Hello. My name is Laura Worfolk and I am the scientific and clinical research manager for Diagnostica Stago. It is my pleasure to provide this presentation titled Effects of Responsive Reagents on PT and APTT Testing.

Title: Objectives

At the conclusion of this presentation, the viewer should be able to:

- Discuss the impact of responsive reagents on PT and APTT testing
- Identify important reagent characteristics
- Recommend protocols for a smooth transition to a more responsive reagent system

At the conclusion of this presentation I hope that the viewer will be able to discuss the impact of responsive reagents on PT and PTT testing, recognize important reagent characteristics, and recommend protocols for a smooth transition to a new reagent system.
Once the decision to switch to a new reagent system has been made, what are some of the technical considerations? Since the PT and PTT are often ordered to monitor anticoagulant therapy, it is important to know the responsiveness of the PT reagent to warfarin and the PTT reagent to heparin.

Other technical characteristics of importance are reagent factor sensitivities, the effect of interfering factors such as the heparin effect on the PT reagent, and lupus anticoagulant sensitivity. And this information can often be found in the package insert.

I will begin now with prothrombin time testing. The PT is by far the most commonly ordered test in the coagulation laboratory. Although there are no published references, my guess is that the majority of the samples received in the lab are ordered for monitoring of oral anticoagulant therapy.
Warfarin therapy is a widely used anticoagulant in this country and is indicated for the prevention and treatment of thrombosis. Warfarin therapy inhibits the formation of fully functional vitamin K dependent proteins such as prothrombin, factor VII, IX, and X. Unfortunately, though, because of its narrow therapeutic index, its delayed onset of action, and individual variability and response due to diet and drug interactions, laboratory monitoring is required. And since the PT test is sensitive to three of the four pro-coagulant vitamin K dependent proteins, it is the test utilized to monitor oral anticoagulant therapy.

Although the PT test is a test utilized, it was long ago recognized that PT reagents vary markedly in their responsiveness to the effects of warfarin. This meant that results in seconds were not comparable between laboratories, instruments, and reagents making monitoring difficult at best, specifically if a patient was tested at multiple facilities. The problem has been markedly reduced by reporting the PT ratio as an international normalized ratio.
The international normalized ratio is a mathematical correction of the PT ratio which corrects for differences in the sensitivity of the thromboplastin reagents.

The standardization system relies upon reference thromboplastins with known sensitivities to the effects of warfarin therapy which are then used by the reagent manufacturers to calibrate their PT reagents. The INR system allows for comparison and results between laboratories and standardized PT reporting.

**Title: INR equation**

The INR calculation is shown on slide number eight. The INR is the patient PT divided by the laboratory geometric mean normal PT, raised to the power of the ISI value. The ISI, which is assigned by the reagent manufacturer, is the international sensitivity index and is unique to a reagent instrument system.

The ISI value reflects the sensitivity of the reagent to the warfarin mediated reduction of the vitamin K dependent clotting factors. The higher the ISI value, the less responsive the reagent is to the effects of warfarin. Conversely, a low ISI reagent is a much more responsive reagent.
The reagent influence on the PT and PT ratio is illustrated on the next slide. In this example blood obtained from a patient receiving warfarin therapy is tested with five different thromboplastin reagents of varying sensitivities.

As expected, the time in seconds is vastly different between the reagents due to the reagent variability and responsiveness to the effects of warfarin. In contrast, the mean normal values obtained with each reagent varies little.

Now because of this variability in responsiveness, both the PT time in seconds and the PT ratio would be unsuitable for monitoring. Now what happens when we take into account reagent sensitivities and calculate the INR?

The variability is reduced when the INR is calculated as shown on the next slide. Please keep in mind that this is a theoretical exercise and that while the INR system is a great improvement, there’s not always 100% INR agreement between systems due to a number of factors.
An example of a real life reagent correlation is shown on slide number 11. The graph on the left is a PT correlation plot between Dade Innovin and Stago Neoplastine CI Plus. The Dade Innovin reagent has an ISI value of approximately 1.0 and the Stago reagent has a value of approximately 1.3. Due to differences in reagent sensitivities, one would expect the results in seconds to differ, and they do. Although the overall correlation is good as evidenced by a correlation coefficient of 0.99, there is a definite bias as the slope value significantly deviates from 1.0. The INR correlation for this data set is shown on the graph on the right. Converting the results to INR eliminated the bias thereby reducing the differences observed when the results were reported in seconds.

