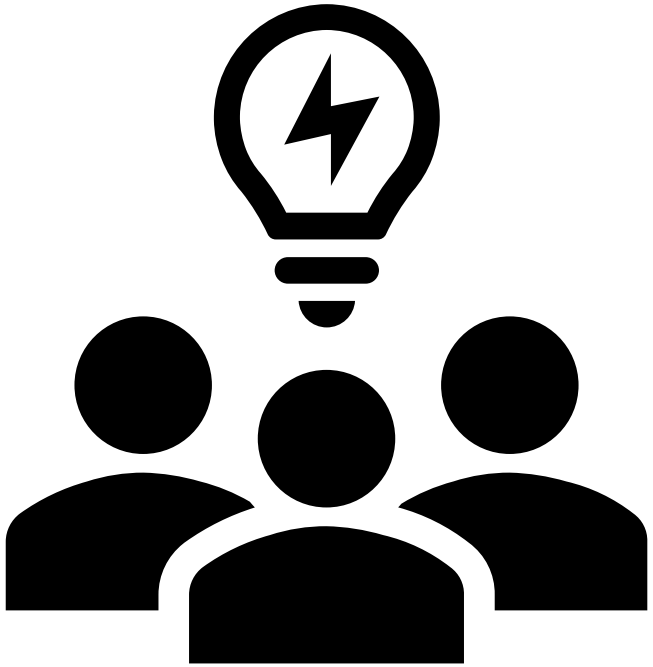
(Avoid additional 5-14% Ultrasounds / Chest CTs)

*Righini et al. JAMA 2014 – **ADJUST PE***
*Van der Hulle Lancet 2017 - **YEARS***
*Kearon NEJM 2019 - **PEGeD***
*Kearon BMJ Open 2022 – **4D***

What are the implications of D-dimer assay quality on clinical practice?

- Are D-dimer assays interchangeable?
- How are exclusion thresholds established?
- What do we know about inter-assay performance?

Are D-dimer assays interchangeable?



Multiple Assays, Multiple antibodies

- 30 available assays using > 20 different monoclonal D-dimer antibodies
- Target different epitopes in FDP fraction

- <u>D-dimer assays are not standardized</u>
  - *As of yet, there is no international reference preparation (IRP) or Universal D-dimer Standard.*
  - *Calibration materials vary by manufacturer*
- <u>D-dimer assays are not harmonized</u>
  - *Assay variability may be reduced by using international reference material from pooled patient plasma to create a "standard D-dimer value" to create a "correction factor"*

Dempfle CE et al. Thromb Haemost 2001
Meijer P et al. Thromb Haemost 2006
Lippi et al. Semin Thromb Hemost 2015
Longstaff et al. Thromb Res 2016
García de Guadiana-Romualdo et al. J Thromb Thrombolyis July 2021

Multiple Assays, Multiple antibodies

- 30 available assays using > 20 different monoclonal D-dimer antibodies
- Target diff

D-DIMER RESULTS ARE ASSAY-SPECIFIC

- D-dimer as
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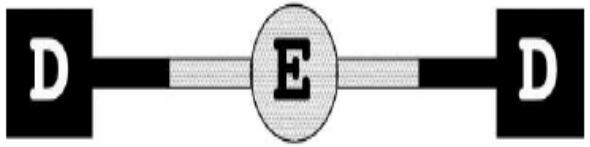
Multiple Reporting units

Types of Units reported

DDU – 195 kDa

FEU – 340 kDa

D-dimer Unit (DDU): 195 kDa



Fibrinogen Equivalent Units (FEU): 340 kDa

Magnitude of Units reported

- 500 **ng / mL**
- 500 **µg / L**
- 0.50 **mg / L**
- 0.50 **µg / mL**
- Less frequent: g/L, g/mL, mg/dL

CLSI Guideline. H-59A. 2011
Olson et al. Arch Pathol Lab Med 2013
Lippi et al. Semin Thromb Haemost 2015
Longstaff et al. Thromb Res 2016

