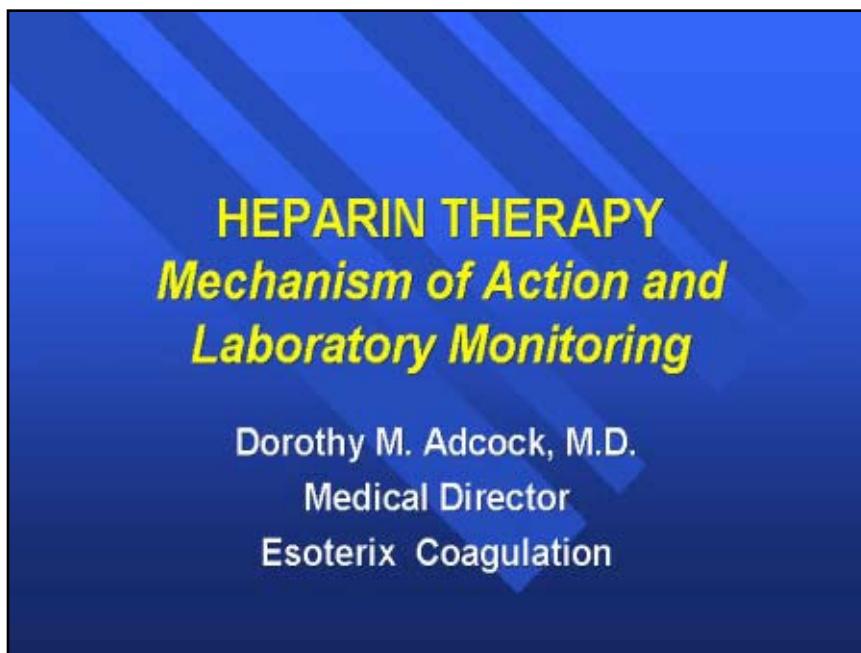


# Heparin Therapy Monitoring

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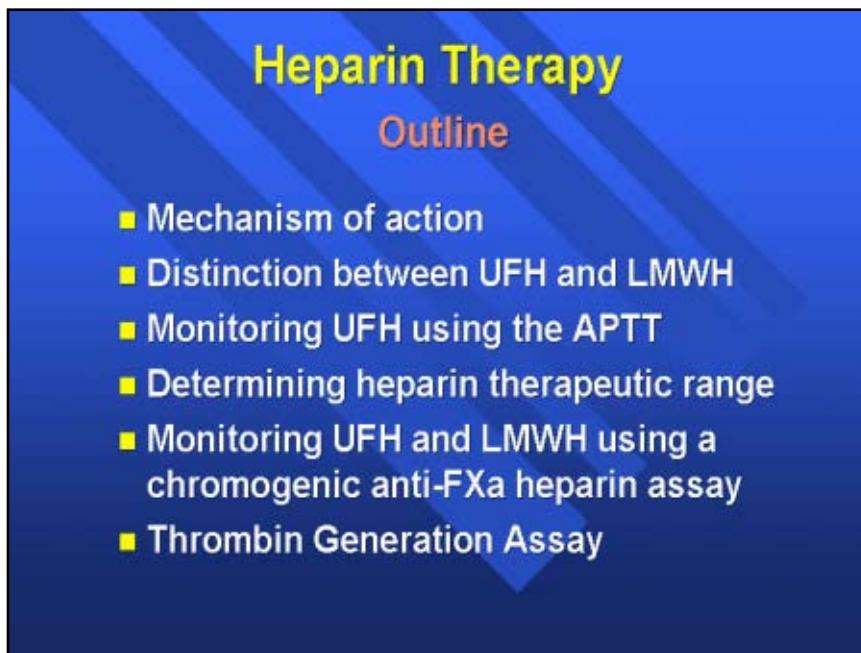
Title: Heparin therapy - Mechanism of action and laboratory monitoring



I want to say thank you to Laura and also thank you to Diagnostica Stago for putting on this conference today, it's really delightful to be associated with an organization that is dedicated to education. So, thank you very much. As Dr. Worfolk mentioned, my topic today is Heparin Therapy: Mechanism of Action and Laboratory Monitoring.

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Title: Heparin therapy - Outline



What we will discuss in the next 45 minutes or so is the mechanism of heparin's anticoagulant action. We'll talk about the distinction between unfractionated and low molecular weight heparin, and I'll have these abbreviated UFH and LMWH.

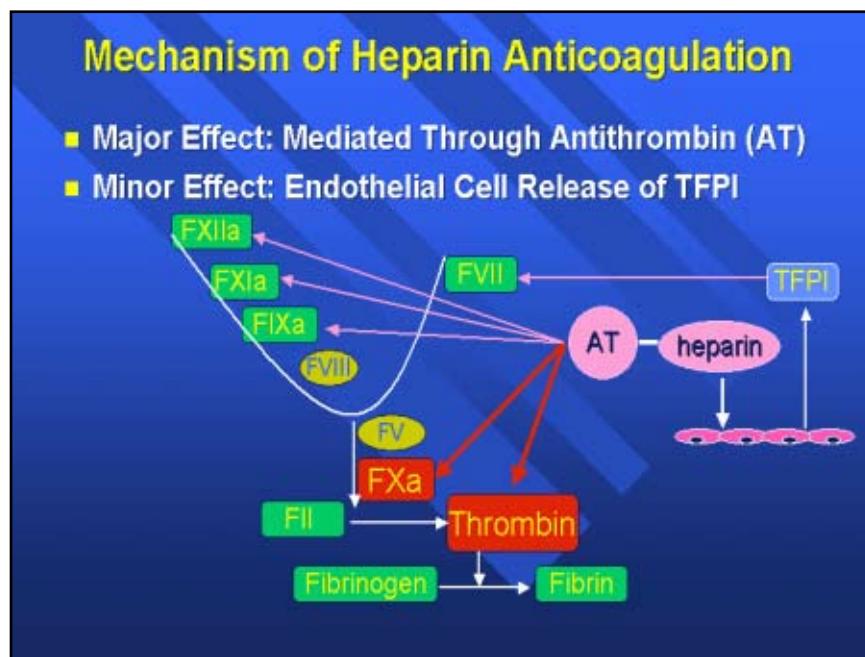
And then we'll talk about monitoring unfractionated heparin using the PTT, how to determine heparin therapeutic range, and then using a chromogenic anti-factor Xa heparin assay, and how that can be advantageous for monitoring heparin therapy. And then finally, I'll introduce what might be a new assay to some of you, which is the thrombin generation assay.

## Heparin Therapy

- Heparin - commonly administered anticoagulant drug
- Indications for use:
  - Prevention & treatment of thrombosis
  - Immediate anticoagulant effect when administered intravenously
    - » Drug of choice when rapid anticoagulant effect required

Now, heparin is a very commonly administered anticoagulant drug, and it is used to both prevent thrombosis as well as treat thrombosis. And one of the greatest advantages of heparin therapy is its almost instantaneous effect when administered intravenously.

So this of course is the drug of choice when rapid anticoagulant effect is required. Now, the manner in which heparin acts as an anticoagulant is largely through its promotion of antithrombin's activity. Antithrombin is a very important naturally-occurring anticoagulant, and antithrombin inhibits a number of the procoagulant factors.

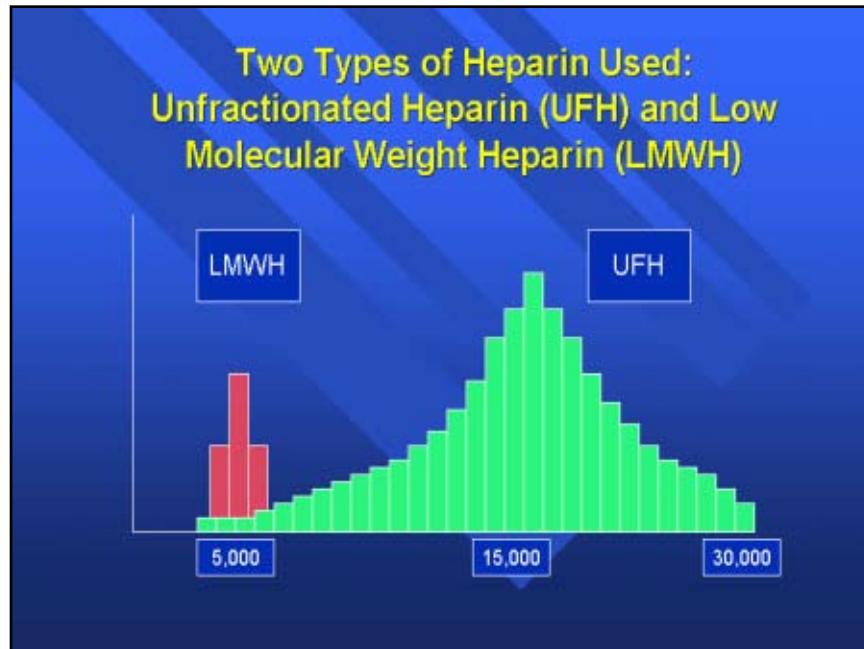


In the presence of heparin, antithrombin's activity is enhanced, and this is a very important mechanism of how heparin acts as an anticoagulant, predominantly through its inhibition of activated factor X and thrombin.

Heparin also acts as an anticoagulant through its ability to release tissue factor pathway inhibitor from the endothelium. And, tissue factor pathway inhibitor is the prime inhibitor of the extrinsic pathway.

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## Title: Two types of heparin used: Unfractionated heparin (UFH) and low molecular weight heparin (LMWH)

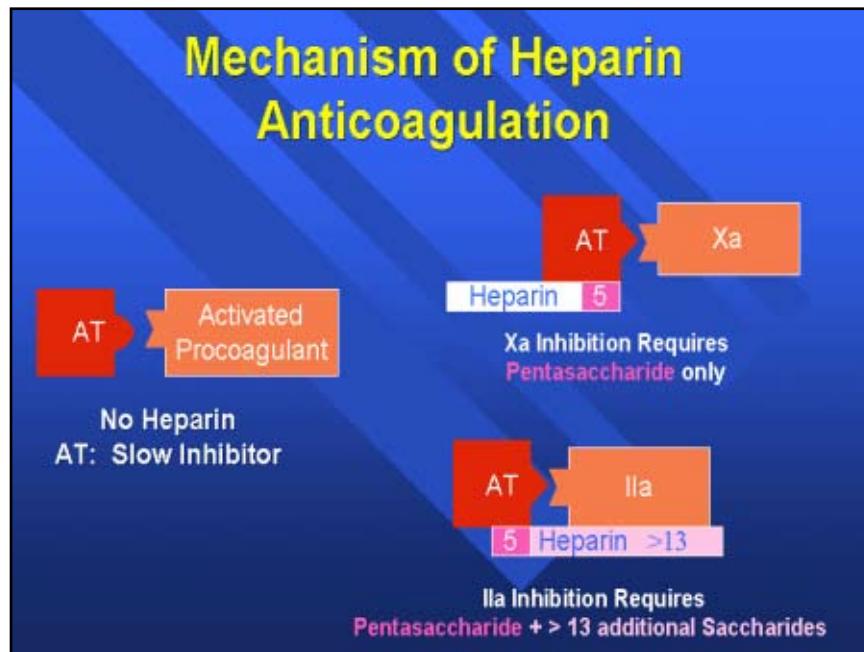


Now, we're talking today about the two different types of heparin therapy. There's both unfractionated and low molecular weight heparin. And really, low molecular weight heparin is a subset of unfractionated heparin.

Unfractionated heparin is a very heterogeneous grouping of polysaccharides that have a very broad molecular weight distribution. Low Molecular Weight heparin is a much smaller subpopulation of polysaccharides.

