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ONTARIO MEDICAL ASSOCIATION

QUALITY MANAGEMENT PROGRAM–
LABORATORY SERVICES

D-dimer Assay Issues and Standardization: QMP-LS Studies

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Mayo/NASCOLA Coagulation Testing Quality Conference

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Disclosure

Relevant Financial Relationship(s)

None

Off Label Usage

None

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Toronto — QMP-LS

Learning Objectives

- Recognize problems involved with D-dimer testing due to lack of standardization
- Distinguish the value of a normal/negative D-dimer result
- Provide examples of four basic D-dimer assay formats
- Review challenges associated with choosing a D-dimer assay
- Discuss EQA D-dimer initiatives to standardize the evaluation model

Lack of Standardization

- **D-dimer assay lacks standardization!**
- **Introduced in absence of a:**
  - Single reporting convention
  - Standardized unit of measure
  - Unique monoclonal antibody (Mab)
  - Standard calibrator
- **Lack of standardization means:**
  - Results, reference intervals and clinical cut-off or threshold values cannot be extrapolated between methods

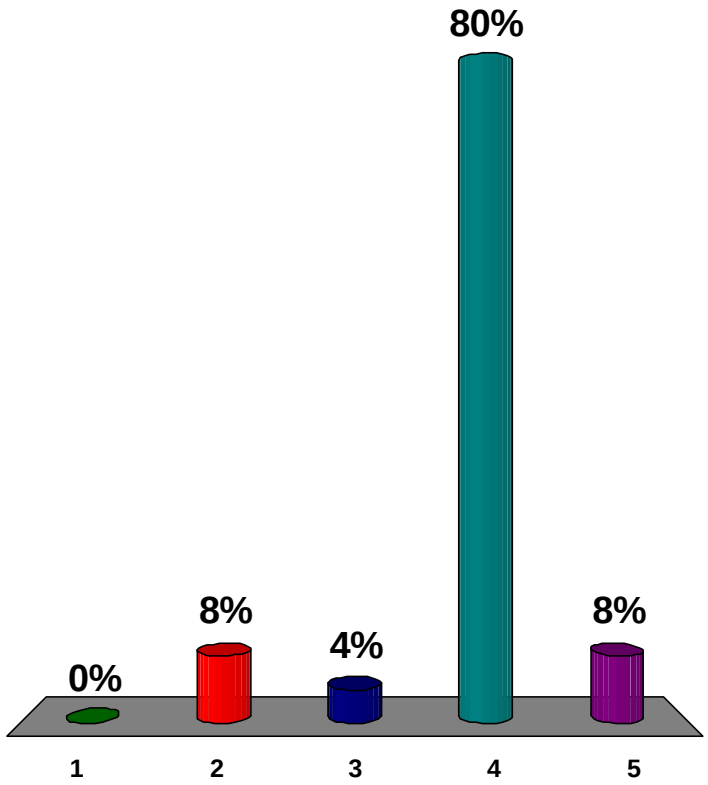
Add to This Mayhem

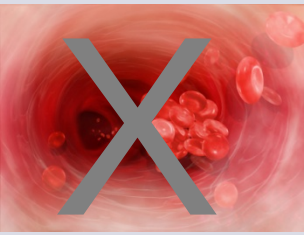
- Errors due to incorrect mathematical conversion of units
- Lack of technical understanding as to which unit of measure a kit is based
- Variation of instrument technology
- Interference by anticoagulant therapy
- Misuse of statistically generated D-dimer reference interval (RI) obtained from healthy individuals but used as clinical cut-off value

Leaves even an optimist thinking, what a mess!

An elevated D-dimer does NOT:

1. Indicate intravascular coagulation
2. Indicate breakdown of fibrin
3. Indicate plasmin lysis of fibrin
4. Determine patient has a VTE
5. Indicate cross-linking by Factor XIII





Value of the D-dimer Assay

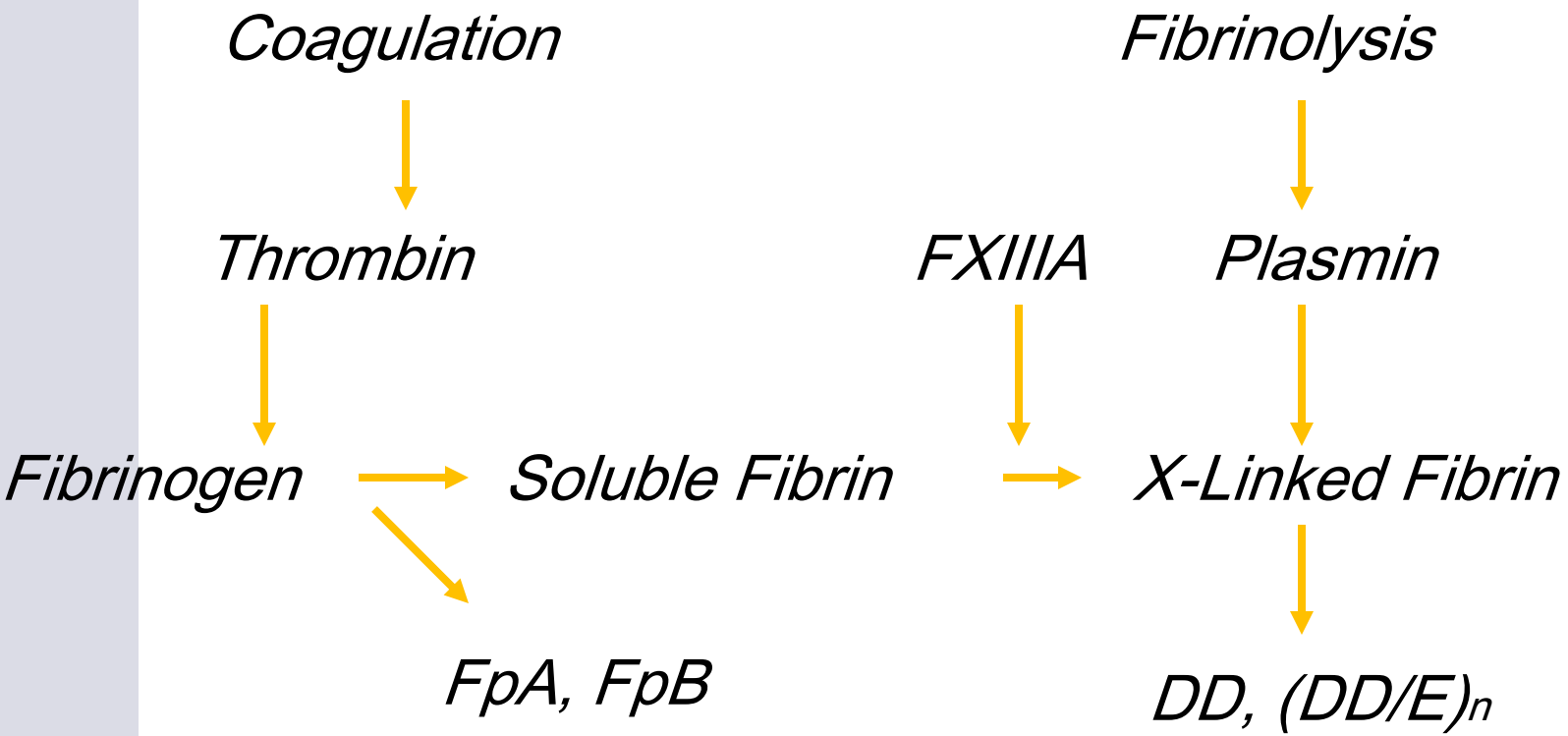
- D-dimer is elevated in systemic and local fibrinolytic states
  - e.g., disseminated intravascular coagulation (DIC) and acute venous thromboembolism (VTE) including deep vein thrombosis (DVT) and pulmonary embolism (PE)
- Value is to exclude VTE in patients whose history and physical exam yields a low preset probability of disease
- Used to evaluate and monitor treatment of patients with DIC