Multiple Reporting units

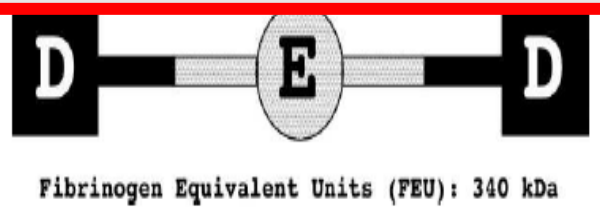
Types of Units reported

DDU ~ 105 kDa

Magnitude of Units reported

Issues with Multiple Reporting Units:

- **Clinicians not realizing that the value is the same although number varies**
- **Mathematical conversions (‘fudge factors”) by Labs leading to high error rates**
- **Non adherence by labs to manufacturer recommended units**
- **Book chapters / Peer-reviewed articles on D-dimer don’t mention units !**



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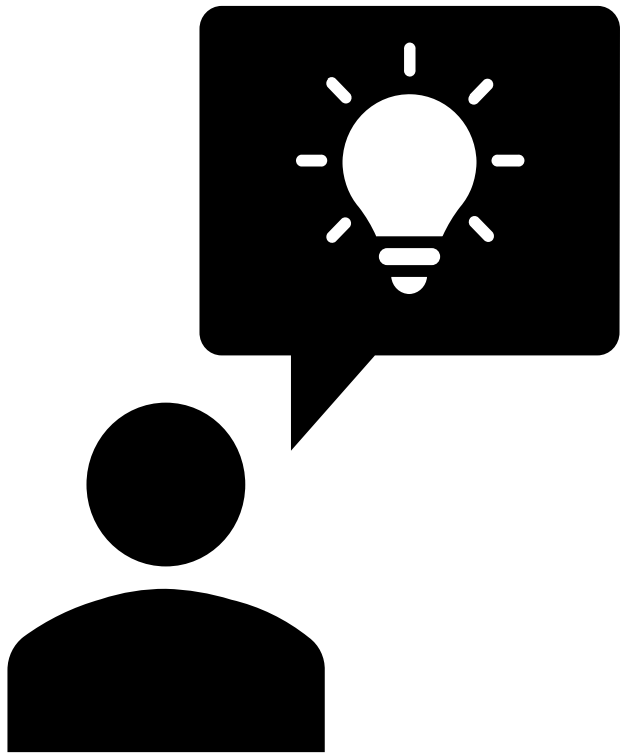
How are exclusion thresholds established?



2 levels of FDA clearance for D-dimer assays

"Exclusion of VTE" cut-off

Management study using D-dimer + Pre test probability assessment

Minimum 3 study sites

Statistically significant number of consecutive, eligible outpatients with suspicion of VTE (> 10% prevalence for both DVT and PE)

Comparison of D-dimer method to:	**VTE proven via imaging techniques + 3 month patient follow up of negative imaging results**
Sensitivity	**≥ 95%**
NPV	**≥ 97% (lower CI ≥ 95%)**

Clinical and Laboratory Standards Institute (CLSI): Quantitative D-dimer for the Exclusion of Venous Thromboembolic disease. Guideline H-59A. 2011

2 levels of FDA clearance for D-dimer assays

"Aid in the diagnosis of VTE" cut-off

NOT A MANAGEMENT STUDY

Minimum 3 study sites

Using outpatient SAMPLES with VTE diagnosis (> 10% prevalence for both DVT and PE)

Comparison of D-dimer method to:	**Predicate D-dimer method**
Sensitivity	**Not defined**
NPV	**≥ 97%**

Clinical and Laboratory Standards Institute (CLSI): Quantitative D-dimer for the Exclusion of Venous Thromboembolic disease. Guideline H-59A. 2011

VTE Exclusion Cut-offs – Patterns of practice

2011-2012 CAP Survey of D-dimer practice

Reviewed package inserts from 10 commonly used assays (10 or more labs)

- 5 inserts - “Exclusion of VTE”
- 4 inserts - “Aid in the diagnosis of VTE”
- 1 method – No threshold for VTE evaluation stated !
- 3 method inserts – Type of units not reported !