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## Title: Mechanism of action - Anticoagulation



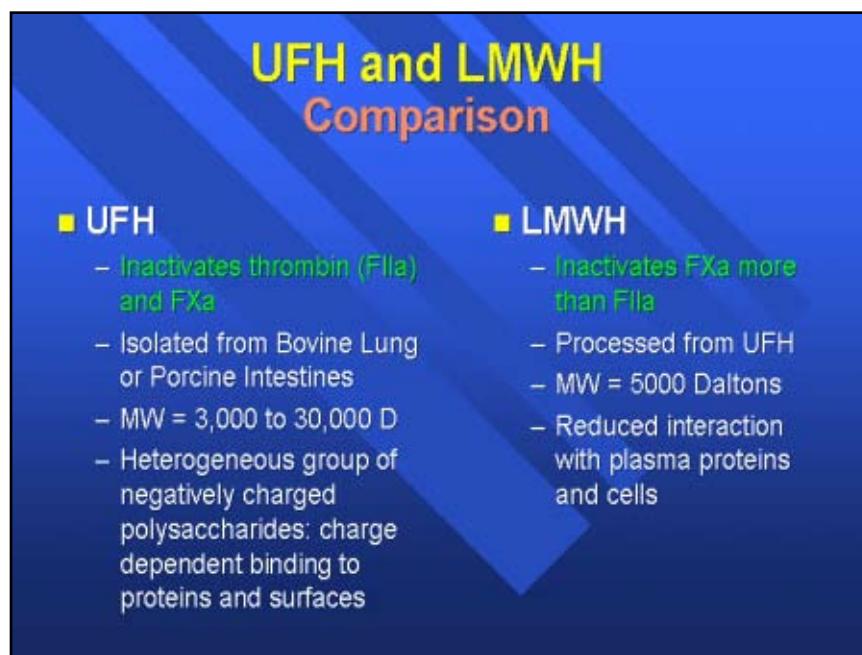
Now, as I mentioned, heparin works by helping antithrombin. In the absence of heparin, antithrombin is a very slow inhibitor of serine proteinases. All right. Now, heparin's activity in part depends on its molecular size. When you have small heparin fragments, such as in low molecular weight heparin, it's most effective at inhibiting factor Xa.

Because in order to inhibit factor Xa, the only thing that antithrombin needs, excuse me, the only thing that the heparin needs to bind to antithrombin is this pentasaccharide sequence, a five sugar sequence, okay? So if the heparin molecule has this five sugar sequence, it binds to antithrombin, induces a conformational change that allows it to inhibit Xa.

And in fact, this pentasaccharide sequence is what is also known as fondaparinux. Fondaparinux is a new anticoagulant agent that consists of this pentasaccharide only. To inhibit thrombin, the heparin molecule must be larger.

It must include the pentasaccharide plus at least 13 additional saccharide units. And this is because, in order to inhibit thrombin, we must have these two sitting together on the heparin cofactor. So, the size of the heparin molecule helps determine how it inhibits, its ability to inhibit these serine proteases.

#### Title: UFH and LMWH comparison



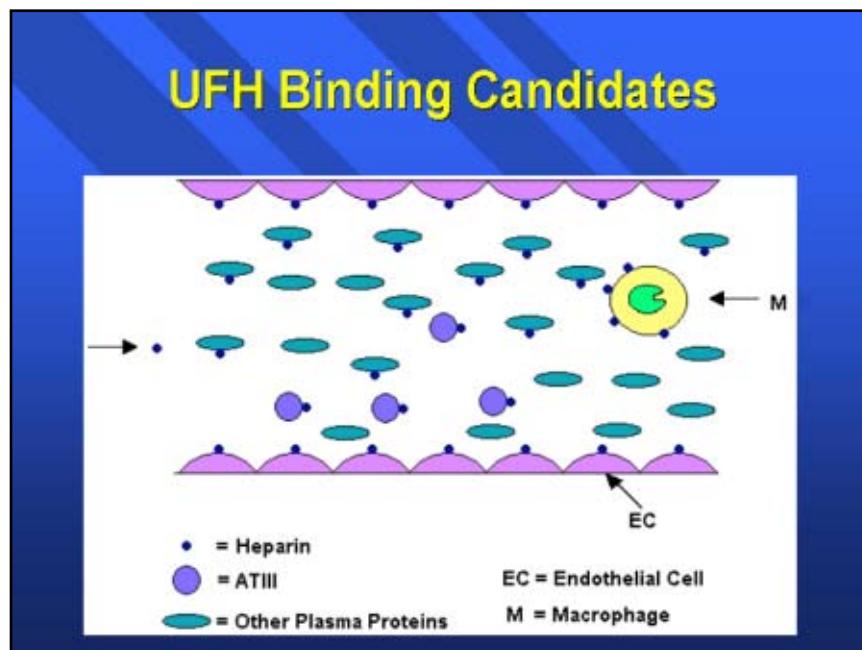
So, as I mentioned, unfractionated heparin inhibits thrombin as well as Xa. This is pretty much in a one-to-one relationship. Whereas low molecular weight heparins inactivate factor Xa much more so, or more, in a greater amount than IIa. And, the ratio of Xa to IIa varies with the different low molecular weight heparin fractions.

Now, in addition, unfractionated heparin is isolated from either cow lung or pig intestines. It has, as I mentioned, a very heterogeneous molecular weight distribution, and it's a very negatively charged molecule, and as Dr. Kessler mentioned, very sticky.

And as a sticky, negatively-charged molecule, it binds to a number of different things in the circulation, either to proteins or surfaces, cell surfaces. Now, low molecular weight is processed, heparin is processed from unfractionated heparin.

It's much more homogeneous in its molecular weight distribution. As a smaller molecule that's more homogeneous, it has significantly less interaction with plasma cells, plasma proteins in cells.

#### Title: UFH binding candidates



Now, what we're looking at here is unfractionated heparin, this is supposedly an unfractionated heparin moiety, and this is coming into the circulation.

Unfractionated heparin is a very sticky, negatively-charged polysaccharide, will bind a number of different items in the circulation. Now, it should bind antithrombin, because that's how it's going to function as an anticoagulant. But, it also binds plasma proteins.

And many of these plasma proteins that it binds are acute phase reactive proteins. And these proteins elevate when the patient is ill. For instance, when the patient has an acute thrombosis, these proteins will elevate. So, the more proteins, acute phase proteins we have, the more the heparin will bind to these acute phase proteins rather than antithrombin.

All right. Now, the second thing to keep in mind is that heparin also binds a variety of cell surfaces, such as leukocytes and endothelial cells. And this is really a very important property, because this enhances heparin's clearance, unfractionated heparin.

In fact, this is the prime way in which unfractionated heparin is cleared, is through binding to cells, being ingested by the cells, and then degraded. And this is actually, this ability to take heparin in and degrade it is dose dependent. So, the higher the dose, the greater is the clearance.

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#### Title: UFH therapy - Unpredictable dose response

**UFH Therapy**  
**Unpredictable Dose Response**

- **Binds plasma proteins**
  - Including acute phase reactants
  - Competes with antithrombin
- **Binds cellular constituents**
  - Endothelial cells, macrophages and platelets
  - Predominant mechanism of clearance of UFH
    - » Dose dependent and non-linear
    - » Renal clearance minor role

So, as I mentioned, heparin binds plasma proteins including acute phase reactant proteins, and these compete with antithrombin. Heparin also binds cellular constituents, and this is the predominant mechanism of clearance of unfractionated heparin. And this is dose dependent and nonlinear. Renal clearance of unfractionated heparin plays a relatively minor role.

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#### Title: Monitoring UFH therapy - Rationale

**Monitoring UFH Therapy**  
**Rationale**

- **Unpredictable dose-response**
  - Clearance varies up to 12 fold
  - Standard fixed dose cannot be used
- **Delayed or Over-anticoagulation**
  - Increased rate thrombus progression or recurrence
  - Increased risk of hemorrhage

**When used in therapeutic doses, UFH must be monitored**

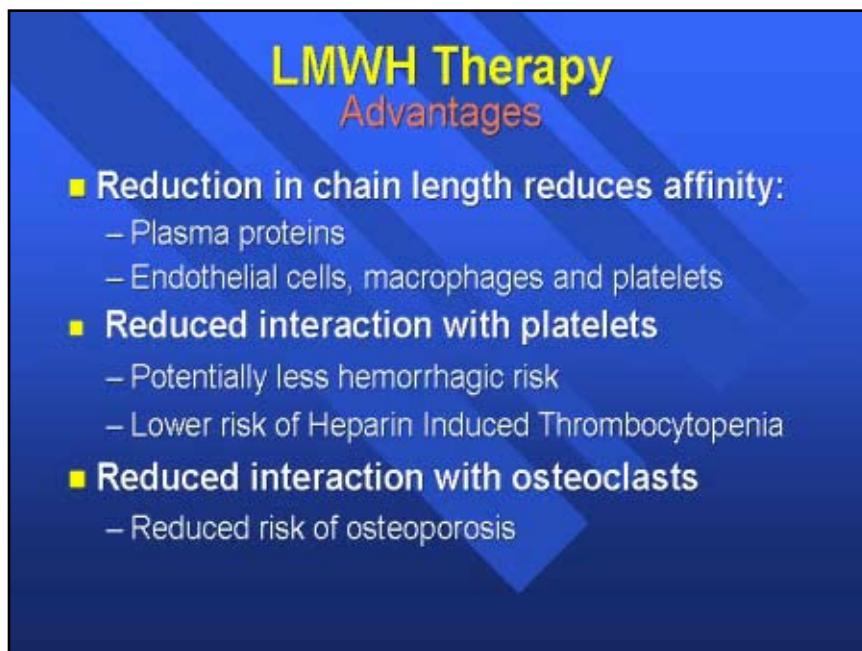
Because of these variables, heparin has a very unpredictable dose response. And in fact, the clearance varies up to 12 fold between different patients. So you can give one patient a dose of unfractionated heparin, give the same, to another patient the same dose and have a very different response.

So, it's very important that a standard fixed dose cannot be used. You must monitor the effect of unfractionated heparin to determine how it is interacting in that particular patient.

It's also very important to keep this in mind because it's clearly been shown through many different studies that if you have delayed anticoagulation, then you have an increased rate of thrombus progression or recurrence. If you have too much anticoagulation, there's a risk of hemorrhage. So, unfractionated heparin has to be monitored clinically when it's used in a therapeutic dose.

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**Title: LMWH therapy - Advantages - Reduction in chain length reduces affinity**



Now, low molecular weight heparin has many advantages over unfractionated heparin. Because it has reduced chain length, it also has reduced affinity for plasma proteins and the cellular constituents that I talked about.

And, one of the other cellular constituents that unfractionated heparin binds to is platelets. Because it has this reduced interaction, low molecular weight heparin has potentially less hemorrhagic risk because it interacts with platelets less.

It has a lower risk of heparin-induced thrombocytopenia. And it shows less interaction with osteoclasts and it has a reduced incidence of osteoporosis, which can be a significant problem with unfractionated heparin when it's administered for prolonged periods, for instance, when patients receive it for greater than 1 month at a time.

## LMWH Therapy Advantages

- Better pharmacological features:
  - More predictable dose response
  - Weight-based dosing
  - Longer plasma half-life
  - Better bioavailability
  - Renal clearance

Laboratory monitoring unnecessary  
except...

Because low molecular weight heparin does not show all of this nonspecific binding because it is not cleared through the cellular mechanism, it has much better pharmacological features. It has a much more predictable dose response. You can give a weight-based dose or a standard fixed dose of low molecular weight heparin, and in a majority of patients, get a known response.