Exclusionary Test

- VTE is one of the most common cardiovascular disorders in industrialized countries, affecting 5% of people; failure to diagnose can result in death
- VTE signs and symptoms such as pain and swelling for DVT or breathing difficulty for PE are common to other illnesses
- PE is an important medical problem causing 10% of deaths in hospitalized patients, 22% PE patients die prior to diagnosis or treatment
- Because of non-specific signs and symptoms many patients are investigated do not have VTE, diagnostic algorithms and D-dimer result < the clinical cut-off value can eliminate unnecessary imaging studies

Wells PS. Integrated strategies for the diagnosis of venous thromboembolism. Journal of Thrombosis and Haemostasis, 5 (Suppl. 1):41-50.

Formation of D-dimer



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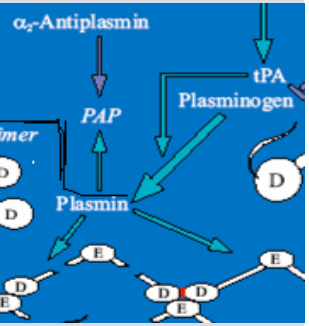
Use of D-dimer



- Specific marker of breakdown of fibrin clot by plasmin and indirect marker of clot formation
- Plays important role in detection of a variety of hypercoaguable states. Compromising components will alter rate of fibrinolysis
- Sensitive but non-specific - elevated in VTE, MI, infection, malignancy, pregnancy, recent surgery, bleeding, trauma, DIC, sepsis, sickle cell crisis, RA, and phlebitis. D-dimer increases with age
- Lack of specificity causes inappropriate testing of very ill hospitalized patients because of expected high positive results

Value

Thinking “Positive” D-dimer will help determine a diagnosis is wrong!

Value is in the absence of an elevated D-dimer!

How D-dimer Tests Evolved

- Early test systems used polyclonal antibodies generated to a variety of fibrin and fibrinogen split products
- Today, methods rely on use of Mabs which recognize epitopes specific to cross-linked D-dimer fragment
- Assays are readily available, economical, non-invasive, rapid and easy to perform

Assays are Not Created Equal

- 30 assays using >20 Mabs
  - Varying sensitivity/specificity contributing to inter-laboratory variation
- D-dimer molecular structure is not homogeneous
- Mabs bind fibrin degradations products of varying MW, identifying them all as containing D-dimer
- There is no international D-dimer calibrator
- Several assays have been evaluated for the diagnosis of VTE
- **Four basic assay formats:**
  - Whole blood agglutination
  - Enzyme-linked immunosorbent (ELISA)
  - Latex agglutination
  - Immunochromatography

Whole Blood Agglutination

- e.g. SimpliRED - Shows a **qualitative** positive reaction when an agglutination reaction occurs in comparison with the negative control