- 2430 labs reported using D-dimer for VTE exclusion
  - Only 54% used the manufacturer’s defined threshold
  - 10% used a threshold from the literature
  - 21% established it locally
  - 15% - Other, Don’t know, No data

Olson et al. Arch Pathol Lab Med 2013

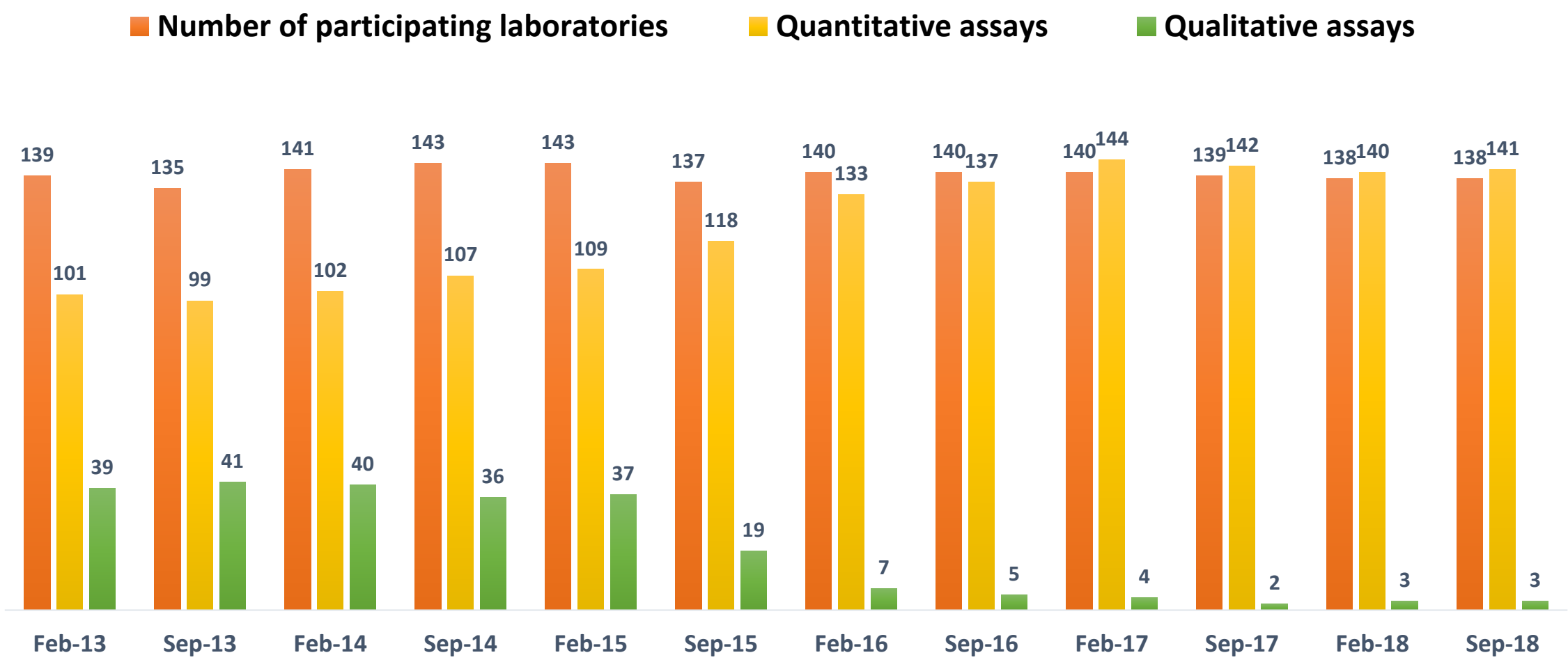
D dimer: Clinical impact of heterogeneity among assays