It has a longer plasma half-life, better bioavailability, and is generally cleared through the renal system rather than through a cellular mechanism. So, in general, laboratory monitoring of low molecular weight heparin therapy is unnecessary, because patients respond in a similar fashion from one to another.

## LMWH Monitoring Indications

- Body weight extremes
  - < 50 or > 160 Kg body weight
- Impaired renal function
  - Creatinine clearance < 30 mL min<sup>-1</sup>
- Pregnancy
  - Especially 3rd trimester, placental clearance, volume of distribution
- Prolonged periods of therapy

Now, of course, we can never say never, and there are certain times when low molecular weight heparin therapy should be monitored. As I mentioned, when low molecular weight heparin therapy is administered, it's administered based on the patient's weight.

However, if your patient is at an extreme of body weight, less than 50 kg or greater than 160, it should be monitored to determine if the patient is getting the appropriate response. If your patient has impaired renal clearance, it's also important to monitor the therapy, because the drug may accumulate and lead to an increased risk of bleeding.

It's also important that low molecular weight heparin be monitored in pregnancy. And, in fact, low molecular weight heparin is one of the preferred drugs in pregnancy, even over unfractionated heparin, because of the risk of osteoporosis and heparin-induced thrombocytopenia.

But in pregnancy, pregnancy is really a special scenario, because with pregnancy, we have increased blood volume, so we have increased distribution of the drug, and there's also probably some factor in the placenta that causes clearance of heparin.

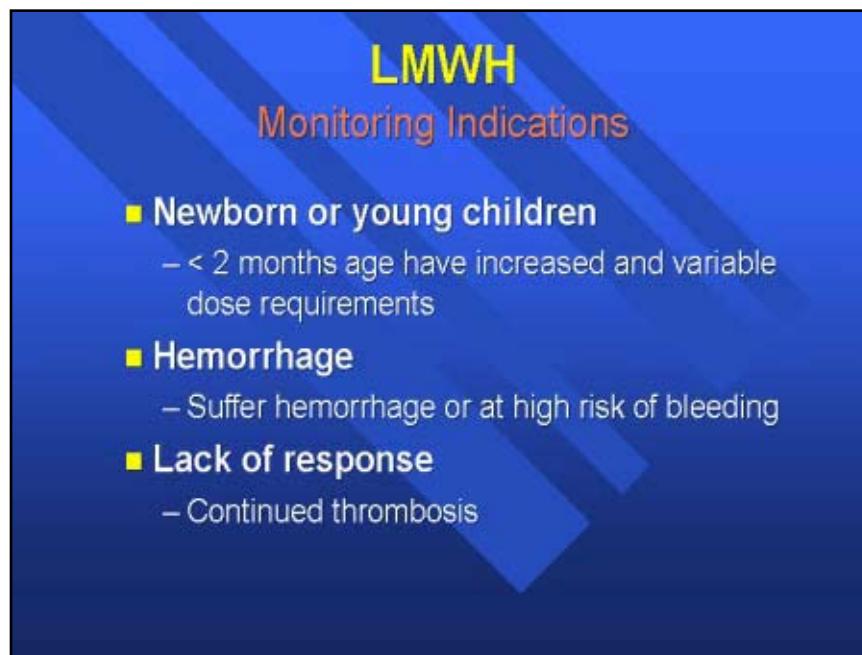
So, in pregnancy, if the patient is on low molecular weight heparin, they should be monitored, at least every month, and in some instances, every week. When a patient is on low molecular weight heparin for a prolonged period of time, this is another indication for monitoring.

Sometimes when the patient has an underlying malignancy, they don't respond as well to warfarin therapy. They have a chronic DIC-like state.

And in that situation, heparin therapy is often preferred, and the low molecular weight heparin is preferred for the reasons I talked about, reduced instance of heparin-induced thrombocytopenia and osteoporosis. So, in this instance, when the patient is on long-term low molecular weight heparin, it should be monitored.

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**Title: LMWH - Monitoring indications - Newborn or young children**



Another indication is in the newborn period. In those babies that are less than 2 months of age, they have increased heparin requirements and also variable dose requirements. This maybe in fact because babies have lower antithrombin levels than adults do. But, it's critically important in this population that low molecular weight heparin be monitored.

And then also, it's common sense that if your patient is on an anticoagulant and they're either bleeding or thrombosing, you might want to see what level the patient is at. Or, if your patient is on therapy and they have to undergo an operative, an emergency operative procedure, might be another time when you want to find out where the patient is at.

So, although in general low molecular weight heparin does not need to be monitored, as laboratorians, we all know that at some point, the clinician is going to call us and say, I really need to find out where my patient's at.

And, there are clearly some conditions where it should be monitored. Now, how should we monitor heparin therapy?

## Monitoring Heparin Therapy

### Laboratory Methods

- Activated Partial Thromboplastin Time (APTT)
  - Can be used to monitor UFH only - not LMWH
- Heparin Assay (Anti-FXa)
- Thrombin Generation Assay

Can be used to monitor both UFH and LMWH therapy

As many of you know, the PTT is the standard means to monitor unfractionated heparin. It is not a good means to monitor low molecular weight heparin therapy, because it doesn't respond in a dose-dependent manner. So, we really can't use the PTT.

The assays, the other assays that I'm going to talk about today that can be used to monitor heparin therapy is something called a heparin assay, which is an anti-factor Xa assay, and also a thrombin generation assay.

As was mentioned this morning, we can also monitor heparin with an anti-factor II assay, IIa assay, antithrombin assay, but this is not in as common use as the Xa assay, so I won't be talking about that.

## Monitoring UFH Therapy

### Using the APTT

- Most common method used
- Global measure of coagulation
- Indirect measure of heparin anticoagulation
- Variety of interacting conditions and/or factors that alter APTT
- Therapeutic range in seconds is dependent on the APTT reagent used

When we use the PTT to monitor unfractionated heparin, and we have to realize that this is the most common coagulation assay used to monitor heparin therapy. It's a global measure of coagulation, and actually an indirect measure of the patient's heparinization.

There are a whole variety of interacting factors or conditions that alter the PTT and therefore alter the PTT's ability to monitor heparin therapy. We'll talk about the fact that the therapeutic range in seconds is dependent on the PTT reagent.

## Monitoring UFH Therapy Using the APTT

- Historically expressed as a ratio
  - 1.5-2.5 patient/control (patient ÷ control)
- “Control” not consistently defined
  - Lab mean normal or patient baseline?
- Is NOT based on reagent sensitivity
- Is NOT appropriate for all reagents

Now, when a patient is on therapeutic heparin therapy, most of, most medical students are taught that a patient is adequately heparinized if their PTT is 1.5 to 2.5 times control. And, I think many of us have used this range in the laboratory. The big question however is, what do we use as control? Do you use the baseline, the patient's baseline PTT?

Do you use the mean normal PTT in your laboratory? Or the control value that day? That is one of the difficulties with using this ratio to determine therapeutic range, because control is not well defined. The other problem with this ratio is that it is not based on the reagent, the PTT reagent sensitivity. And this ratio is not appropriate for all reagents, and I'll show you some data that validates that.

## Monitoring UFH Therapy Using the APTT

- APTT reagents vary considerably in their responsiveness to heparin
  - Phospholipid content and activator used
- Laboratories should calibrate a therapeutic range for their APTT reagent
  - Variety of methods available
- Current recommendation - expressed as correlation to a direct heparin level\*
  - 0.2 - 0.4 U/ml protamine titration
  - 0.3 - 0.7 U/ml anti-FXa (heparin assay)

\*Chest 2001;119:64S-94S

As many of you know, PTT reagents vary considerably in their responsiveness to heparin, and this is because different reagents vary in their phospholipid content and in the activator used in the reagent. And it's been determined that the laboratory should calibrate a therapeutic range for their PTT reagents.

And currently, there are various ways to do it, but it can be done by either doing a protamine titration or by using a heparin assay. Has anybody here ever done a protamine titration? No. And nobody offers it. It's a very laborious, relatively difficult test. Heparin is a negatively-charged polysaccharide, and protamine is a positively-charged molecule.

And so, what you actually do with protamine titration is a charge neutralization. So depending on the amount of protamine that needs to be added to a patient's sample, you can determine how much heparin is in the patient's sample. But, it's not an automated technique, and it's not terribly exact.

So, it's not often performed. Really, the recommended method is correlating the heparin, PTT to a heparin assay, an anti-factor Xa assay.

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**Title: Heparin assay - Anti factor Xa assay**

**Heparin Assay**  
**Anti Factor Xa Assay**

- $AT + H = AT-H$
- $AT-H + FXa \text{ (excess)} = AT-H-FXa + FXa \text{ (residual)}$
- $FXa \text{ (residual)} + \text{substrate} = \text{signal}$   
(color or fluorescence)

Some reagents contain AT and some rely on patient AT

AT = antithrombin    H = Heparin    FXa = activated factor X

We're going to be talking about this assay quite a bit, so I'm just going to introduce the manner in which this assay works. This is the anti-factor Xa assay and in this assay, antithrombin and heparin interact to form a complex. And in the presence of this complex, excess activated factor X is added.

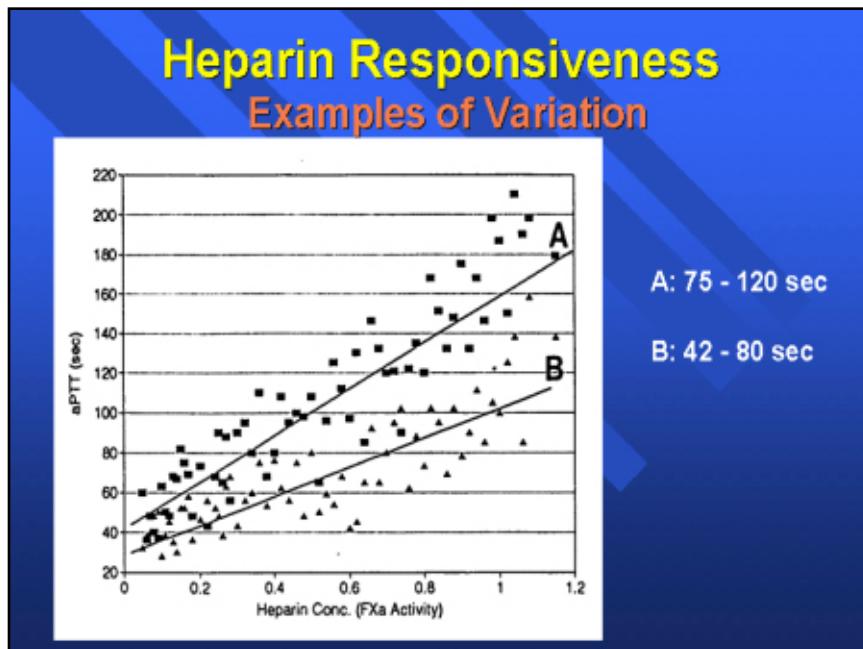
The antithrombin heparin binds factor Xa proportional to the amount of antithrombin and heparin in the sample. And then, there's a certain amount of residual Xa, and this residual Xa is identified with some sort of a substrate and then the signal is measured. And the amount of heparin is inversely proportional to that signal. Okay.

One of the things you need to know about Xa assays is that some reagents include antithrombin and some reagents rely on patient antithrombin. Okay. So if your patient is antithrombin deficient and you were to use a reagent that added antithrombin, you may overestimate their heparin level.

Okay. So that's just an important consideration. This is, this factor is particularly important if you're monitoring babies, because babies don't have enough of their own antithrombin.