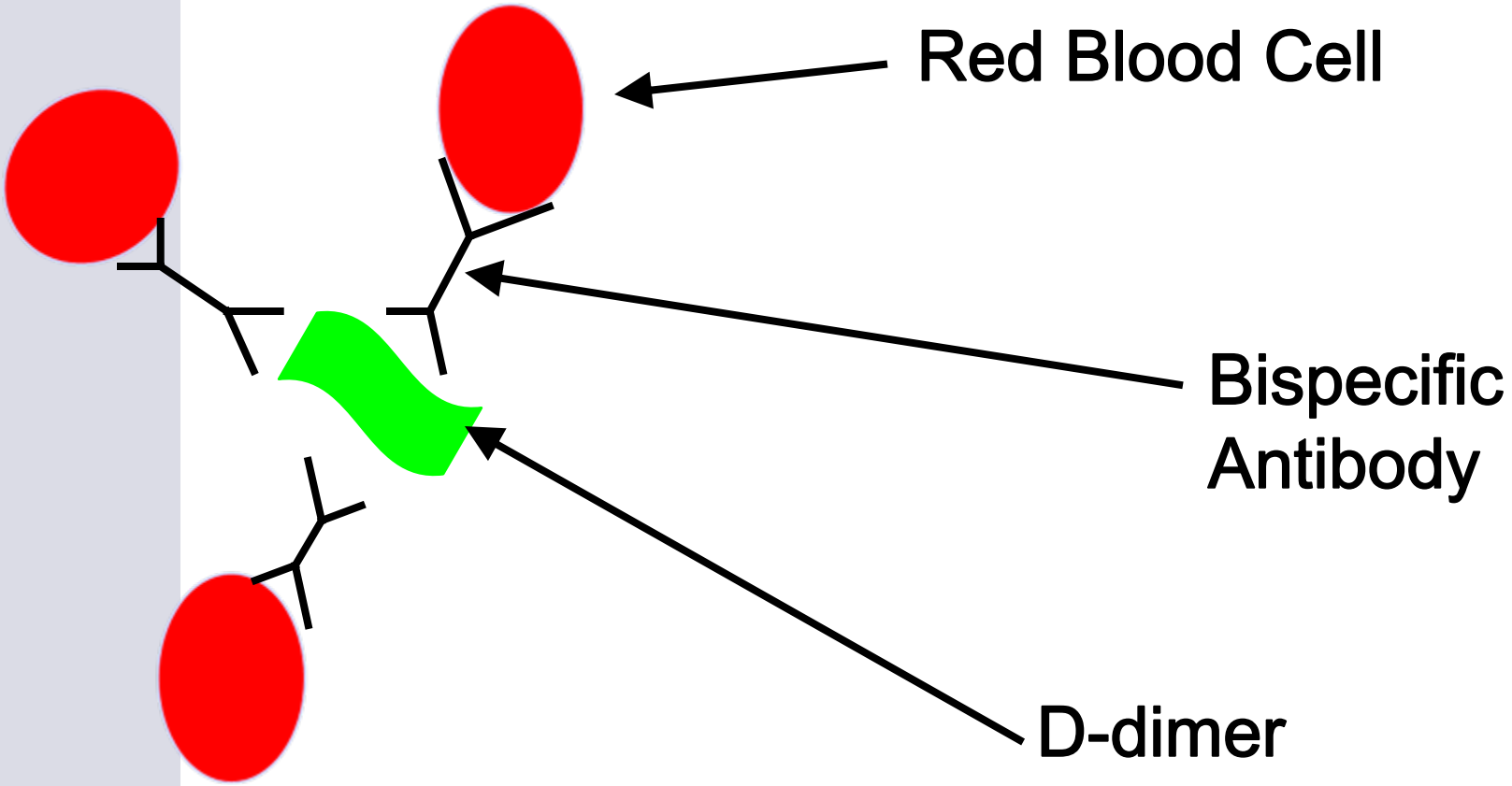
Advantages

- No plasma preparation required (10µl of blood obtained from venipuncture or finger stick)
- Point of care testing (can be performed at the bedside)
- Results within 5 minutes
- Better specificity than other assays (66-77%).
- Sensitivity generally around 80%

Disadvantages

- Qualitative
- Manually read (may vary between readers)

Whole Blood Agglutination



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SimpliRED

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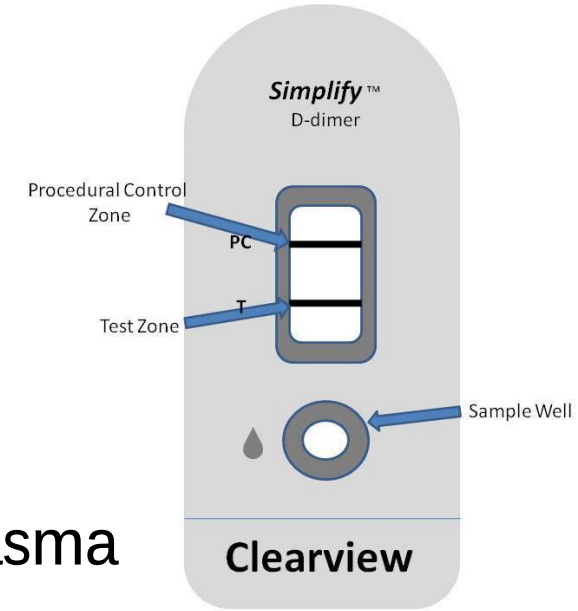
Immunochromatography

- e.g. Clearview Simplify D-dimer
- A rapid **qualitative** immunochromatography test
- Murine Mab is conjugated to colloidal gold particles
- Antibody–gold conjugate binds specifically to patient's D-dimer
- Concentration of complexes causes a pink/purple line to appear
- 34 uL whole blood and 20uL plasma
- High sensitivity



