

# Update: D-dimer Testing



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# Introduction

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- Not since the PT was introduced in the 1930s has a coagulation test, the D-dimer, had such a profound effect on the quality of patient care
- Much of the impact of this assay is due to the ability of modern testing techniques to detect extremely small amounts of this circulating fibrin split product
- This presentation is about the practical aspects of the clinical laboratory medicine of the D-dimer assay and not an exhaustive examination of its use in clinical medicine
- How the clinical laboratory interfaces with their physician customers is extremely important in regards to the proper use of such an assay as the D-dimer
- Proper use is directly related to the provision of quality patient care within a framework of reasonable cost



# Goals of this presentation

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- Provide an overview of the hemostatic mechanisms emphasizing the role of fibrinolytic system
- Discuss the chemistry of fibrinolysis and how to relate its biochemistry to select abnormal bleeding and thrombotic states
- Review past and current laboratory tests used to quantitate fibrin(ogen) split products with emphasis on the D-dimer
- Show how modern D-dimer tests can be used to aid in the diagnosis of disseminated intravascular coagulation (DIC) and help to aid physicians in the diagnosis of possible DVT/PE
- Discuss how we selected our current test and applied it in our institution



# The hemostatic response

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- The vertebrate hemostasis system has evolved over hundreds of millions of years and is designed to arrest the loss of blood from the vasculature
- Human biology and medicine is a systems science
- It is composed of three interacting elements: the **vasculature** in particular the vascular endothelium, the **blood coagulation protein-enzymes and their inhibitors**, and circulating **blood platelets**
- These systems interact with other systems, e.g. immune and neurological-endocrine to orchestrate overall somatic or body function

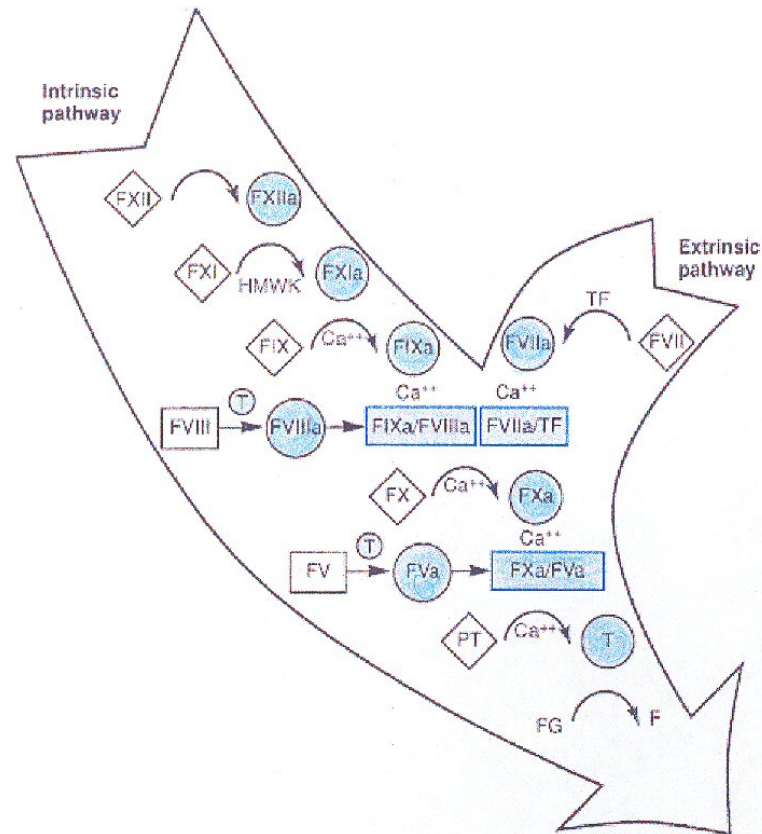


# Blood coagulation

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- The intrinsic, extrinsic, and common pathways generate thrombin (Figure 1)
- Thrombin converts circulating fibrinogen into fibrin
- Fibrin interacts with the primary platelet plug to arrest bleeding
- Once thrombin is generated, if it is not controlled, it can systemically clot the body
- All of the protein-enzymes and their inhibitors are produced in prodigious or “supra amounts” to meet possible massive loss of blood from the vasculature
- Consumption of these factors can lead to significant bleeding
- Improper control of hemostasis can lead to a thrombotic tendency