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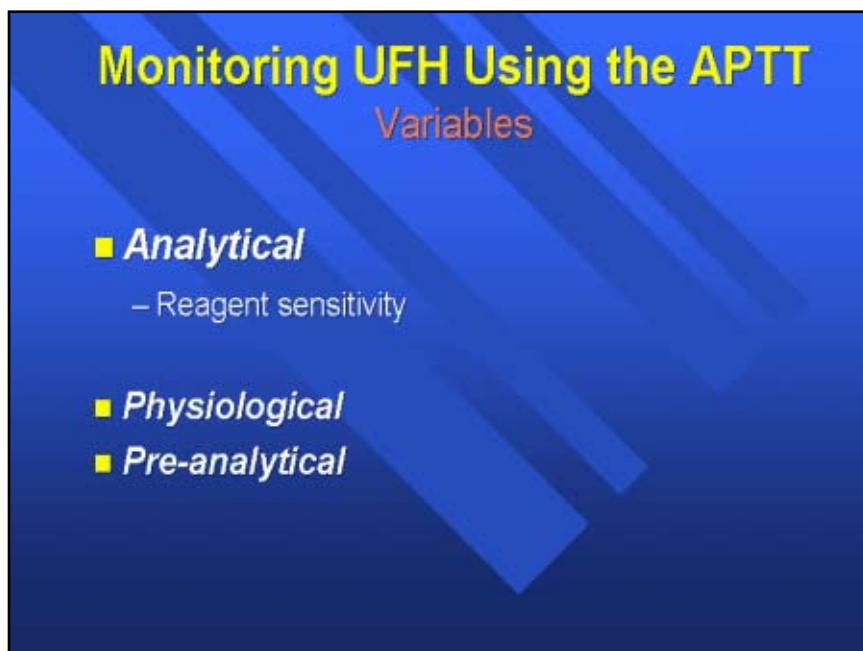
Title: Heparin responsiveness - Examples of variation



Now, this is just an example of how different reagents may respond to heparin. All right. Some reagents are not very responsive to heparin, while other reagents are. And, here we're correlating the heparin concentration to the PTT. So, heparin responsiveness varies considerably between reagents.

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Title: Monitoring UFH using the APTT - Variables



Now, when we're monitoring unfractionated heparin using the PTT, there are a number of important variables. We've talked about reagent sensitivity which is a very important variable, but there are physiological as well as pre-analytical variables that should be kept in mind.

## Monitoring UFH Therapy Physiological Variables

- **APTT Only Affected**
  - Increase in FVIII and fibrinogen
  - Prolonged baseline APTT value
  - Diurnal variation in APTT despite constant heparin infusion rate
- **APTT and Heparin Assay Affected**
  - Antithrombin deficiency
  - Concurrent thrombosis - larger doses of heparin required with larger clots

When we talk about physiological variables, there are some that will affect the PTT only and some that will affect both the PTT and the heparin assay.

As Donna talked about this morning, some patients in an acute phase reaction have an increase in factor VIII and fibrinogen, and this will shorten a patient's baseline PTT. Anything that either shortens the PTT or prolongs the PTT will interfere with the PTT's ability to accurately monitor heparin therapy, and we'll give you some examples of that in a minute.

Another thing to keep in mind is that the PTT shows a diurnal variation to a constant infusion of heparin. If you take a patient and you've got them on stable heparin therapy, the PTT varies in a diurnal fashion, such that it is very, it can be shortened in the very early morning hours, even despite a constant infusion.

So the PTT shows a physiological response to heparin. Now, there are also a number of factors that can interfere with both the PTT and the heparin assay. The one thing I mentioned is antithrombin deficiency. Although not very common, antithrombin deficiency can be a cause of heparin resistance.

If you don't have enough antithrombin, then heparin cannot function adequately. In this instance, the PTT may be short, and if you've got a heparin assay without added antithrombin, it will also be low. If, however, you have a heparin assay where antithrombin is added by the reagent manufacturer, your PTT and heparin assay will appear discordant.

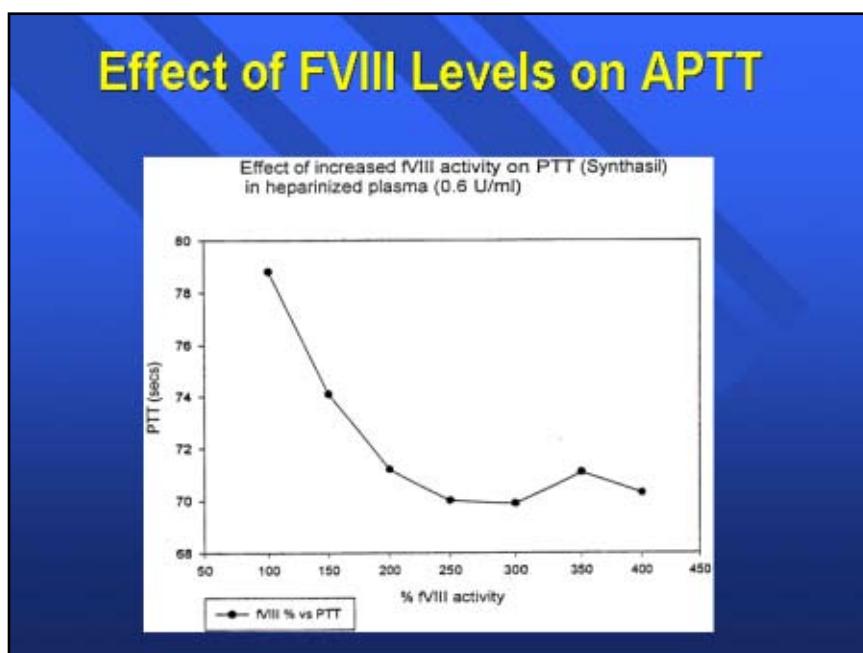
If your patient is not responding to heparin therapy or they consistently have a shortened PTT, it's very important to measure a heparin assay, and it's also important to get a baseline antithrombin level; although uncommon, it's an important cause of heparin resistance.

And it's also been shown that with concurrent thrombosis, the larger that thrombosis, the greater the amount of heparin required and the harder it is for the patient to come into therapeutic range.

## Monitoring UFH Using the APTT Increase in Acute Phase Reactants

- Factor VIII and fibrinogen elevate
  - Can shorten the APTT in a clinically significant manner
  - Increase in factor VIII from 100% to 250% can shorten APTT by 10%
- Occurs in pregnancy
- Under-estimates level of heparinization
- Cause of *in vitro* heparin “resistance”

Now, as I mentioned, both factor VIII and fibrinogen can elevate as an acute, as acute phase reactant proteins, and these can shorten the PTT. And let me just go ahead and show you that slide.



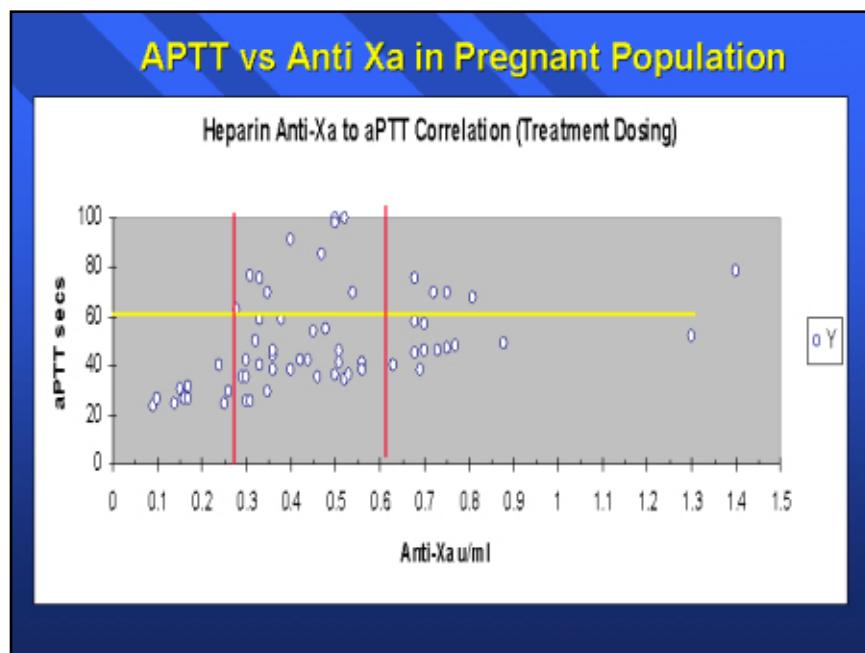
This was the study we did at the VA. And, what we did is, we took a patient sample, a patient that was on heparin therapy, and we added factor VIII concentrate, and the patient started out with a baseline factor VIII of about 100%, and we added factor VIII.

And, as you can see here, when the patient's factor VIII level increased from 100% to 250%, which is a very common range for factor VIII to elevate as an acute phase reaction, the PTT dropped significantly. The PTT lowered by about 10 seconds in a patient on unfractionated heparin therapy.

## Monitoring UFH Using the APTT Increase in Acute Phase Reactants

- Factor VIII and fibrinogen elevate
  - Can shorten the APTT in a clinically significant manner
  - Increase in factor VIII from 100% to 250% can shorten APTT by 10%
- Occurs in pregnancy
- Under-estimates level of heparinization
- Cause of *in vitro* heparin “resistance”

So, as I've shown you, when the factor VIII increases, the PTT can shorten. Elevation of factor VIII and fibrinogen is very common in pregnancy. This is a cause of *in vitro* heparin resistance. Individuals that have a shortened baseline PTT have underestimated levels of heparinization.



And we did a study on a pregnant population. And what we did in this pregnant population, these were all, these were women that were all on unfractionated heparin therapy. And when we were monitoring their heparin anticoagulation, we obtained a PTT as well as a heparin assay, an anti-factor Xa assay.

And with the PTT reagent that we were using in order for patients to be heparinized, their PTT had to be greater than 60 seconds, so that's why we've got the yellow line here at 60 seconds. The recommended heparin therapeutic range based on the heparin assay is in the realm of 0.3 to 0.7 units.

Now, as you can see from this diagram, there were a number of patients that were adequately heparinized based on the heparin assay; however, their PTTs appeared subtherapeutic, right, they're all below the yellow line. So if you were to monitor this population with a PTT only, you may increase the level of heparinization and overheparinize these patients.

All right. So, this is true of a pregnant population, but it may very well be true of any patient that has significant acute phase reaction, reactive proteins elevated.

## Monitoring UFH Therapy Elevated Baseline APTT

- Numerous factors may elevate the APTT:
  - Concomitant warfarin therapy
  - Lupus anticoagulant
  - Factor deficiency
  - Liver disease
- APTT response to heparin therapy is exaggerated

Now, there are also numerous factors that can elevate the PTT and I know you are all familiar with these, concomitant warfarin therapy, lupus anticoagulants, factor deficiency, liver disease.

And, in this instance, when the baseline PTT is elevated, the response to heparin is exaggerated. So, this population runs a risk of being underheparinized, because the PTT seems too elevated.

## Monitoring UFH Therapy Pre-analytical Variables

- APTT Affected
  - Sodium citrate: 3.2 % Vs 3.8 %
- APTT and Heparin Assay Affected
  - Centrifugation speed and duration
    - » platelet poor
  - Conditions of storage
    - » whole blood versus centrifuged
    - » Fresh plasma vs frozen

Now, there are also a number of pre-analytical variables that play a role when we monitor heparin, and it's important to know these so that we can account for them. I think all of you are aware that citrate concentration can affect the value of the PTT, and this is because the greater the citrate concentration, the more reagent added calcium is bound and the longer the clotting time.

So, we should all be using one citrate concentration in our laboratories, and it's recommended that 3.2% is the citrate that be used. Now, it's also very important when we're treating patients with unfractionated heparin that we process the samples properly. And, we must look at centrifugation and conditions of storage.