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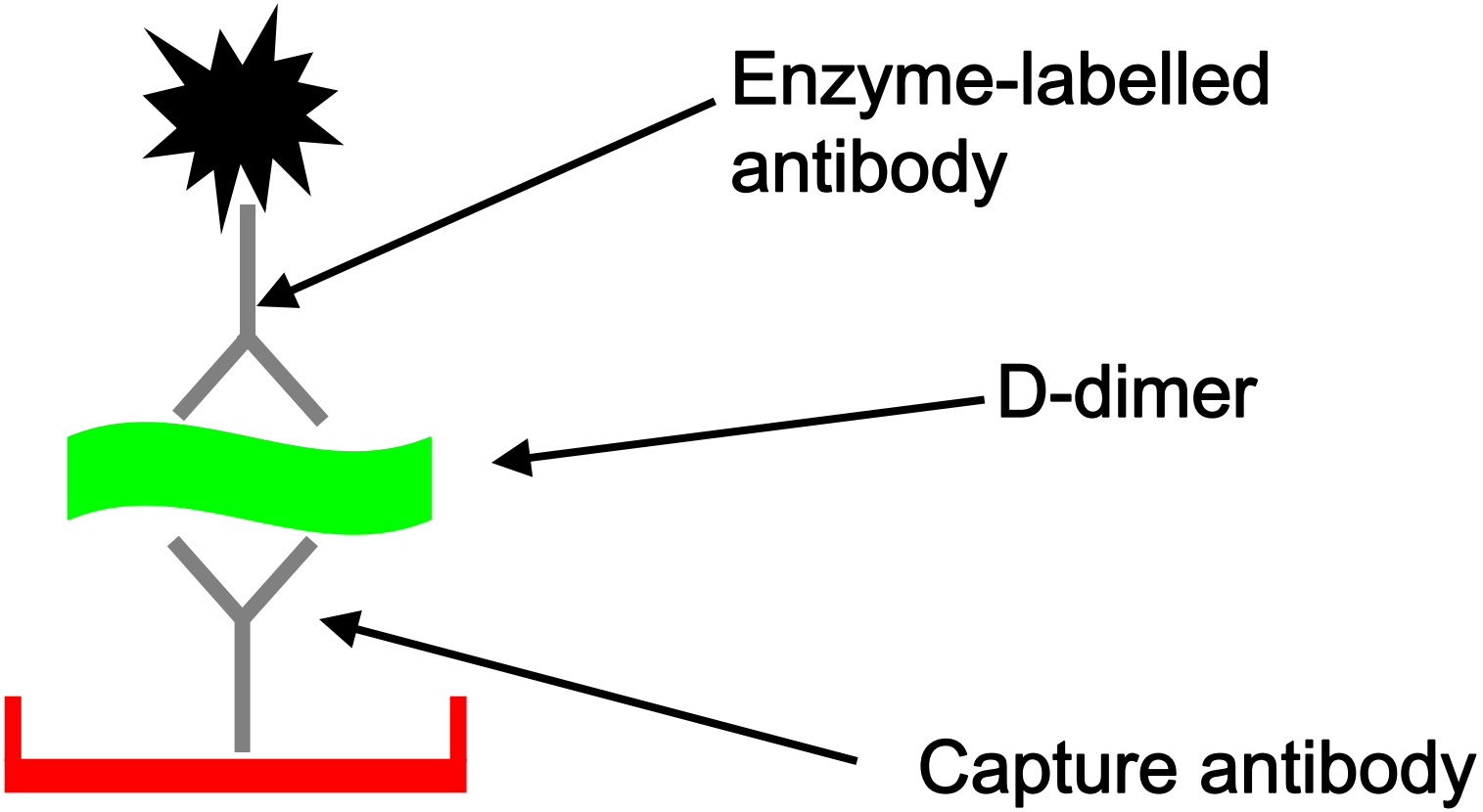
ELISA

- **Microplate ELISA Quantitative Assay**
  - Considered reference standard. Not useful in routine emergency since labor intensive
  - High sensitivity at 97% at a cut-off of 500 ng/mL and medium specificity of 50%
- **Rapid ELISA/ELFA Quantitative Assay**
  - Combines ELISA with final detection in fluorescence
  - Automated immunoanalyzer provides numeric result for quantitative assay
  - VIDAS – extensively studied and used in diagnosis of VTE
  - Similar sensitivity and specificity to classic ELISA with shorter TAT (~35 min.) therefore suitable for real-time use
  - High sensitivity at 98% and medium specificity of 55%

ELISA



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Latex Agglutination

- Use Mabs specific for cross-linked D-dimer, coated onto latex particles
- Single-step reactions in which agglutination of Ab-coated particles is used for detection
- Latex-enhanced photometric assays are either turbidimetric or colorimetric
- Less expensive and more rapidly available than the ELISA (7-15 minutes).
- Modifications that allowed turbidimetric quantification increased sensitivity to 98% (similar to conventional ELISA)

Latex D-dimer

1st Generation

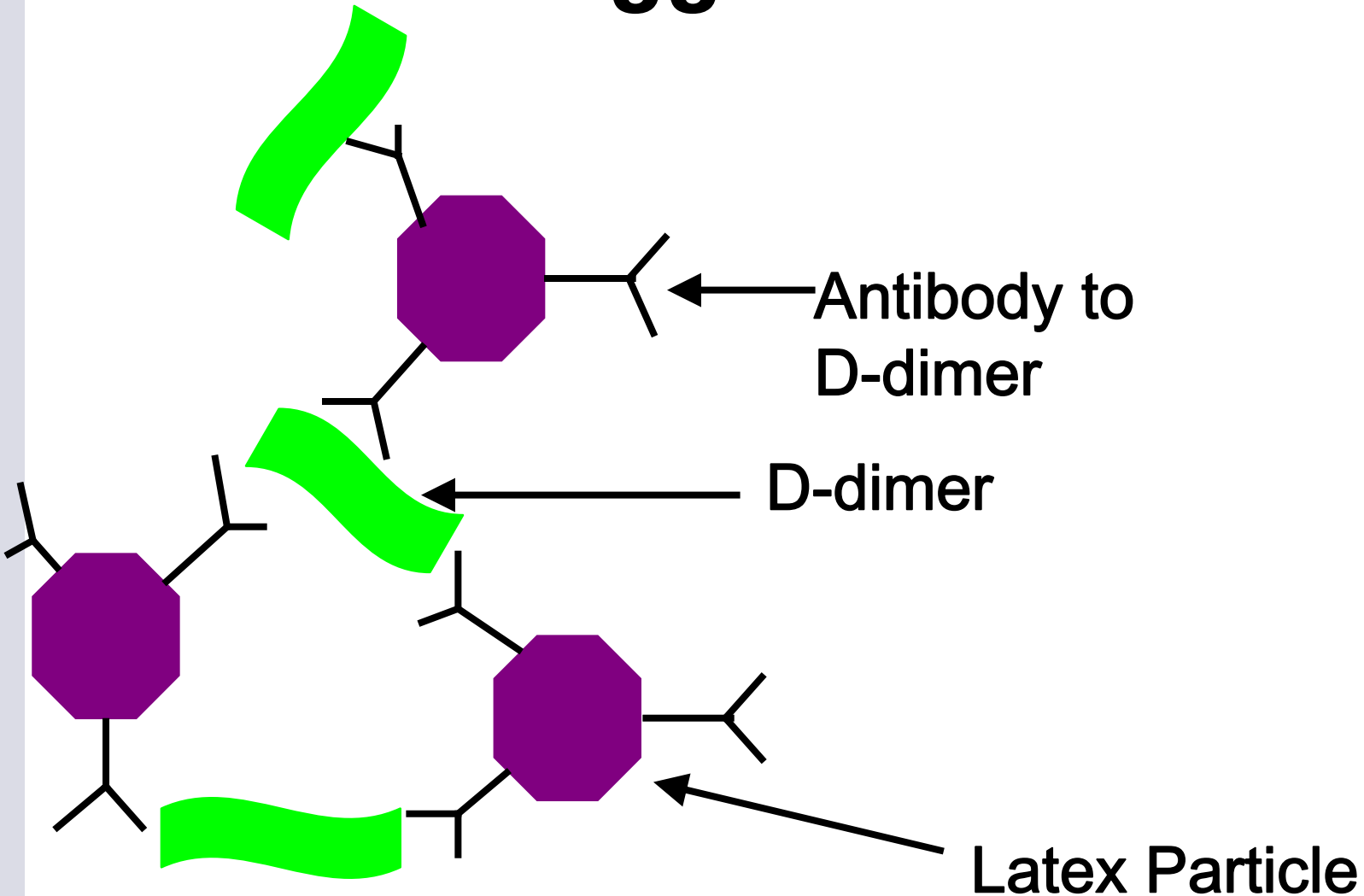
- Manual
- **Semi-quantitative**
- Inexpensive
- Rapid (<5 minutes)