# Figure 1-Blood Coagulation System





# Biologic control of blood coagulation

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- Biologic control of blood coagulation has evolved a system of naturally-occurring inhibitors that effectively down-regulate certain key proteins and cofactors
- Antithrombin-III will directly bind to thrombin
- The protein C-S inhibitors bind to and control the two cofactors- Factor V and Factor VIII
- The fibrinolytic system controls fibrin deposition by directly cleaving deposited fibrin into split products

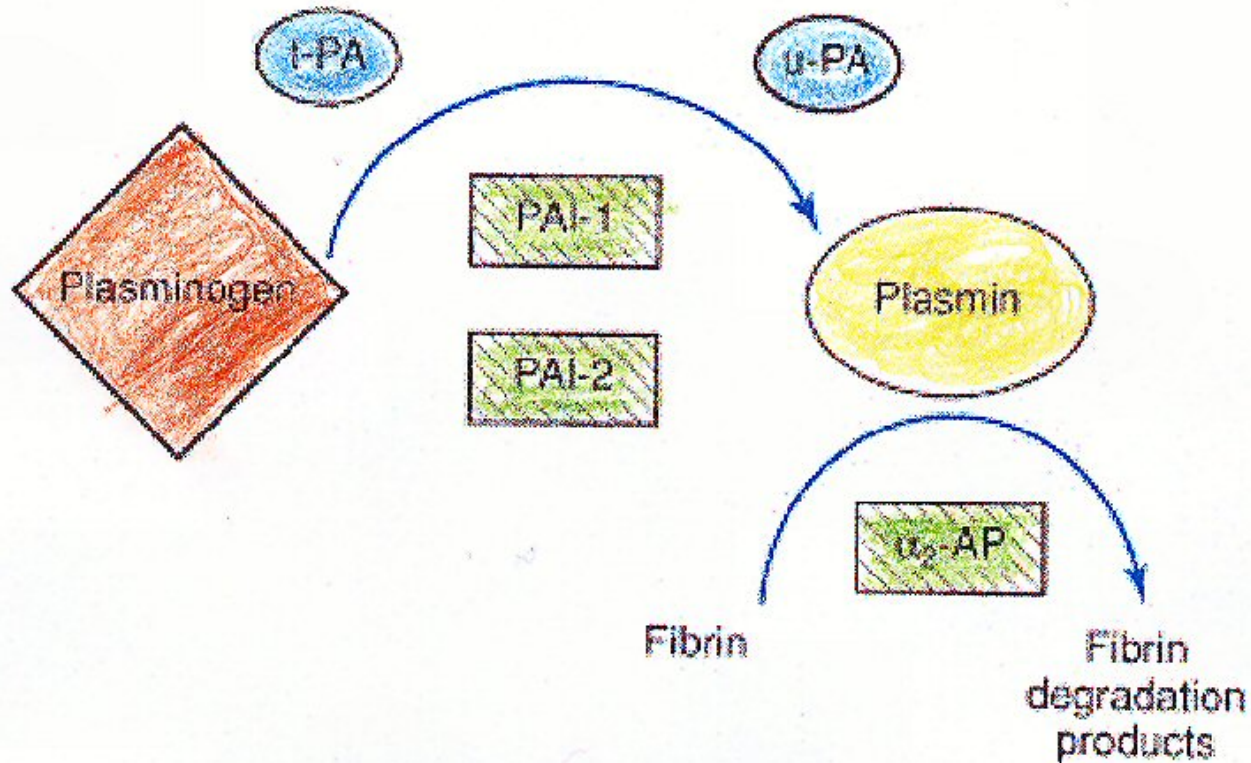


# The fibrinolytic system

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- As the name connotes, the fibrinolytic system is about the business of the lysis or chemical destruction of deposited fibrin (Figure 2)
- This is accomplished by the generation of the enzyme, plasmin
- Activated plasmin is derived from a circulating precursor called plasminogen
- Plasminogen is in turn derived from tissue source