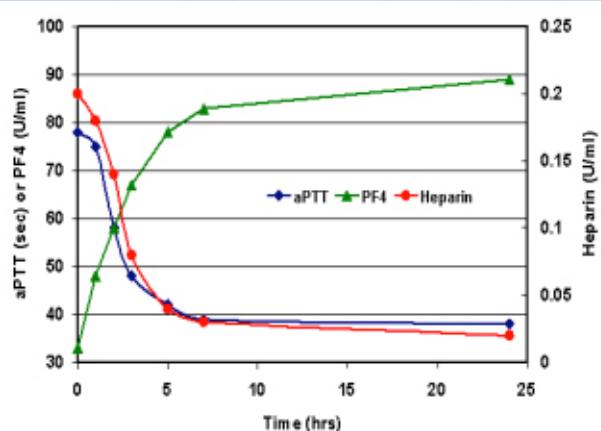
## Monitoring UFH Therapy by APTT Role of PF4

- Platelet Factor 4 (PF4) is a platelet specific protein released from activated platelets
- PF4 is a potent heparin-binding and neutralizing protein
- Effect of PF4 can be diminished by adequate centrifugation and rapidly removing platelets from plasma

Now, the reason for this is that platelets contain a platelet-specific protein called platelet factor 4. And, this is released from platelets as they sit in a test tube. Platelet factor 4 is a very potent heparin neutralizing protein.

And, the effect of platelet factor 4 can be diminished if we centrifuge samples, and I'll show you a number of studies that we performed about 10 years ago.

## Effect of Time on Heparin Levels Role of PF4



What we did, and actually, the way all of this started is, when I was working at Kaiser Permanente, one of the techs told me, that we, we had a pregnant patient on unfractionated heparin and she was being monitored as an outpatient.

And the tech very wisely noticed that if she got to the sample rapidly and processed it, processed the sample, the patient appeared adequately heparinized. However, if she got busy and let the sample sit, the patient always seemed as though she was nontherapeutic with her heparin level.

So, we actually began to look at this and try to figure out why this was happening, because it was a very reproducible occurrence. And so, what we found out was that as a sample from a patient on unfractionated heparin sat over time, platelet factor 4 was released from the platelets, and as platelet factor 4 was released, heparin was neutralized.

In here, we've got our heparin concentration. And, as the heparin was neutralized, the PTT decreased. So, this was really a very nice study showing the effect of platelet factor 4 release, neutralization of heparin, and normalization of the PTT.

Monitoring UFH Therapy by APTT			
Effect of Storage and Temperature			
<u>Group</u>	0 hrs	4 Hrs	24 Hrs
Spun-Ice	63.1	62.7	58.9
Spun- RT	61.5	60.8	53.0
WB- Ice	67.7	58.8	40.0
WB- RT	65.1	53.0	38.4

Based on this, we performed a number of other studies, and we wanted to evaluate the stability of samples at different conditions. So what we did is, we took samples from patients on unfractionated heparin and we either spun them within an hour or left them as whole blood, and then we maintained them on ice or at room temperature.

And we monitored the patient's PTT and we also performed heparin levels at different points of time. And as you can see, if those samples are maintained as whole blood, the PTTs decrease significantly over time. And this decrease is most apparent in those samples maintained at room temperature. If, however, the samples were spun in a timely fashion, they were relatively stable over time.

Sample	Fresh	Fresh	Frozen	Frozen
	APTT	Anti-Xa	APTT	Anti-Xa
A	131	0.86 IU	124	0.86 IU
B	93	0.33 IU	81	0.32 IU
C	123	0.59 IU	111	0.58 IU
D	72	0.20 IU	61	0.20 IU

We also looked at the effect of freezing and thawing samples. And, a fairly typical response to freezing a heparinized sample is that compared to a fresh PTT, the frozen thawed PTT is shorter, and in general, the heparin Xa level is generally shorter as well, although they do correlate a little bit better than the PTT samples.

## Monitoring UFH Therapy by APTT

### Sample Processing and Storage

- Centrifuge within one hour of phlebotomy
- Centrifuge adequately
  - Platelets  $< 10 \times 10^9/L$
- Centrifuged samples stable up to 4 hrs
  - Room temperature or refrigerated
  - NCCLS recommends only 2 hrs room temp

So, based on this, it's very important how you process samples. Samples from patients on heparin therapy should certainly be centrifuged within one hour, and you must centrifuge to remove essentially all of the platelets.

Once the sample is centrifuged, it's stable whether maintained at room temperature or refrigerated, but remember that NCCLS recommends room temperature storage for only two hours.

## Monitoring UFH Therapy by APTT

### Analytical Variables

- aPTT values may vary significantly:
  - Reagent manufacturer and lot
  - Type of instrumentation and clot detection method used
- CAP surveys reveal 300 different APTT instrument/reagent systems in the U.S.

Now, we've talked about the fact that your PTT values may vary significantly depending on the reagent that you're using. And I think many of you are very familiar with this, because this has been a very important topic in the coagulation literature recently.

So, your PTT values vary depending on your reagent as well as your instrumentation. And fairly recently, CAP survey revealed that there are at least 300 different PTT instrument systems in the U.S., and that's really an extraordinary number.

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Title: Variation in APTT by reagent manufacturer and lot

Variation in APTT by Reagent Manufacturer and Lot				
Reagent	APTT Ratio 1.5–2.5(sec)	Heparin Conc (U/mL)	APTT (sec) related to Hep Conc 0.3–0.7 U/mL	APTT Ratio
A	53–88	0.28–0.75	57–82	1.6–2.3
B	50–83	0.25–0.40	60–145	1.8–4.4
C	47–78	0.26–0.52	54–105	1.7–3.4
D-1	48–80	0.17–0.28	94–130	3.0–4.2
D-2	45–75	0.15–0.25	90–132	3.0–4.4
E	42–70	0.18–0.30	70–114	2.5–4.1

In another study that we performed, we looked at, we wanted to see what the variability is between some of these PTT reagents. So we looked at five different reagents, and in fact, we looked at two lots of one reagent in particular. And we determined what the therapeutic range would be based on the 1.5 to 2.5 ratio, and we used as our denominator the mean of a normal population.

And then we compared that to a heparin concentration. And I hope you can see here that given the 1.5 to 2.5 ratio, the heparin concentration is in the realm of 0.2. This reagent actually goes up to 0.7, but in most instances it's not higher than 0.5.

And heparin therapeutic range is 0.3 to 0.7. So, if we were to use this ratio of 1.5 to 2.5, the vast majority of patients would be underheparinized. And this is really clinically significant, because this is the patient that is going to suffer propagation of the thrombus or recurrence.

So, you're really not serving the patient in a very good fashion if you rely on this ratio. So then what we also did with these reagents is, we determined what the PTT range would be if we used the heparin concentration of 0.3 to 0.7 and then determined the aPTT ratio, which you can see for most of the reagents is much greater than 2.5.

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Title: Monitoring UFH therapy - Recommendations and regulations

Monitoring UFH Therapy Recommendations and Regulations	
■ CAP Commentary 02:3712:	<ul style="list-style-type: none"><li>– <i>Laboratory tests are commonly used to monitor anticoagulant therapy. The laboratory should provide recommendations concerning test(s) to use and the range of test results that indicate control of anticoagulation.</i></li></ul>
■ Laboratories should calibrate a therapeutic range for their APTT reagent	<ul style="list-style-type: none"><li>– Variety of methods available</li></ul>

So, based on studies just like this, the CAP came up with the new recommendation in their checklist. And this states that laboratories who use various tests to monitor anticoagulant therapy must provide the range of test results that indicates control of anticoagulation. And this is critically important for heparin therapy for the reasons that I mentioned.

## UFH Therapeutic Range Establishment and Verification

- Therapeutic range needs to be established and then re-evaluated with any change in:
  - *Instrumentation*
  - *Reagent manufacturer*
  - *Reagent lot*

And what most of us do when we set up this range is, we make certain that the range is established for all of our instruments in the laboratory, so anytime we have a change in instrument, reagent manufacturer or reagent lot, we must reestablish this therapeutic range.

## UFH Therapeutic Range Procedure using Anti-Xa Assay\*

- Perform aPTT and heparin assay
- Plot values:
  - Heparin anti FXa on X-axis
  - APTT on Y-axis
- Calculate statistical “best fit” using regression analysis
- Determine APTT range that correlates to 0.3 - 0.7 U/ml heparin concentration

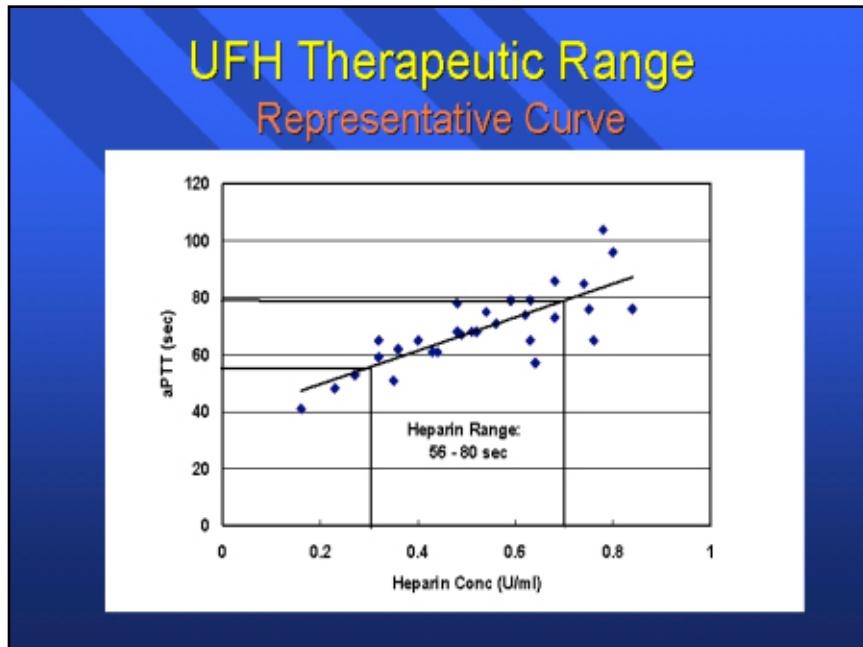
\*Brill-Edwards. Ann Intern Med. 1993;119:104

And most of us use the method by Brill-Edwards, and this is the correlation of the PTT to a heparin assay using the anti-factor Xa methodology. And in this instance, you take patients on unfractionated heparin therapy and you perform both a PTT and a heparin assay.