- Ongoing use of inappropriate assays to exclude VTE
- Failure to adhere to manufacturer recommended thresholds (still more than 30% of labs in 2021 CAP survey reporting a higher threshold)
- Confusion between magnitude and type of reporting units (FEU or DDU) - inaccurate mathematical conversions, wide variation in reported units
- Inadequate D-dimer reporting in peer reviewed literature and textbooks !
    - *Peer-reviewed publications often do not identify assay name, type, or even manufacturer!*
    - *Inadequate reporting of type of units, magnitude of units, cut-offs, analytical performance of assay*

Assumption that D-dimer assays are interchangeable

Clinical and Laboratory Standards Institute;2011. Document H59-A
Olson et al. Arch Pathol Lab Med 2013
Thachil J et al. J Thromb Haemost. 2020

Use of quantitative, high sensitivity assays is increasing – Ontario 2013-2018

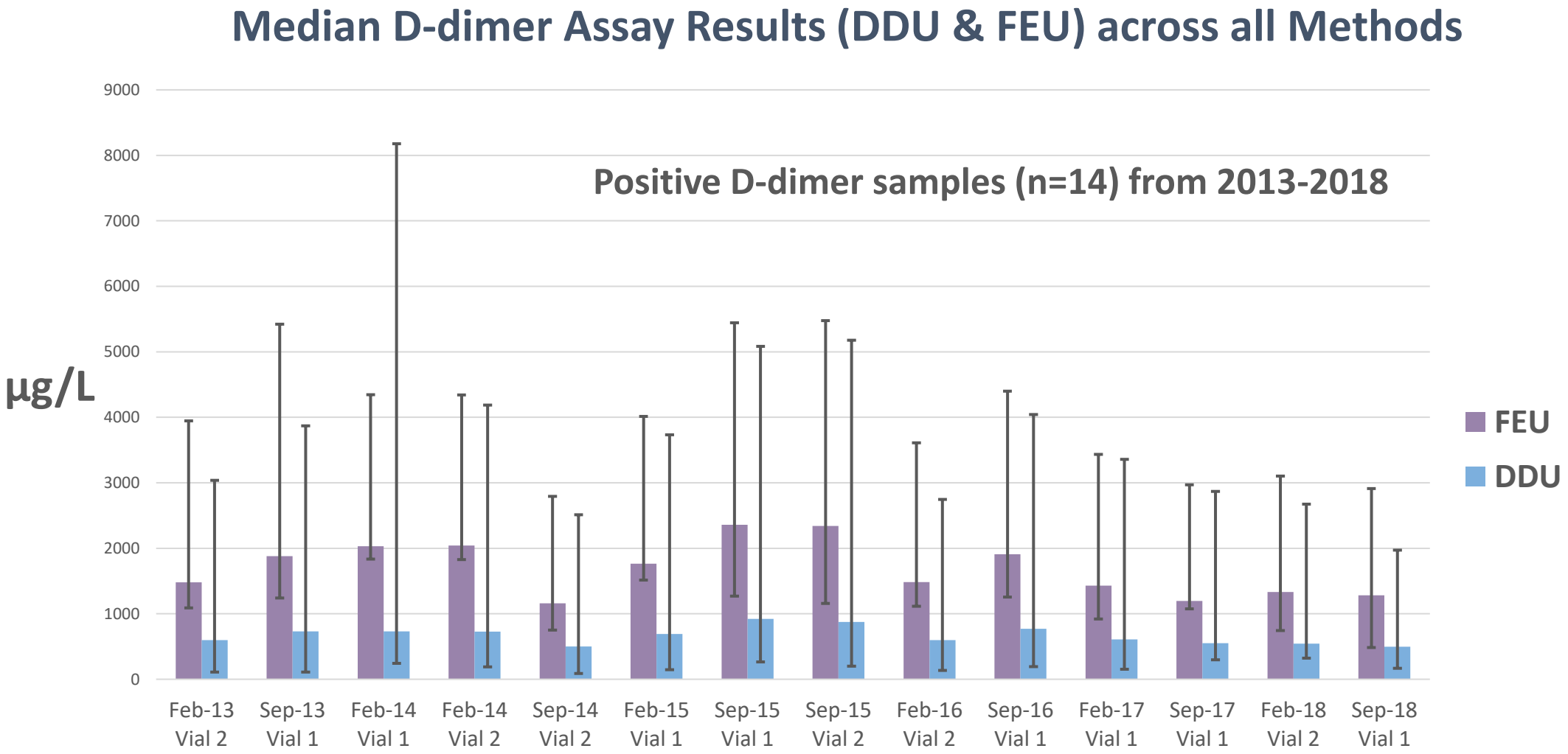


Elbaz, Selby et al. ISLH May 2019

What do we know about inter-assay performance?

Variability in positive “numeric” D-dimer values across methods

IQMH D-dimer Survey data 2013-2018



Elbaz, Selby et al. ISLH May 2019

Excellent agreement on qualitative interpretation only

IQMH D-dimer Survey data 2013-2018

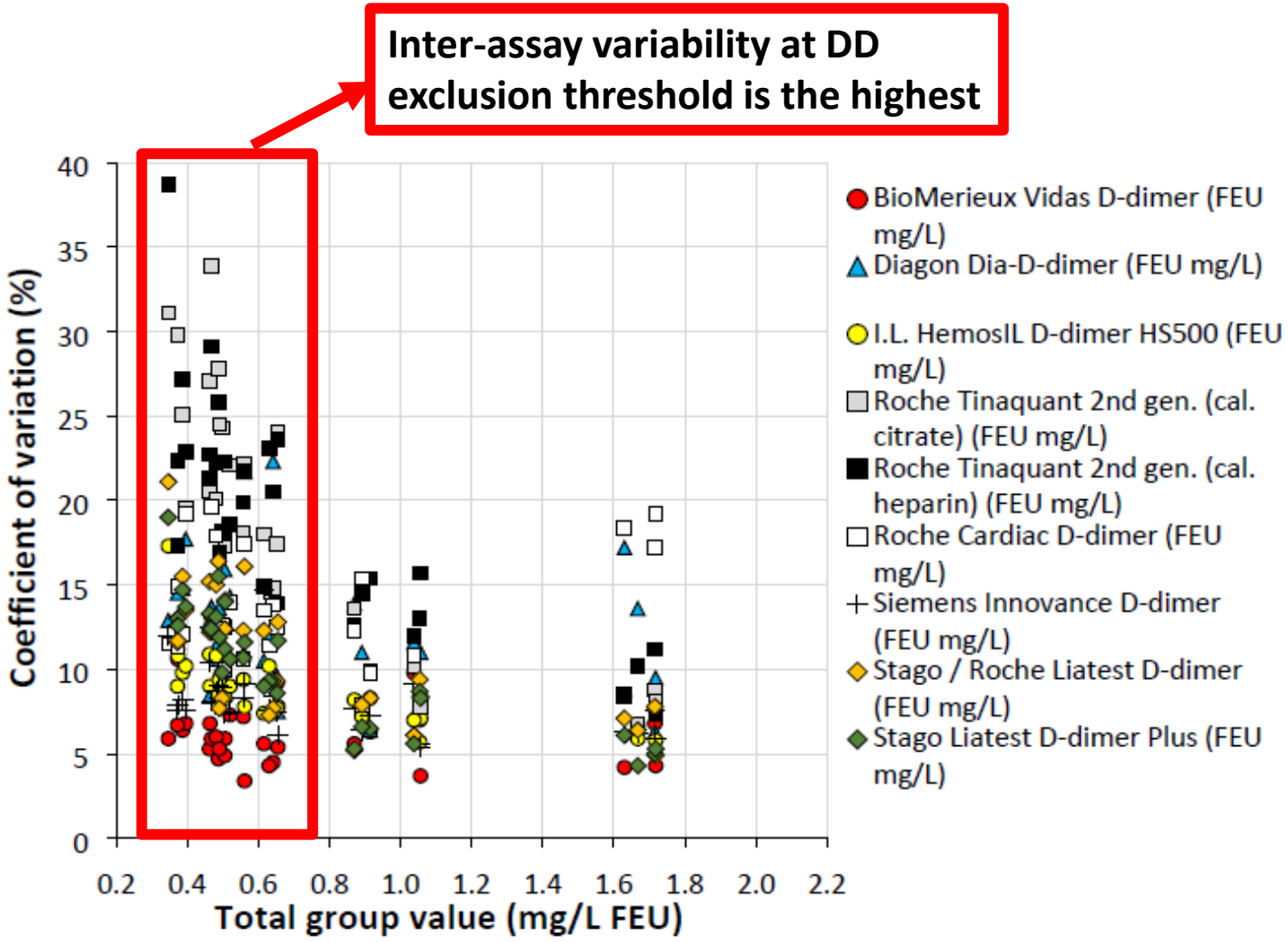
Sample assignment	Qualitative interpretation by laboratories	
	Negative	Positive
Positive samples (n=14) (Normal plasma spiked with D-dimer)	0.75%	99.25%
Negative samples (n=10) (Pooled normal plasma)	97.91%	2.09%