# Figure 2-Fibrinolytic system





## Fibrinolytic system-range of action

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- Many physicians think that normal patients have no circulating split products of any type-this is not true
- This is dependent on what kind of test is used and its sensitivity to extremely low amounts of a particular split product
- It is important to note that the fibrinolytic system is a control mechanism constantly acting to remove small amounts of deposited fibrin, a natural part of maintaining normal blood vessels
- In other words, there is a constant low amplitude signal of these products and this is completely normal and if not occurring the patient may be at risk for thrombotic diseases



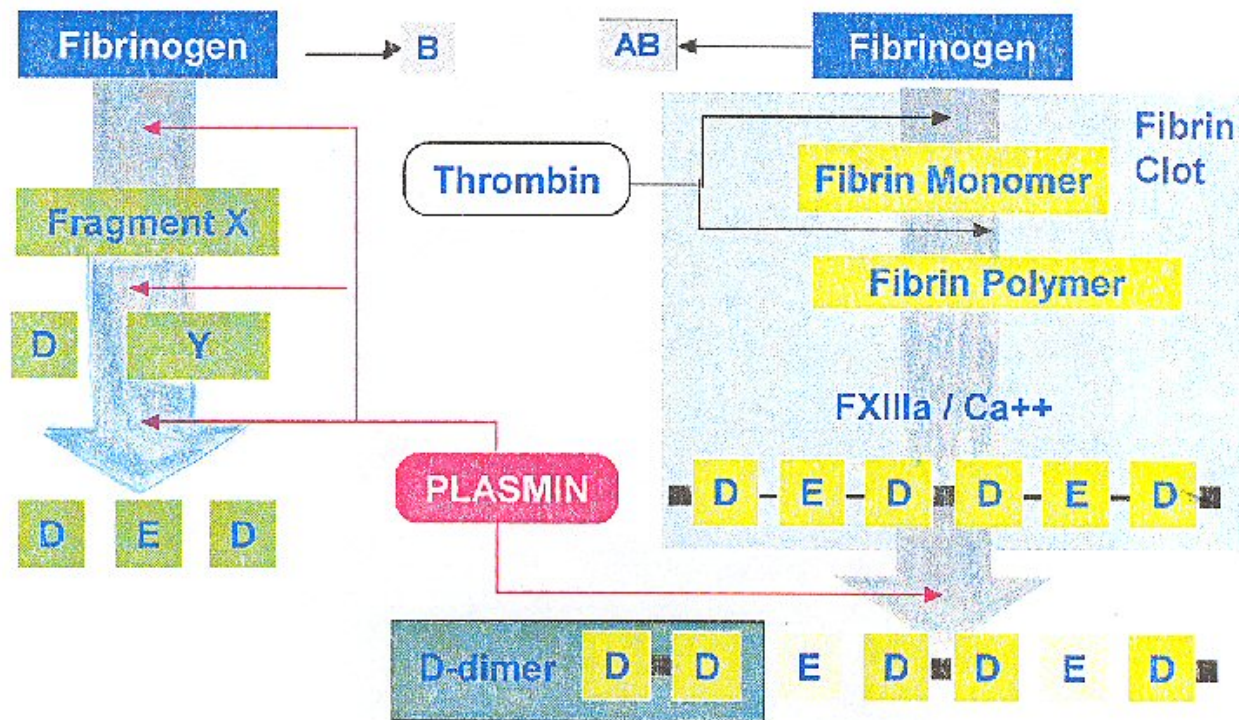
# Action of plasmin on fibrin

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- Plasmin is a proteolytic enzyme that sequentially degrades fibrin but in certain conditions can also destroy circulating fibrinogen
- It enzymatically cleaves fibrin(ogen) into smaller pieces called split products
- These products can be detected and quantitated using a variety of laboratory tests; what we used to call FDPs
- The D-dimer, a fibrin specific split product, indicates that thrombin has been generated and plasmin activated