- e.g. Minutex D-dimer
- Low sensitivity

2nd Generation

- Automated
- **Quantitative**
- Relatively inexpensive
- Rapid (10 minute processing)
- e.g. HemosIL, STA Liatest D-Di, Innovance
- Increased sensitivity

Latex Agglutination



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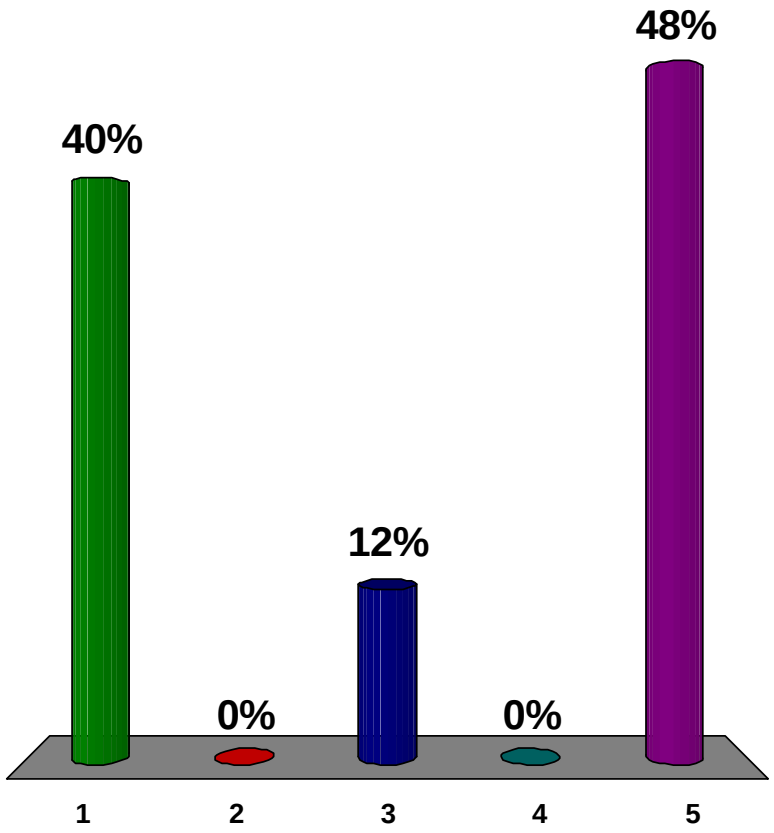
What is common to D-dimer assays?

1. Monoclonal antibody
2. Calibrator
3. Specificity/sensitivity
4. Cut-off value
5. Requires validation



Calibrators

- Manufacturers of assays choose the type of calibrator that fits best with their assay
- Majority are calibrated with material obtained by controlled lysis of fibrin clots while some use more purified standards
- Specificity of the Mabs differs depending on if the mixture of fragments in the sample or calibrator are different

Three Types of Calibrators

- Protelotyic fragments of fibrin clot
- Plasma clot proteolytic fragments
- Fibrin fragment D-dimer purified by gel filtration chromtography

FEU vs. D-dimer Units (DDU)

- Calibrators' concentrations often expressed as *amount of fibrinogen present in the mixture used to prepare the calibrator material*, fibrinogen equivalent units (FEU)
- According to respective MW (340 and 195 kDa), D-dimer values expressed in FEU should be 2x as high as those expressed in D-dimer units (DDU)
- However, fibrin digests do not only contain D-dimer fragments but HMWF depending on the lysis procedure so the 2x rule does not always apply
- Along with the different unit of measure, manufacturers provide variety of reporting units
- Same value reported in different units:
  - µg/mL – 0.5 µg/mL
  - µg/L – 500 µg/L
  - mg/L – 0.5 mg/L
  - ng/mL – 500 ng/mL

Reference Interval/Cut-off Value

- RI established by assaying specimens that are obtained from healthy individuals
- Cut-off value established by reviewing high risk candidates that undergo objective testing. According to results, a D-dimer cut-off is chosen that optimizes sensitivity and specificity, making it clinically useful
- Cut-off value for the majority of assays will be > the upper limit of the RI
- Recommended each lab establish own cut-off value and not rely on manufacturer's recommended value or another lab's validation
- Main criteria for establishing cut-off is that it must have high negative predictive value

Cut-off Value

- If full validation is not an option, review literature to confirm manufacturer performed Receiver Operator Curve (ROC) analysis using a sufficient population and then verify recommended cut-off in-house with smaller population
- Different assay or same assay for investigation of different disease states may have different cut-off values (DVT vs. DIC)
- Some labs tweak the cut-off to increase the specificity of the assay or use different cut-off for different groups of patients such as pregnant or elderly patients
- Drs cannot be expected to understand principle of assay, or context within which it was validated, labs should report interpretative comment along with the result