And then you plot your values, the heparin value on the X axis and the aPTT on the Y, you calculate a best-fit regression, and then you determine that range that correlates to 0.3 to 0.7 IU/ml

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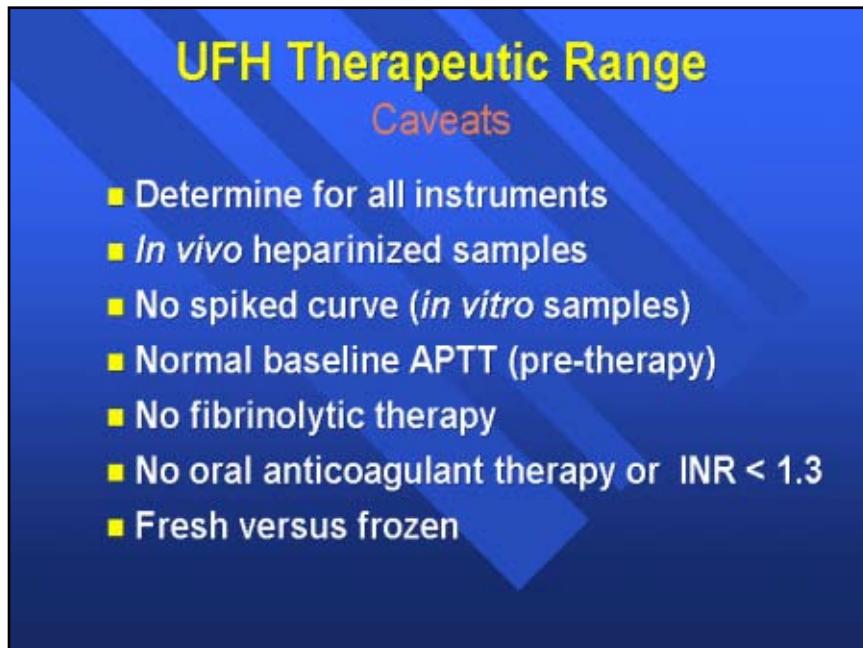
Title: UFH therapeutic range - Representative curve



So, if I can show you here, we've taken our values and we've plotted it with the heparin concentration on the X, the PTT on the Y. We've drawn a best-fit statistical regression line. And then you take the range of 0.3 to 0.7 and determine what range in PTT that correlates to. Now, if you've ever performed this, you know that it can be very difficult to do. It sounds easy, but it can actually be difficult to perform.

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Title: UFH therapeutic range - Caveats



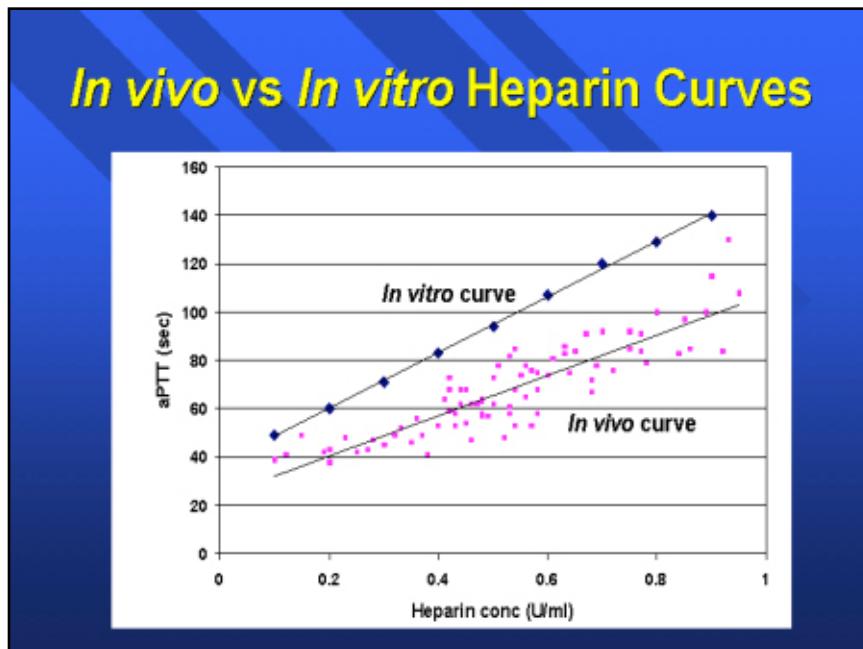
It's important if you do determine a heparin therapeutic range that you determine this range for all of your instruments, even your backup instruments. It's really important that those be included as well. You should use samples that are not spiked with heparin, but rather samples from patients that are truly heparinized physiologically.

Ideally, patients would have a normal baseline PTT pre-therapy. They shouldn't be on fibrinolytic therapy. You should probably try to get patients with INRs less than 1.3, although it's very difficult. Because in most scenarios, when a patient is started on heparin therapy, they're also started on oral anticoagulant therapy.

And then, when you run your PTTs, in my opinion, if you run your PTTs fresh on a normal basis, you should perform this correlation such that your PTTs are run fresh and then perhaps freeze your samples for the heparin assays at a later time.

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Title: In vivo vs in vitro heparin curves

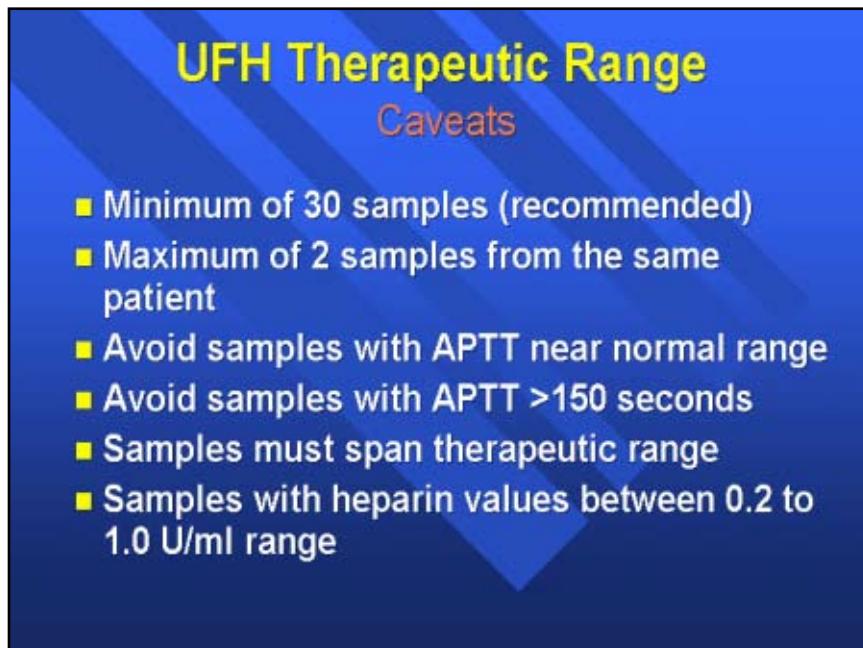


But I wouldn't freeze them and then thaw them and do your PTTs. Now this is the study we did comparing an in vivo heparin curve to a curve where we took normal plasma and we spiked it with known concentrations of heparin.

And these curves do not correlate. So if you perform a heparin, if you do a study to determine your heparin therapeutic range and you spike your samples, you will not have equivalency to those patients that are actually therapeutically heparinized.

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Title: UFH therapeutic range - Caveats



So when you perform this correlation, it should be done using a minimum of 30 samples. You should have no more than two samples from each patient. You should avoid PTT samples that are near normal or that are extensively prolonged. And you should have PTT samples that span, and heparin samples, that span the therapeutic range.

## Heparin Assay Advantages

- Direct measure of heparin activity
- More accurate measure of heparin activity
- Minimal interference due to biologic variables
- Automated and STAT availability
- Anti-Xa Assay can be used to monitor both UFH and LMWH therapy

I think as you can see, based on all the problems that we have with the PTT, there are many advantages to a heparin assay. A heparin assay based on the anti-factor Xa methodology, is a more direct measure of heparin activity. It's a more accurate measure of heparin activity as well.

And it shows minimal interference to the biological variables that I talked about. This assay is also automatable and available on a stat basis. And it's advantageous because it can be used to monitor both unfractionated as well as low molecular weight heparin.

## Heparin Assay Advantages – Monitoring UFH

- Patients monitored with heparin assay reached therapeutic levels more rapidly
  - Failure to achieve therapeutic anticoagulation within 24 hours is associated with both poor outcome and increased risk of recurrence
- Heparin levels are better able than APTT to differentiate those at risk of bleeding
- Overall, heparin assay may be more cost-effective than the APTT

Now, some of the clear advantages of using this assay in patients on unfractionated heparin is that it's been shown when patients on unfractionated heparin therapy are monitored using the heparin assay, they reach therapeutic levels of heparin more rapidly.

And this is really critical because failure, as I mentioned, to achieve therapeutic anticoagulation within 24 hours of therapy is associated with a poor outcome and an increased risk of recurrence. So this is really the crux of it in my opinion. In addition, the heparin level is also better able to predict who is at risk of bleeding.

And this has been shown in patients that appear to be heparin resistant. Because you remember that pregnant population, we would have overanticoagulated that population if we relied on the PTT only. So, overall, given these very two important considerations, the heparin assay may actually be more cost effective than a PTT in this population.

## Heparin Assay Monitoring LMWH

- Only readily available assay to monitor LMWH
- Prophylactic and therapeutic ranges are established in the literature
- Clinical studies do not support routine laboratory monitoring to improve efficacy of LMWH in terms of thrombus extension and recurrence or safety in terms of major bleeding

Now, if we use the heparin assay to monitor low molecular weight heparin, it's important to keep in mind that this is really the only readily available assay that we have to monitor low molecular weight heparin; we cannot use the PTT.

Prophylactic and therapeutic ranges are published in the literature. And then, it's also important to keep in mind that it's really not recommended to monitor routinely low molecular weight heparin, because it has not been shown to improve outcome.

However, we discussed that whole realm of patients that should be monitored. And so, clearly there are indications for monitoring low molecular weight heparin, and when that's the case, we really need the anti-factor Xa assay.

## Heparin Assay Laboratory Caveats

- Must use the appropriate standard
  - Different standard for UFH and LMWH
- Must know if patient is receiving UFH or LMWH therapy
- Must report appropriate range based on type of heparin administered
  - LMWH or UFH
    - » Prophylactic
    - » Therapeutic

If you use the factor Xa assay in your laboratory to monitor heparin therapy, you need to have a different standard for unfractionated heparin and low molecular weight heparin.

Therefore, when your sample comes in to the laboratory, you must know which type of heparin therapy your patient is on. And, you must also report a different therapeutic range, because they vary between the two types.



**Heparin Assay**  
**Anti Factor Xa Assay**

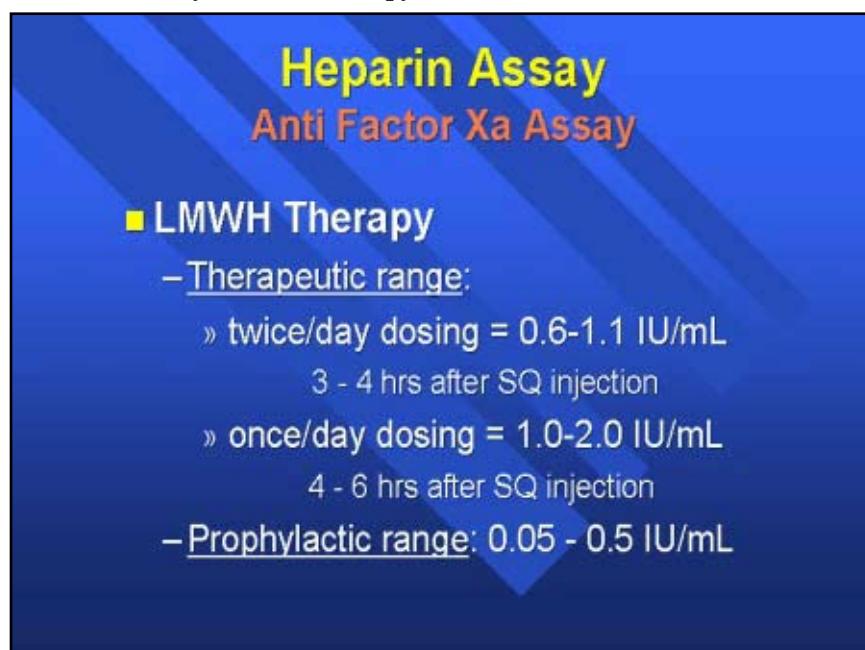
■ **Unfractionated Heparin Therapy**

- Therapeutic Range - 6 hrs post injection or change in dose
  - » Fresh Plasma: 0.3 - 0.7 IU/mL\*
  - » Frozen Plasma: 0.3 - 0.47 IU/mL\*\*
- Prophylactic Range
  - » 0.1 - 0.2 IU/mL

\* Chest 2001;119:64S-94S.  
\*\*Kitchen, S. Blood Coag and Fibrinolysis. 2000;11:137.