Elbaz, Selby et al. ISLH May 2019

Quantitative D-dimer: ECAT surveys – 2017 to 2020

Variability on <u>same positive sample</u> between assays (n=32)

- Annually - 578 to 640 labs, 37 countries, RR: 88-95%
- 2020 - 28 unique D-dimer assays
- 65% of participants used 3 quantitative, automated immunoassays – Siemens, IL, Stago (all FEU)
- Only 3% used VIDAS ELISA (considered “gold standard”)



Elbaz C, Hollestelle MJ, Meijer P, Selby R. Presented ISTH 2020

Figure by: Martine Hollestelle, ECAT

Okay, okay….so D-dimer assays are not interchangeable…..BUT is that clinically important?

Clinical scenario 1: Suspected DVT in ER

- The ER physician is evaluating a 36 year old female with leg pain for a week since flight from Australia, Takes hormonal contraception
- Calculated Pre-test probability (PTP) using Wells score is “Low”
- D-dimer is **Positive - 468 ng/mL DDU (“Negative” for this lab’s D-dimer assay is <230 ng/mL DDU)**
- **The ER physician treats this D-d as NORMAL or NEGATIVE (The other hospital he works at has a cut-off of “500”)**
- **Low PTP + “normal” D-dimer = DVT ruled out**