Differences Among D-dimer Assays

- To Recap: D-dimer is complex variety of cross-linked fibrin derivatives and not single molecule
- Heterogeneity in performance characteristics is due to:
  - Mabs with different specificities for fibrin, and their derivatives
  - Differences in calibrators
  - Differences in reference intervals
  - Differences in cut-off values
  - Differences in patient populations evaluated
- When choosing an assay review the “intended use” statement in manufacturer’s package insert
  - e.g. “for the exclusion of DVT and as an aid in the diagnosis of PE”

Choosing a D-dimer Method

- Difficult to decide which D-dimer assay to use
- No perfect test method
- Varying levels of sensitivity and specificity, in general, those with the highest sensitivity tend to be less specific

Choice Dependent On:

- Prospective evaluations (sensitive and specificity data)
- Local diagnostic strategies
- Published data regarding data accuracy
- Method characteristics (simplicity, time to result)
- Direct costs (materials, instruments, personnel)
- Indirect costs (additional investigations required)

Evaluation of D-dimer Assay

Before introducing D-dimer assays into practice, they must be thoroughly evaluated

Evaluation involves 3 stages:

Diagnostic Criteria

- Differentiate negative/positive, reproducible

Accuracy Studies

- Evaluation of test validity by comparing to reference standard which is assumed to be correct
- Accuracy of the new test is determined by calculating the sensitivity and specificity
  - Conducted under optimal conditions but may not be similar to clinical practice

Management Studies

- Evaluation safety of managing patients suspected of having disease according to results of the new test
  - Appropriate in VTE - anticoagulants are effective and inadequate management results in a high rate of recurrence which can be objectively documented

Quality Management Program– Laboratory Services

- Inter-laboratory comparison evaluates 3 aspects of laboratory testing:
  - Quality of Material
  - Reliability of Method
  - Reliability of Operator
- In PT/EQA, quality of material is known (through homogeneity, stability, determination of AV)
- The techniques and/or methods are being evaluated

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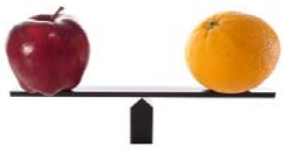
Pilot Survey

- Normal plasma spiked with pooled human-derived D-dimer
- Reporting dependant on method e.g. qualitative, semi-quantitative or quantitative results and RIs (but no interpretation).

Findings

- Use of different units
- Misunderstanding of unit of measure of kit
- Variation in results
- Use of inappropriate RIs and/or cut-off values for method

Pilot Survey



- Reasonable within method consensus but poor between method consensus
  - Different kits produce different results on same sample due to variable Mabs
    - Sensitivity and specificity
  - Difficult to compare methods with this material
  - Potential for viral transmission

EQA/PT Testing Material

- To remove variation associated with material (allow comparison of technique and method) researched testing material and moved to purified D-dimer
- Normal plasma spiked with human-derived D-dimer from purified protein components
  - Predominantly single product of lysis should produce similar results between kits IF target is D-dimer
  - Allows result comparison within method and between method and over time
  - Overcomes problem of varied results due to method and breakdown product present
  - Allows for consistent evaluation using PT/EQA

D-dimer Survey Model

- **Material:** Normal plasma spiked with human-derived D-dimer from purified protein components *(Affinity Biologicals™ Inc, Hamilton, ON)*
- **# Participants**: 135
- **Survey Frequency**: 2 per year
- **No Samples**: 2 per survey
- **Range:** Low, medium and high values of D-dimer to mimic normal, VTE and DIC, respectively.

D-dimer Survey Model

- **Quantitative**: Value (specify FEU or DDU in µg/L) and interpretation (+ or -)
- **Analysis:** Robust statistics using measure of uncertainty (allowable performance limit/standard error)
- **Qualitative/Semi-quantitative**: interpretation (+ or -)
  - Participant consensus (>=80%)
- **Quantitative**
  - **Assigned Value**: Value AMM (by unit of measure) ±40%
  - **Interpretation:** Participant consensus (>=80%)

Model Improvement Over Time

- Removed variability associated with material
- Recommended standardized reporting units

  (Fibrin D-dimer DDU nnn μg/L

  or

  Fibrin D-dimer FEU nnn μg/L)
- Emphasized need to validate reference intervals and cut-off values
- Recommended use of interpretative comment
- Educated participants on use of D-dimer testing

Crowther MA et al. Human-Derived D-dimer for External Quality Assessment. *Am J Clin Pathol* 2008:130:805-810.

Current Findings

- With removal of variability associated with pooled TM, use of standardized units and sorting results by method, now able to compare methods and assess technique and practice
- February 2009 D-dimer Survey
- 2 samples, target value 605 μg/L (HemosIL D-dimer), mimic DVT/PE
- 5/135 reported a qualitative and semi-quantitative

Types of Assays

- **Qualitative**
  - BBI SimpliRED (N=50)
  - Trinity Biotech Minutex D-dimer (N=2)
- **Semi-quantitiave**
  - Trinity Biotech Minutex D-dimer (N=6)
- **Quantitative**

  D-dimer Units (DDU)
    - IL HemosIL D-dimer (N=45)
    - Siemens D-dimer PLUS (N=5)

  Fibrinogen Equivalent Units (FEU)
    - Diagnostica Stago STA Liatest D-Di (N=27)
    - Roche Cardiac D-dimer (N=3)
    - Siemens Innovance D-dimer (N=1)
    - bioMerieux Vidas D-dimer Exclusion (N=1)

Qualitative – SimpliRED (N=50)

- 100% reported DD-1 and DD-2 as Positive
- 52% provide interpretive comment
- Reasons for testing: Unknown/exclusion of DVT/PE and diagnosis/monitoring of DIC

Example of Interpretive Comment:

> "D-dimer screen, this test is only used to exclude a VTE (DVT/PE) with patients who have LOW clinical probability. This test should NOT be used for the assessment of patients for DIC."