If your patient is on unfractionated heparin therapy, the therapeutic range should be measured either six hours post initial injection or after any change in the dose. For fresh plasma, the range of heparin assay should be 0.3 to 0.7. And there was a fairly recent study by Dr. Kitchen which suggested that there may actually be a different range for frozen plasma.

This is the only study that I've seen to-date that shows these data, and I hope that there are more studies in the future, because it suggests that the range is probably a little bit tighter if we use frozen plasma. Now, here's a prophylactic range. When patients are on low-dose heparin, they should have a heparin Xa assay in the realm of 0.1 to 0.2 international units per mL.



**Heparin Assay**  
**Anti Factor Xa Assay**

■ **LMWH Therapy**

- Therapeutic range:
  - » twice/day dosing = 0.6-1.1 IU/mL
    - 3 - 4 hrs after SQ injection
  - » once/day dosing = 1.0-2.0 IU/mL
    - 4 - 6 hrs after SQ injection
- Prophylactic range: 0.05 - 0.5 IU/mL

Now, for low molecular weight heparin, the time that you draw a therapeutic level depends on whether the patient is receiving injections once a day or twice a day. And if you're reporting out a recommended therapeutic range, you should probably have one for once a day injection or twice a day injection.

If the patient is receiving drug twice a day, the levels should be drawn three to four hours after injection, and the therapeutic range is 0.6 to 1.1, although it varies a little bit between some investigators. Once a day dosing should be drawn four to six hours after injection with the range of 1 to 2 international units per mL. And then I've listed prophylactic range for you as well.

## Preparation of LMWHs

- Chemical or enzymatic depolymerization of UFH
- Mean molecular weight of the LMWH varies with different methods of preparation
  - Influences the anti-factor Xa to anti-factor IIa activity
- The FDA treats each LMWH preparation as a separate drug
  - FDA approval for a specific indication requires appropriate clinical trial

Now, I want to talk just a little bit about low molecular weight heparins. And the low molecular weight heparin is made, it's a subset of unfractionated heparin, and it's made by a variety of different methods, either chemical or enzymatic depolymerization of the unfractionated heparin.

The mean molecular weight of the low molecular weight heparin fraction varies depending on how it's prepared. And as I told you earlier, the molecular weight predicts its Xa to IIa activity.

So each low molecular weight heparin, depending on how it's produced, has a slightly different molecular weight distribution. And based on that molecular weight distribution, it has a slightly different Xa to IIa ratio of activity.

So, the FDA actually treats each low molecular weight heparin preparation as its own drug. So it's, they're considered distinct drugs. So, when the FDA cites approval for a specific indication, as you know that the drug has to go through clinical trial, and then the FDA will approve the drug for that indication depending on the clinical trial.

## Therapeutic Equivalence of Different LMWH Preparations

- Largely unknown
- Evaluation of clinical trials limited by:
  - differing clinical conditions and diagnostic methods
  - differing drug administration times and doses
  - differing outcome measurements

Enoxaparin: Lovenox, Clexane

Dalteparin: Fragmin

Tinzaparin: Innohep, Logiparin

There are at least three different types of low molecular weight heparin therapy that are being used clinically, and these are the generic names, and then the trade names are listed. These drugs are FDA approved for different clinical indications. All right.

So for instance, let's say enoxaparin was studied using a trial of prophylaxis for hip replacement surgery. All right. Lovenox or enoxaparin may be approved for that indication.

But say for instance, it's not been, it has not undergone a trial for knee replacement surgery but dalteparin has, the point of this is that whether or not these drugs are interchangeable clinically is really not known, because there are many variables that go into these trials so that the trials cannot be compared one drug to the next.

For this reason, your hospital pharmacy may have more than one low molecular weight heparin in use, because they're being approved for different indications. All right. So your hospital pharmacy may have all three drugs in their pharmacy because of the different indications that the FDA has provided approval.

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**Title: Monitoring LWMH therapy by anti-FXa assay**

## Monitoring LWMH Therapy by Anti-FXa Assay

- Ideally standard curve should be specific for the type of LMWH being measured
- Not always practical!
- Standard curves between different preparations of LMWH actually compare well

When you're monitoring low molecular weight heparin using the factor, anti-factor Xa assay, it's been recommended that you should use a standard curve that is specific for the type of low molecular weight heparin that you're using.

Well, this is really not practical, because we don't always know what kind of therapy the patient is on, let alone what type of low molecular weight. And so, we've done a study looking at standard curves based on different low molecular weight heparins.

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**Title: Comparison of LMWH and UFH by anti-Xa assay**

## Comparison of LMWH and UFH by Anti-Xa Assay Standard Curve

	UFH	Dalte	Enox
UFH	0.22	0.36	0.32
	0.48	0.65	0.62
	0.68	0.79	0.77
Dalte	0.11	0.16	0.17
	0.23	0.35	0.30
	0.68	0.82	0.78

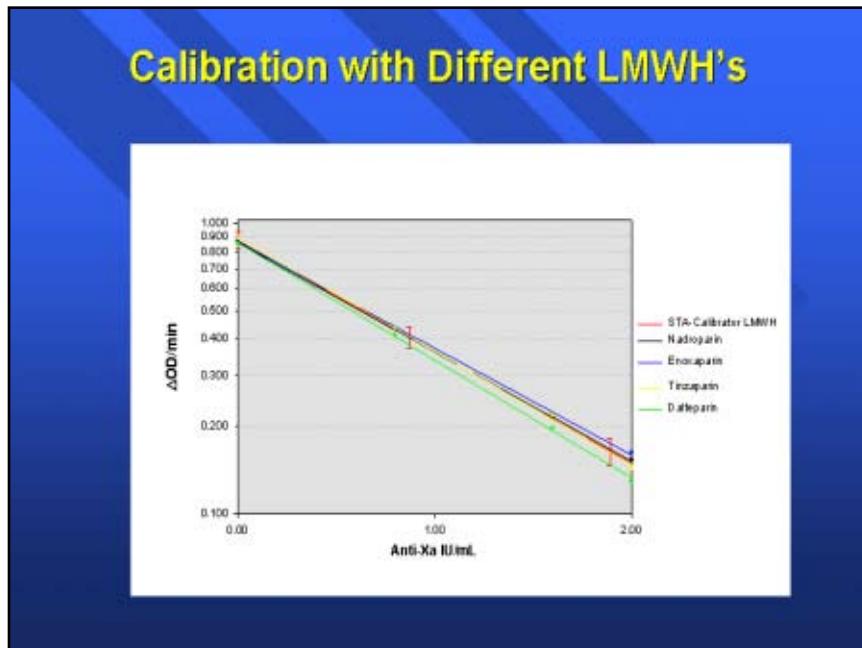
And what we did here is, we took a patient that was on unfractionated heparin and a patient what was on dalteparin, and we had three different curves set up in our assay system. We had an unfractionated curve, a dalteparin curve, and enoxaparin curve.

And what you can see here is that when a patient is on unfractionated heparin, the values differ fairly significantly from, when measured against an unfractionated heparin curve versus a low molecular weight heparin curve.

And likewise, when a patient's on dalteparin, the values when determined based on an unfractionated heparin curve vary when compared to those values read off a low molecular weight heparin curve.

The other thing you might notice is the dalteparin and the enoxaparin curves are actually quite similar. And we've done a number of studies like this, and actually the low molecular weight heparins tend to respond fairly well similar.

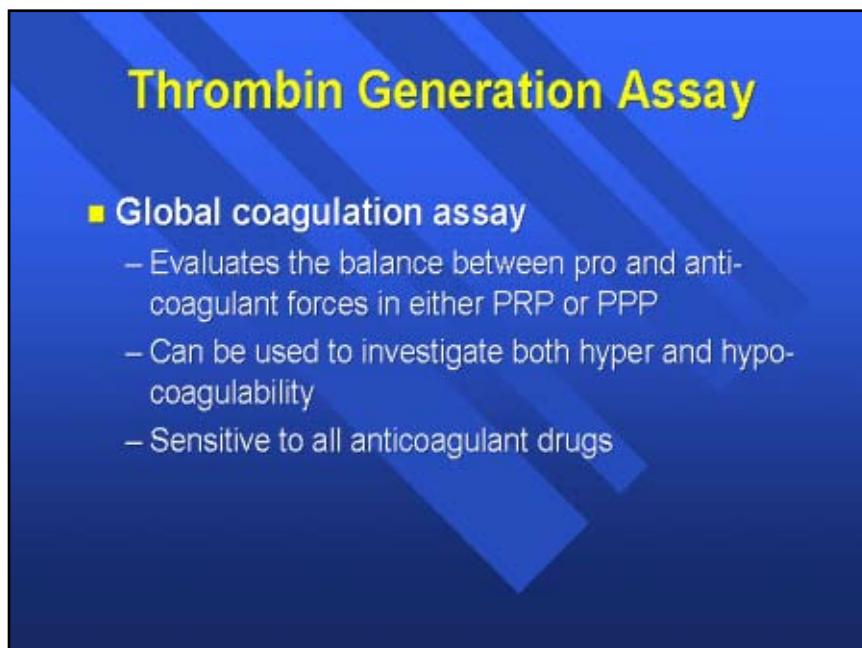
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**Title: Calibration with different LMWH's**

Now, this was a study published in the literature by Dr. Woodhams, and although it's a little bit difficult to see. This study was done using a standard, and you can buy commercially prepared low molecular weight standards.

And the standard on this curve is the red line which falls right in the middle. And then the other lines are four different low molecular weight preparations. And what this study showed is that if you used the appropriate standard, you could actually use one standard for all low molecular weight heparin therapies.

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**Title: Thrombin generation assay - Global coagulation assay**

Now, the last thing I'm going to talk about is the new assay, and I think it will be a new assay to some of you. And it's really a very exciting assay, and it's called the thrombin generation assay. How many of you have ever heard of a thrombin generation assay? Oh good, all right. So it'll be something new.

Thrombin generation assay is an assay that's been used in Europe for a number of years, and it's gaining increasing use here in the US. It is a global coagulation assay. And what it does is it evaluates the balance between procoagulant and anticoagulant factors.

And this assay is really very unique because it can be performed using either platelet-rich plasma or platelet-poor plasma. And when you perform a global assay using platelet-rich plasma, you're actually really looking physiologically at a very complete picture, because platelets play a very important role physiologically in the process.

This is a fascinating assay because it can be used to evaluate both hypocoagulability and hypercoagulability. And, what I think is really amazing about this assay is that it will also be able to evaluate almost all anticoagulant drugs.

So many of us know that there are a whole variety of new anticoagulant agents that are being brought to market, and our big question in the laboratory is how are we going to monitor these? Well, this is an assay that really has a lot of promise for such an evaluation.