# Figure 3-Fibrin(ogen) split products or fibrin degradation products

## Plasmin induced lysis of fibrinogen & fibrin





## Laboratory testing used to detect these products

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- The various split products are proteins and as such can be used to stimulate the production of antibodies
- Take these proteins, inject them into an animal to which they are foreign and they will form specific antibodies
- These antibodies can be used in various test systems to detect and/or quantitate the amount of split product



# First generation tests to detect split products

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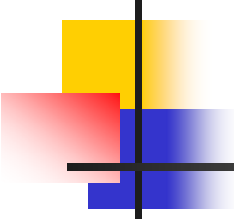
- The early test systems used polyclonal antibodies generated to a wide variety of fibrin and fibrinogen split products
- The initial widely available assays were relatively crude **latex agglutination** tests (antibody attached to latex bead)
- These tests could not differentiate fibrinolysis from fibrinogenolysis
- Semi-quantitation of the relative amount of circulating product was accomplished by titration of test plasma



## Problems with early latex agglutination tests for FDP

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- As initially designed, the tests were not sensitive to low circulating levels of these products
- Non-automated card assay with potential inter-technologist variation
- They were used, in combination with other global coagulation tests, to aid in the diagnosis of the consumptive coagulopathies, in particular disseminated intravascular hemolysis (DIC)



## A new and better approach to assessment of fibrinolysis; the D-dimer

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- Researchers and clinicians refined this testing to detect fibrin specific degradation products that directly indicate the presence of the breakdown of deposited fibrin
- This would help define secondary fibrinolysis as is found in disseminated intravascular coagulation (DIC)
- Techniques were developed using **monoclonal antibody** and adapted to a fibrin specific degradation product, the D-dimer
- A positive D-dimer tells the physician that thrombin has been generated with subsequent activation of plasmin, the pathophysiologic indicators of secondary fibrinolysis
- It is the best test we have to aid in the diagnosis of DIC and its attendant potentially fatal bleeding



# Quantitative assessments of circulating D-dimer

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- The key to fully utilizing the diagnostic capabilities of the D-dimer lies in developing and using analytical techniques that accurately identify this fibrinolytic product while having good sensitivity across a wide range of concentrations
- You want to be able to use the test to detect large and small circulating amounts of this fibrinolytic end product
- Must have a good reagent coupled with a automated test system with wide analytical range



# Quantitation-continued

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- Analytical goals- automated, analytical sensitive to match a particular clinical need, and least expensive
- Use a refined latex agglutination (turbidimetric) assay with a high sensitivity detection system-cheaper and quicker
- Use an ELISA technique to accomplish the same goal-slower and more expensive



# Overview of DIC (Death is Coming!

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- In tertiary care, DIC is common and a serious cause of morbidity and mortality
- DIC is a secondary manifestation of a primary disease state (Table I) that activates systemic, usually microcirculatory in nature, blood coagulation followed by bleeding
- Systemic release of tissue factor with Factor VII activation
- Bleeding is due to ongoing consumption of procoagulants with loss of biological control of the blood coagulation system
- From the pathophysiologic perspective, thrombin is generated and plasmin activated to induce fibrinolysis
- The D-dimer is positive in this form of secondary fibrinolysis
- The level of D-dimer is not only diagnostic but also can be used to assess the severity and possible clinical direction of DIC



# Table I- DIC triggers

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- Massive trauma, especially head
- Sepsis-endotoxemia
- Obstetrical problems, e.g. dead fetus, abruptio placenta
- Acute hemolytic transfusion reactions
- Cancer
- Sickle cell crisis
- Snake venoms