Semi-quantitative –
Trinity Biotech Minutex D-dimer (N=6)

- 100% reported DD-1 and DD-2 as Positive
- 100% provide interpretive comments
- Use of 2 unit values and reference intervals:
  - <500 μg/L FEU and <250 ng/mL DDU
- 1 established reference interval
- No lab validated cut-off value
- Reasons for testing: Unknown/exclusion of DVT/PE and diagnosis/monitoring of DIC

Example of Interpretive Comment

"D-dimer elevated. This could be due to acute thrombosis (DVT/PE), or increased fibrinolysis associated with DIC, liver disease, recent surgery, sepsis, malignancy, etc."

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Quantitative DDU – HemosIL D-dimer (N=45)

	Mean	SD	CV	Min	Max	LL	UP
DD-1	595	78	13	396	761	357	883
DD-2	612	87	14	385	951	367	857

- 96% reported DD-1 and DD-2 as Positive (4% did not report interpretation)
- 71% provide interpretive comments
- Variation in RIs: <230/240/256/278 DDU μg/L (N=32) and <230 ng/mL DDU (N=13)
- 73% report a RI but only 33% established it in-house
- 84% report a DVT/PE cut-off but only 45% validated it
- Majority reporting RI and Cut-off use identical values

Quantitative DDU cont'd

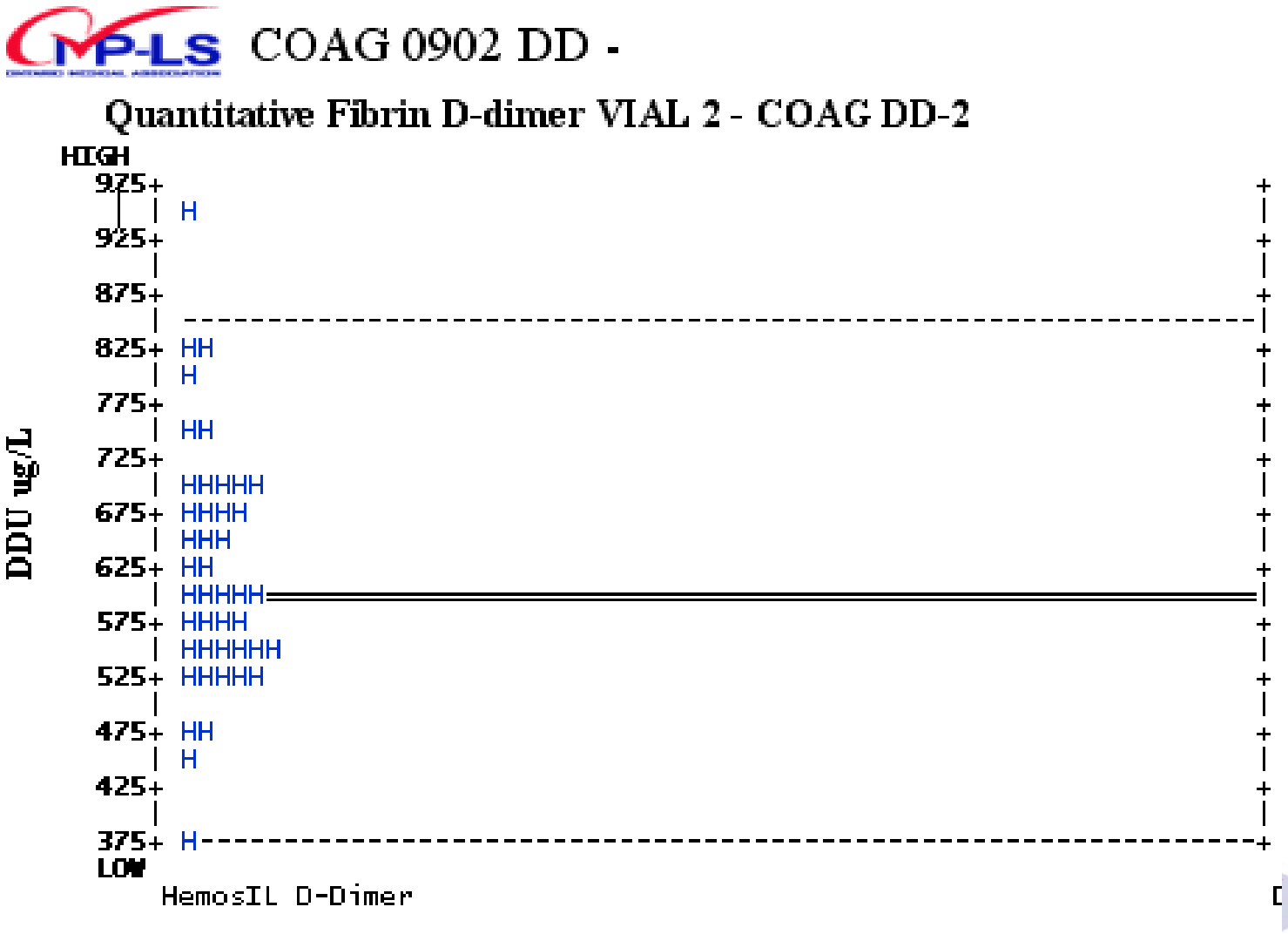
- Reasons for testing: Unknown/exclusion of DVT/PE and diagnosis/monitoring of DIC/Predicative value

Examples of Interpretive Comment

(RI only) – "A D-dimer result should never be used in isolation for Dx of DVT/PE and must be interpreted in combination with clinical probability. D-dimer **<240** µg/L is only useful to exclude Dx of DVT/PE in patients with LOW clinical suspicion. It is recommended that all other patients undergo additional investigation. D-dimer result of >2000 ug/L is compatible with a Dx of DIC in right clinical setting."

(Cut-off only) – "D-dimer result should never be used in isolation for Dx of DVT/PE and must be interpreted in combination with clinical probability. D-dimer of **<400** µg/L is only useful to exclude Dx of DVT/PE in patients with LOW clinical suspicion..."