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**Title: Thrombin generation assay - Uses a slowly responding fluorogenic substrate that allows...**

## Thrombin Generation Assay

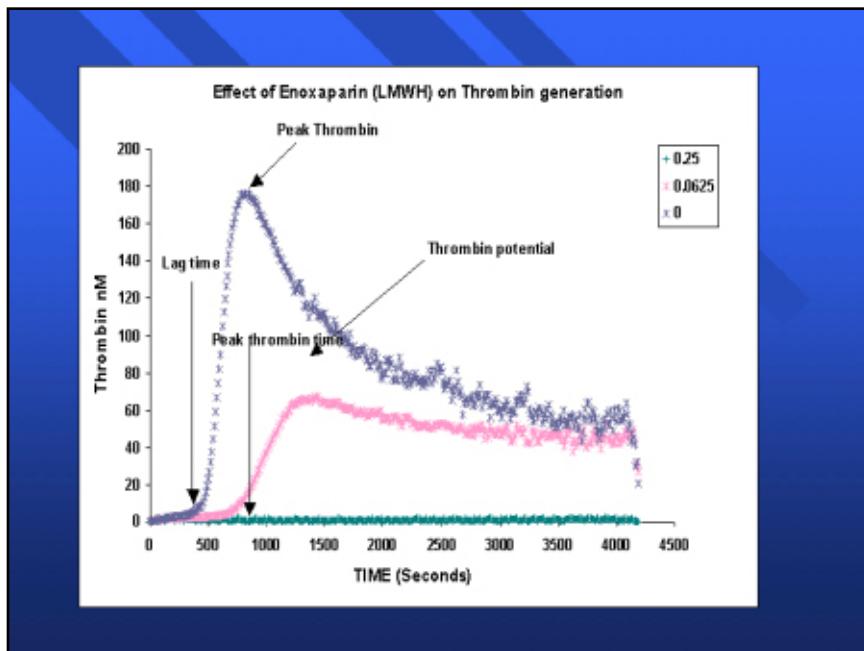
- **Uses a slowly responding fluorogenic substrate that allows continuous measurement of thrombin generation over time**
- **4 parameters of TGA**
  - Lag time, peak height, time to peak, thrombin potential
- **Currently - labor intensive procedure, technically demanding, expensive**

The way this assay is performed is you take patient plasma, either platelet-rich or platelet-poor plasma, and you add an activator. You could either add a PTT reagent or a PT reagent plus calcium. All right.

And then, rather than monitoring clot formation, what this assay does is it includes a very slowly responding fluorogenic substrate that allows continuous measurement of thrombin generation over time. And I'm just going to go to this next slide here and show you what one of these looks like.

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## Title: Effect of enoxaparin (LMWH) on thrombin generation



What this assay does is we add some sort of an initiator of clot formation and then we have our slowly-releasing fluorogenic thrombin substrate within the mixture. And in the presence of clot initiation, we're able to look at not only the amount of thrombin generated, but the period of time over which thrombin generation occurs.

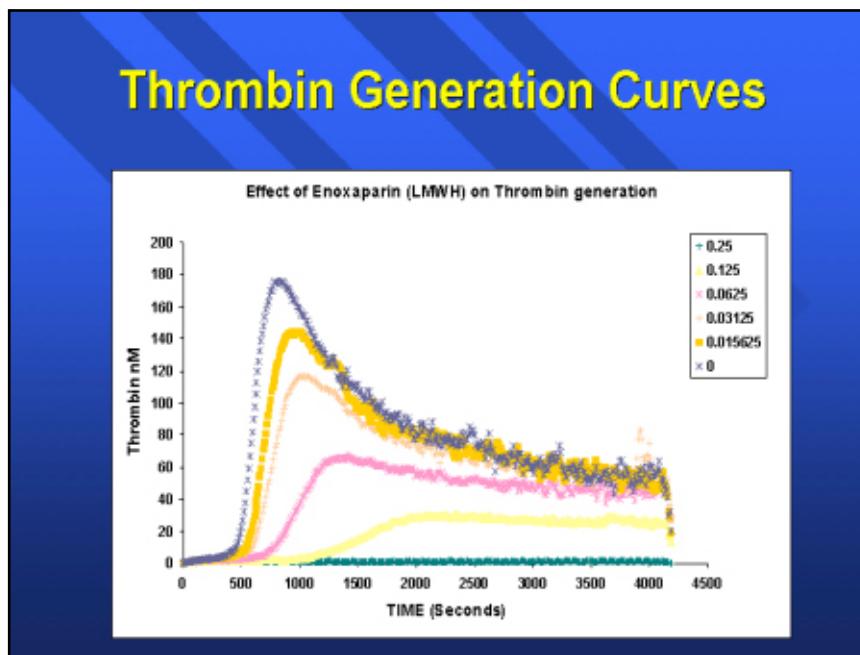
So you're really able to look at the work with which thrombin can perform. All right. How much effect thrombin has. When we look at a thrombin generation assay, there are four different parameters that we evaluate.

The first parameter is lag time, how long does it take before we begin to really see thrombin's action. When clotting is initiated in the laboratory, it only requires about 1% thrombin, which is really pretty amazing. And so when we're looking at this scheme here, the lag time actually correlates to our clotting time.

But what this assay is providing us is all this information here that we're not getting with the clotting time. And what this is showing us is how much thrombin is generated and how long it's active. So, it's looking at the relationship between procoagulants and anticoagulants. All right.

So what we're looking at when we measure a thrombin generation assay is the lag time. How long does it take until we begin to see thrombin. How much thrombin is generated. What is the peak time of thrombin generation which correlates to this peak here. And then, something called thrombin potential, which is the area under the curve.

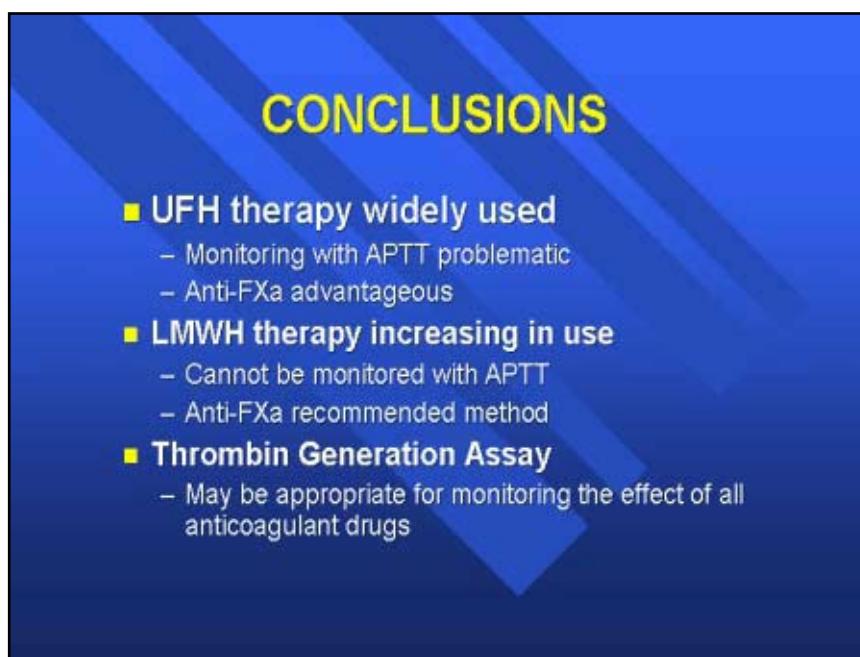
Now currently, this is a laborious assay and it takes quite a bit of time to perform all these calculations. But it's really a very promising assay and it also has the potential to be automated. It's not automated currently in our laboratory. Now, what this is showing you is a normal individual in the taller peak, and then this is an individual that is hypocoagulable, that would have a bleeding potential.



And then the very last slide I have here to show you the thrombin generation is an example of the effect of different concentrations of low molecular weight heparin on plasma.

And you can see here, here's normal plasma, and then we have increasing concentrations of low molecular weight heparin, until eventually we have a complete flat line. And so this assay is really showing significant promise for evaluating different forms of anticoagulant therapy.

And it's also an assay that can predict hypercoagulability as well. And although I don't have an example of that, a patient who has too much thrombin generation, who is hypercoagulable, will have a peak that's narrower and taller. So anyway, this is kind of an exciting assay, and this assay can also be used to evaluate both unfractionated and low molecular weight heparin therapy.



So in conclusion, unfractionated heparin is still widely used, it's still a very popular, effective drug. It's problematic to monitor with a PTT, it must be monitored, and it must be monitored appropriately. The anti-factor Xa or heparin assay is really advantageous.

Now, low molecular weight heparin therapy is increasing in use. You cannot monitor it with the PTT. You really need to use a heparin assay or some sort of a global assay. And then the thrombin generation assay is a new assay that's very exciting, because it may be appropriate for monitoring the effect of all anticoagulant drugs.

And, thank you for your attention, I know it's hard after lunch, and I'd be happy to entertain any questions. (Question from audience.) A: The question has to do with using frozen samples to determine a heparin therapeutic range. My recommendation would be that you should run the PTT fresh and then freeze the sample and then perform the heparin assay on the frozen sample.

I think that's perfectly acceptable. But one study by Kitchen gives a little bit narrower heparin therapeutic range, but in my opinion, it's so important to have the lower end of that range appropriate, because you really don't want to underheparinize the patient.

Actually although there is a risk of bleeding with overheparinization, it's really not as great as we might think it is.

So I think that's the best way to go, is to run the PTTs fresh, especially if you run PTTs fresh in your laboratory, which most of us do, and then freeze the heparin. And I hope to see more studies that look at the frozen heparin therapeutic range. (Question from audience.)

A: It shouldn't. Again, in that one study, the lower range was the same. It was just at the upper range that it seemed to be a little shortened. (Question from audience.) A: That is the, right, right. And, with pregnancy, the ranges are still the same.

And it's really, pregnancy, it's really important in my opinion not to use the PTT. Obviously you can't use it for low molecular weight, but it's really important to use established ranges in a pregnant population. (Question from audience.)

A: The question has to do with determining the heparin therapeutic range that, you know, in any institution, patients are generally on unfractionated or unfractionated heparin as well as oral anticoagulant. That's just the way it is, and that's how all your samples will be.

You should try to get patients who have INRs less than 1.3 to 1.4 although it's difficult. But if you can get them earlier rather than later, you're probably better off. But it's impossible to get somebody not on oral anticoagulant therapy.

(Question from audience.) A: It's, the way we have it set up is a fluorogenic, and so you have to actually have a fluorometer to be able to read the measurements. My understanding is if you use an optical instrument and you have to defibrinate the plasma, and I think that's somewhat problematic. Yes.

(Question from audience.) A: Just spun, excellent question yeah. All we did was just spin it. Yes. (Question from audience.) A: Okay, so the, one of the problems is something called heparin resistance.

And heparin resistance means that a patient is receiving more than, I think it's 40,000 units or 35,000 units in 24 hours, but yet their PTT does not appear to be in a therapeutic range. And this can occur for a number of reasons. The first thing that can happen is that a patient has a very large clot, and they need a lot of heparin.

Okay, that's one thing that can happen. The second thing that can happen is that it's an in vitro resistance, it's related to this elevated factor VIII and elevated fibrinogen. Okay. So, in that instance, your PTT would be low and your heparin level would be in the therapeutic range. Okay.

The third type of heparin resistance which is probably the least common is antithrombin deficiency. Okay. In that instance, your PTT will be subtherapeutic, but your heparin level depends on whether you're using an assay that has AT added or relies on patient's AT.

So, when you're evaluating heparin resistance, you may be best off measuring both the heparin level as well as an antithrombin level. That's what at least clinicians should do, because they don't always know whether the assay has added antithrombin or not.

If your assay does not have antithrombin and is relying on patient's antithrombin, the PT and PTT will be discordant. If your assay has antithrombin, they'll be discordant. Now, babies don't have as much antithrombin as adults do.

So my understanding is that you need to perform assays such that you actually add antithrombin, and that's never made a lot of sense to me, but it's what, those are the typical assays. Do you Craig, do you? (Comment from audience.) Yeah, that's. (Comment from audience.) But how do you know when that patient's really adequately anticoagulated if their endogenous antithrombin is low?

(Comment from audience.) Thank you Dr. Kessler. Yes, question? (Question from audience.) A: Actually the CAP did a survey that Dr. Brandt was involved in, and I believe, John, it was 90% still used the PTT? Do you remember? (Comment from audience.)

We can only hope so. Thank you very much.