CI around a hypothetical true D-dimer value of 0.55 mg/L FEU by method

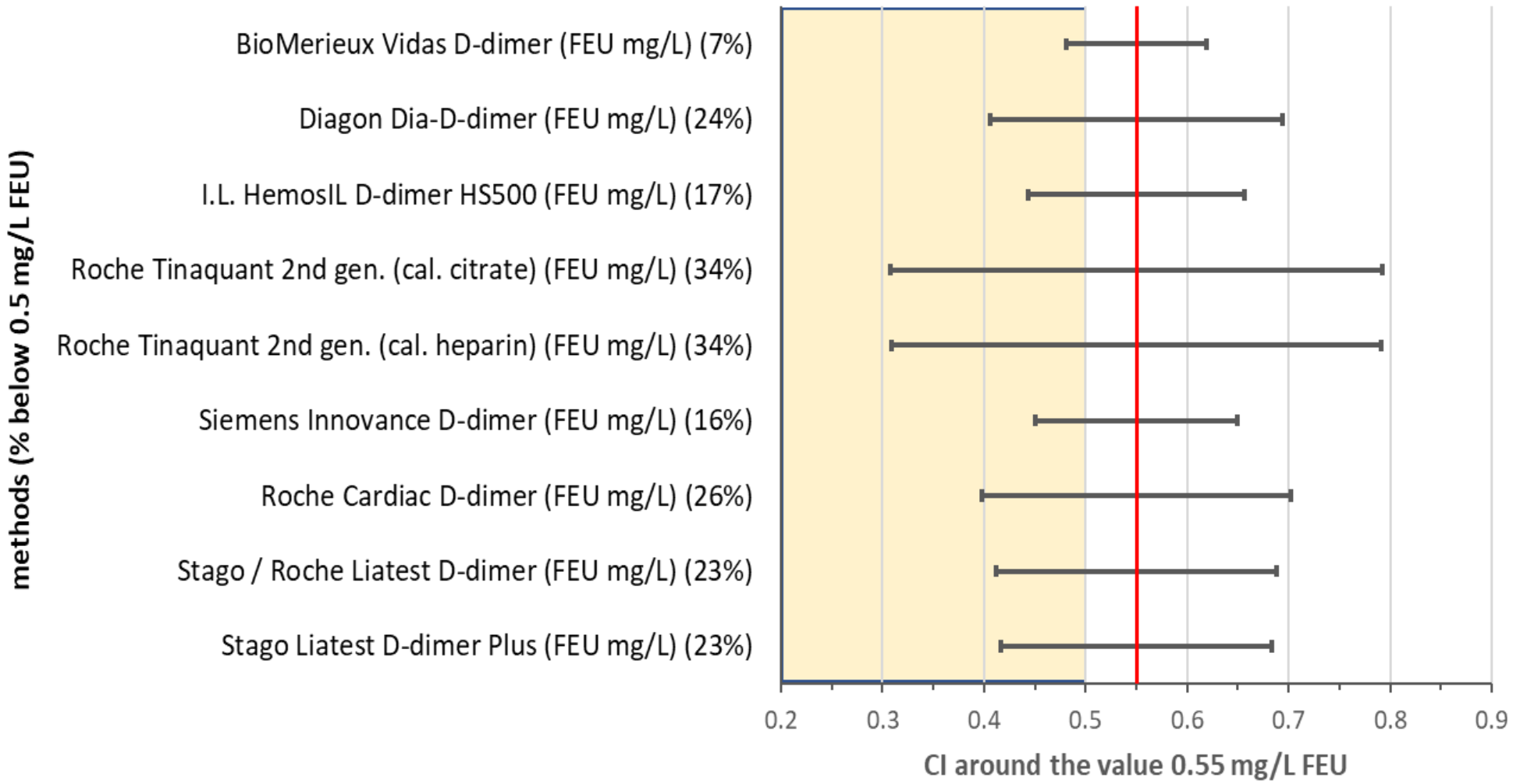


Figure by: Martine Hollestelle, ECAT

Clinical scenario 2 – Predicting Recurrent VTE

- The hematologist is evaluating a 40 year old female who developed an unprovoked left leg DVT and has completed 6 months of oral anticoagulant therapy
- Her BMI - 25 kg/m2
- Her leg has improved but still has **edema**
- D-dimer is **740 µg/L** (local assay cut-off 500 µg/L)
- She calculates her HERDOO2 score:

HER – Hyperpigmentation, **Edema**, Redness of leg
D – **D-dimer > 250 µg/L while on warfarin**
O – Obese, BMI>/= 30 kg/m2
O – Older, Age >/= 65 years

Risk score – 0 or 1 - 3% / annually (1.8-4.8)
Risk score - 2 or more – 8.1% / annually (5.2-11.9)
If continue anticoagulant prophylaxis – Risk 1.6% (1.1-2.3)

Based on HERDOO2 score of 2 (Leg edema and Elevated D-dimer) patient is assessed to be at high risk for recurrence. Long term anticoagulant prophylaxis is recommended after consideration of risk: benefit

Rodger et al CMAJ 2008

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

"HERDOO2" clinical decision rule to guide duration of anticoagulation in women with unprovoked venous thromboembolism. Can I use any D-Dimer?