# Types of DIC

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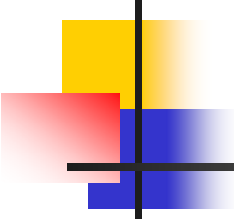
- **Acute fulminating DIC**- uncontrolled or decompensated DIC; patients systemically clot in their microcirculation and then bleed extensively
- **Chronic DIC**-controlled DIC that the body can compensate for



# D-dimer and DIC

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- You do not need a highly sensitive assay to detect the gross amounts of D-dimer being generated in acute DIC
- We use the D-dimer with the PT, aPTT, fibrinogen, and CBC plus platelet count to confirm, what many good clinicians already know, is DIC
- The issue of chronic DIC is less clear e.g. associated with a malignancy where the levels of circulating D-dimer may be lower in concentration



# Can you use the D-dimer to help guide blood component therapy in decompensated DIC?

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- Patients with decompensated DIC can bleed to death if not treated
- Clinicians need to be proactive in dealing with DIC and treat it early in the episode and with great aggressiveness
- We use the D-dimer to make the diagnosis but do not repeat extensively, as we try to re-establish hemostatic balance
- **As the PT goes so goes the patient**
- The more the patient loses extrinsic drive the worse the prognosis



# Deep vein thrombosis (DVT) and Pulmonary embolism (PE)

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- DVT/PE are major causes of morbidity and mortality
- They also cost health care systems billions of dollars/year to diagnose, treat, and prevent
- The venous system is unique in that back flow is prevented by a series of valve cusps
- The problem from a pathologic stand point is that these valves are areas where blood can pool and become static
- Stasis in the venous system in the thigh (proximal) and lower legs (distal) are the primary sites where DIC will occur
- PE results when venous thrombi from the lower body migrate to the pulmonary circulation; PE is associated with significant morbidity and mortality-300,000 patient die/year



# Diagnosis of DVT/PE

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- Symptomatology is nonspecific and may be subtle, especially for PE (Table II)
- DVT is present in approximately 1/3 of patients presenting with complaints associated with DVT
- Physical examination and a history are not sufficient, in and of themselves, to make the diagnosis of DVT
- There are a number of diagnostic approaches and they vary in terms of type, availability, and overall clinical usefulness
- Radiographic assessments are used
- The most effective diagnostic regimens use multiple methods in a cost effective manner



# Signs and symptoms of PE

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- Chest pain (70%)
- Tachypnea (70%)
- Cough (40%)
- Tachycardia (33%)
- Shortness of breath (25%)
- Signs of DVT (10%)
- Syncope (5%)



# Invasive techniques for DVT/PE

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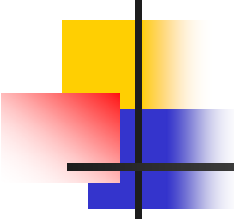
- **DVT**- contrast venography is the “gold standard” for DVT but it is costly and has associated technical problems
- **PE**- pulmonary angiography is the “gold standard” for PE and is also costly and has an associated patient risk of 0.5% mortality



# Non-invasive diagnostic techniques for DVT

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- Impedance plethysmography (IPG)
- Venous ultrasonography
- Compression ultrasonography-duplex ultrasound, CUS, B-mode imaging, color Doppler



## Usefulness of non-invasive techniques in the diagnosis of DVT

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- Compression ultrasonography has high sensitivity for proximal DVT and lower accuracy for distal thrombosis
- IPG is less sensitive than ultrasound
- Doppler flow is no better than ultrasound



# Non-invasive techniques for PE

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- Perfusion-ventilation scan (V/Q) scan, perfusion lung scan
- Spiral or helical CT



# D-dimer and DVT/PE

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- The next evolution in D-dimer testing was its adaptation for low impulse signals coming from an evolving thrombus as might be found in DVT and its fatal cousin, PE
- We all have low circulating levels of D-dimer as the body performs normal surveillance and maintenance of the vascular endothelium
- People that exercise have higher base-line D-dimer levels than those who do not; pregnant females are different
- Harder than you think to normalize the baseline