Quantitative DDU –
IL HemosIL D-dimer (N=45)



Quantitative FEU – Diagnostica Stago STA Liatest D-Di (N=27)

	Mean	SD	CV	Min	Max	LL	UP
DD-1	2096	122	6	215	3620	1258	2934
DD-2	2114	87	4	209	3540	1268	2960

- 93% reported DD-1 and DD-2 Positive and 7% reported Negative
- 70% provide interpretive comments
- Variation in RIs - <400/500 FEU μg/L (N=21) and < 0.4/0.5 μg/mL FEU (N=6)
- 100% report a RI but only 44% established it in-house
- 59% report a DVT/PE cut-off but only 25% validated it
- Majority reporting RI and Cut-off use identical values

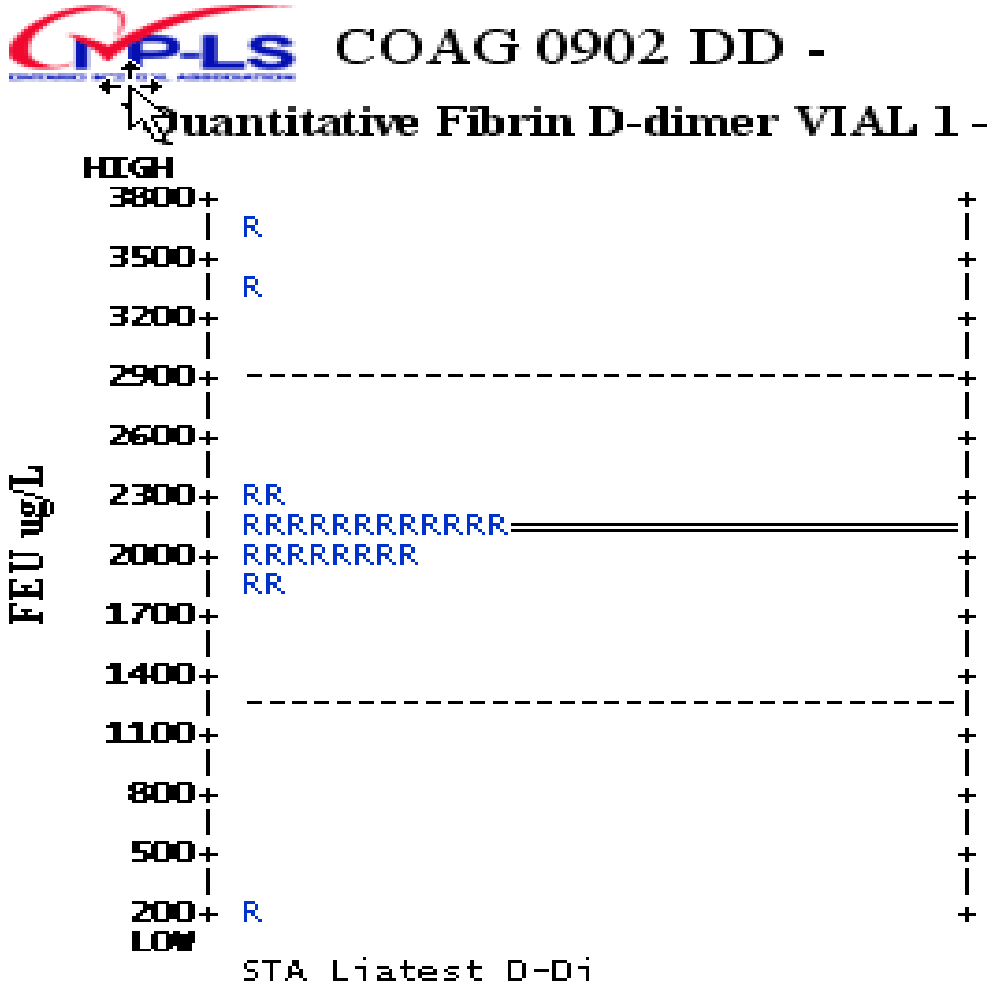
Quantitative FEU cont'd

- Reasons for testing: Unknown/exclusion of DVT/PE and diagnosis/monitoring of DIC/Predicative value

Example of Interpretive Comment

(RI and Cut-off) – "D-dimer should only be used in conjunction with appropriate algorithm for Dx of DVT/PE and must be interpreted in combination with clinical probability. D-dimer of <500 µg/L (FEU) is only useful to exclude Dx of DVT/PE in patient with LOW clinical suspicion. It is recommended that all other patients undergo additional investigation. A D-dimer result >4000 µg/L (FEU) is compatible with Dx of DIC in right clinical setting. Rheumatoid factor positive patients and lipemic patients should be interpreted with caution."

Quantitative FEU – Diagnostica Stago STA Liatest D-Di (N=27)



Establishing RI / Validating Cut-off

Examples

- **Reference Interval** – "Validated existing RI using 30 healthy donors with no history of bleeding problems, not on oral anticoagulant, not on hormone replacement or oral contraceptives, not pregnant, and not having liver disease. Followed CLSI Guideline C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition. 2000."
- **Validation of Cut-off** – "D-dimer results of 25 patients suspected of VTE were compared with radiology results. This was done in collaboration with thrombosis committee."

Work Required

- **Labs need to:**
  - Understand principle of test and target of Mab
  - Understand limitations of test (specificity and sensitivity)
  - Define the use for their test (DIC, VTE)
  - Validate method, reference intervals and cut-off values according to use
  - In addition to numerical value lab should report an interpretative comment
- Data to date indicates methods can distinguish between positive and negative values however, assessment of numeric results on their own is of limited value

Summary

D-dimer testing is work in progress!

- In Ontario, we are working toward standardizing reporting units, understanding unit of measure, educating participants on the appropriate use of reference intervals and cut-off values, however...
- Methods need further refinement by reduction of Mabs, identification of reliable targets, standard calibrator, standardization of unit of measure, proper use of reference intervals and cut-off values

Ottawa

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