- VIDAS ELISA D-dimer assay used in the original derivation and validation study of HERDOO2 rule
- Only prospectively validated CDR - Identifies low risk women with unprovoked VTE who can safely discontinue anticoagulation.
- N=248 women participants - plasma frozen for future research
- Calibration and concordance study conducted between 4 commercially available D-dimer assays and VIDAS
- **Poor agreement between all 4 assays and VIDAS leading to 14-20% HERDOO2 misclassification**

Authors recommendation: Assays other than VIDAS "should not be used" – but <5% use VIDAS (ECAT data)

Rodger et al Thromb Res 2018
Elbaz C, Hollestelle MJ, Meijer P, Selby R. ISTH 2020

"Adjusted" Cut-offs – Implementation issues

- Various D-dimer assays used in studies with adjusted cut-offs – NOT generalizable across all assays
- Manufacturers currently do not provide assay-specific, age or PTP adjusted thresholds
- Local study to validate or even verify an age or PTP adjusted D-dimer threshold by a clinical service lab is not feasible

Which D-dimer assays were used in Adjusted cut-off management studies?

	PEGeD	4D	YEARS	ADJUST-PE	Total studied prospectively	
STA-Liatest	1250	948	1323	389	3910	STA-Liatest
Hemosil HS 500	329	214		185	728	Hemosil HS 500
Innovance	124	67	1100	838	2129	Innovance
Triage	32	270			302	Triage
VIDAS			271	1345	1616	VIDAS
Tinaquant			768	128	896	Tinaquant
Cobas H 232				13	13	Cobas H 232
other	18					other

*Righini et al. JAMA 2014 – **ADJUST PE***
*Van der Hulle Lancet 2017 - **YEARS***
*Kearon NEJM 2019 - **PEGeD***
*Kearon BMJ Open 2022 – **4D***

Slide courtesy of : Dr. Kerstin de Wit

Additional evidence “themes” from Real life studies

- Decision rules **seldom used** in every day practice despite high quality evidence supporting these diagnostic strategies
- D-dimer test done **prior to** applying clinical probability – for e.g. rapid ER triage of chest pain = result biases clinical assessment
- **No reduction in radiological test utilization or yield** despite adherence to clinical decision rule plus D-dimer strategy
- Availability of D-dimer (easy blood test) lowering threshold for suspecting VTE? - **Prevalence of DVT and PE getting lower**

Ingber, Selby et al. Can J Emerg Med 2014
Raja, Greenberg et al. Ann Intern Med 2015
Wang, Bent et al. Ann Emerg Med 2016
Deblois, Chartrand-Lefebvre et al. J Hosp Med 2018

D –dimer Diagnostics: Addressing Knowledge to Action Gap

- **<u>Addressing Variability</u>: International Societies & Manufacturers**
  - *Harmonization of D-dimer Assays and D-dimer Reporting units*

D –dimer Diagnostics: Addressing Knowledge to Action Gap

- **Addressing Variability: International societies & Manufacturers**
  - *Harmonization of D-dimer Assays and D-dimer Reporting units*

- **Addressing Appropriate Use: Hospital and Local Laboratory**
  - ***Appropriate assay selection*** *by local laboratory with clinical input*
  - ***User education*** *– assay heterogeneity, reporting units, lack of generalizability of assays, D-dimer use AFTER decision rule only, education on adjusted cut-offs*
  - ***Mandatory, Clinical decision support algorithms*** *with performance improvement component*
  - ***Appropriate laboratory input*** *for large management trials and publications sent for peer review*

D –dimer Diagnostics: Addressing Knowledge to Action Gap

- **Addressing Variability: International societies & Manufacturers**
  - *Harmonization of D-dimer Assays and D-dimer Reporting units*
- **Addressing Appropriate Use: Hospital and Local Laboratory**
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  - ***Mandatory, Clinical decision support algorithms*** *with performance improvement component*
  - ***Appropriate laboratory input*** *for large management trials and publications sent for peer review*

- **Addressing Effectiveness of Diagnostic Strategy:**
  - *Well designed quality improvement studies assessing real-life impact of selected diagnostic strategy on efficiency and safety of VTE diagnosis*

Acknowledgements

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