## Testing characteristics and adaptation of the D-dimer in DIC

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- Must determine what the mean value (what amounts do John and Jane Doe have) to make potential statements about possible DVT/PE
- Need an assay to detect extremely small amounts of circulating degradation product, ng/ml ( $10^{-9}$  grams)-assay sensitivity
- Assay or marker needs to be specific for the disease to be clinically useful
- Must generate a cutoff value that reflects a clinically significant change in D-dimer level



# Determination of mean values

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- Calculate how much D-dimer does a normal person in the general population have- a model figure that represents adult males and females (not pediatric patients)
- Run a statistically significant number of defined normal individuals and calculate a mean value
- We do this type of descriptive population statistics all of the time
- Use mean and generate a 2 S.D.level



# Detection of low levels of D-dimer in DVT/PE

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- ELISA techniques (gold standard)
- Enhanced latex agglutination tests that have been standardized against the gold standard
- Both tests can detect ng/ml amounts of D-dimer
- Older manual latex tests (FDP) cannot be reliably used



# Sensitivity of the D-dimer tests

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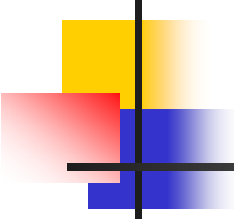
- D-dimer assays are highly sensitive for fibrinolytic activity
- They do not, however, have positive predictive value
- They cannot rule in or differentiate the exact cause of the fibrinolytic activation
- They do have significant ability to aid in the diagnosis of DVT/PE, in other words, they have clinically useful **negative predictive value**



# Other desirable testing attributes

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- Quantitative test result with a cut off value and test result the clinician can understand
- Automated and quick TAT to get negative patients in and out of the door
- Availability 24/7/365



## Cut-off values or what the laboratory must do to introduce D-dimer as a tool in DVT/PE

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- Determine the sensitivity and specificity of the assay of choice
- With FDA-approved testing reagents-test systems use the manufacturer's cut-off
- Must validate the testing in your institution's own environment



# Validation techniques

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- Some of this stuff gets very complicated
- Involves the determination of a ROC analysis (receiver operator characteristics)
- Must statistically analyze the testing results with the actual radiographic diagnosis of DVT/PE



# What does all of this stuff mean?

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- The take home message is all of these methods, including the D-dimer, have their intrinsic strengths and weaknesses
- Must use multiple diagnostic tests in a algorithmic type of assessment to maximize the capabilities of the radiography in combination with the laboratory testing



# How do we use the D-dimer in regards to DVT/PE

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- Calculate a pre-test probability or assessment of risk using, e.g. Well's criteria; patients are stratified as low, moderate, or high risk
- Low risk patients are tested using the D-dimer
- If patient is moderate or high risk then we routinely subject them to radiographic assessments without the D-dimer



# Other potential research opportunities for D-dimer

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- Some researchers are looking at the potential for D-dimer as a marker for metastatic disease; remember that some cancer patients have chronic forms of DIC
- Some OB specialists are assessing the use of D-dimer in abruptio placenta and other obstetrical problems in which fibrinolysis is involved
- Is there a use for D-dimer in assessing fertility related problems where we know that fibrinolysis might be involved in implantation?
- Are patients with high normal values for circulating D-dimer more resistant to thrombosis than those with lower circulating amounts?



# Summary

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- We now have automated highly sensitive assays for the determination of circulating levels of the fibrin split product, the D-dimer
- A positive test for D-dimer coupled with global coagulation testing and the CBC is extremely useful in the diagnosis of DIC, a potentially fatal coagulopathy
- A highly sensitive D-dimer test can be used to aid in the diagnosis of DVT/PE
- There may be other potential future uses of this very important assay



# Thanks for attending!

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If you need more detailed information on the D-dimer, please check with your Dade-Behring representative