The current CAP recommendation is to use a PT reagent with an ISI value of less than 1.7. And one reason for this was that the observation that in North America the use of high ISI reagents led to a much higher requirement of dosing of the drug compared to what was done in Europe. Other benefits of more responsive reagents include a wider therapeutic range and the potential to reduce the INR imprecision.
The ISI impact on the therapeutic range is demonstrated on slide 13. In this study the therapeutic range that correlates to an INR of 2.5 to 3.5 was determined using two different reagents. The reagent with an ISI value of 2.4 yielded a PT therapeutic range of 17.6 to 20.2 seconds.

In contrast, the more responsive reagent with an ISI value of 1.0 yielded a therapeutic range of 30 to 42 seconds. As the ISI value increases the therapeutic range decreased.

Discrimination of the patient PT from normal is reduced with high ISI reagents and there refinement of dosage may become more difficult. And from a practical point of view a target is always easier to hit when it is bigger or wider in this instance.

Another benefit of a more responsive reagent is the potential to reduce the imprecision of the INR. Assuming that there is no error in the ISI value the CV of the INR is equal to the CV of the PT ratio raised to the power of the ISI. Consequently, the CV of the INR determination may be greater when the PT is performed with less sensitive thromboplastins having high ISI values.

However, the CV of the PT ratio is also dependent on the instrumentation. Therefore, this argument may not hold up all the time. In fact, CAP survey data, which are shown on this slide, demonstrate that there’s very little difference in INR precision between reagents with ISI values in the range of 1 to 1.8.
Diagnostica Stago offers two rabbit brain thromboplastin reagents. One with an ISI value of approximately 1.7 to 1.8, and a more responsive reagent with a value in the range of 1.2 to 1.3.

Both reagents contain a heparin neutralizer which will inhibit therapeutic concentrations of both unfractionated and low molecular weight heparins. And due to the high phospholipid content, both reagents are also relatively insensitive to the presence of lupus anticoagulants.

Title: Sample Stago PT ranges

Representative normal and therapeutic reference ranges obtained with Stago’s more responsive reagent is shown on slide 16. The mean lower and upper limit of the ranges obtained at 13 different sites using 10 different lots of reagent is shown.

A typical therapeutic range that corresponds to an INR of two to three was in the range of 23 to 31 seconds. In contrast, the therapeutic range obtained with STA Neoplastine CI, the less responsive reagent, yielded a range of 19 to 23 seconds. However, there was very little difference in the normal reference range.
The PT test is sensitive to deficiencies in prothrombin, factors V, VII, and X, and to a lesser extent by fibrinogen. As different reagents respond differently to the effects of oral anticoagulant therapies, factor sensitivities will vary by reagent as well. Sensitivities of several commercial reagents are shown on slide 17.

This data was published several years ago and since reagents may change over time it is always best to confirm this information.

Now let’s move on to PTT testing. When considering a new PTT reagent system important reagent characteristics include heparin responsiveness, factor sensitivity, specifically to factors VIII and IX, and lupus anticoagulant sensitivity. And I will discuss each of these points in more detail.
The Stago PTT reagent is highly responsive to heparin and factor deficiencies and displays moderate to high responsiveness to the presence of a lupus anticoagulant.

The PTT is by far the most common method that is used to monitor unfractionated heparin therapy. Although there are many drawbacks to using the PTT to monitor heparin therapy which are the subject of another presentation, it is a cheap assay and readily available in all laboratories.
Historically, when the PTT was used to monitor heparin therapy the target therapeutic range was expressed as a ratio, usually 1.5 to 2.5 times some baseline.

Since there is considerable variability in PTT reagent responsiveness to heparin, the ratio approach, which does not take into account reagent variability, was not appropriate for all reagents. The current recommendation is to express the PTT therapeutic range by correlation to a heparin level. And the target range is the PTT time in seconds that corresponds to a heparin level of 0.3 to 0.7 international units per ml.

Unfortunately, the PTT heparin therapeutic range must be established for each reagent and must be re-evaluated if there is any change in the lot number or instrumentation.

The accepted procedure is to collect samples from patients receiving heparin therapy, test the PTT and heparin level, and then plot the data with the heparin level on the X axis and the PTT on the Y axis. Linear regression is then performed to determine the PTT range that corresponds to 0.3 to 0.7 international units per ml.
An example of this procedure is shown on slide 23. The first thing that one notices is the horrible scatter around the regression line. And if you’re a chemist you would not accept this data at all. However, this is coag, it’s a little bit different.

Keep in mind that the PTT is a surrogate marker for heparin activity and there are many other factors that will affect the PTT unrelated to heparin, such as increases or decreases in factors and the presence of other inhibitors such as a lupus anticoagulant.

The heparin response curve on the left was established using the Stago PTT reagent. And the PTT heparin therapeutic range was 64 to 112 seconds. In contrast, the heparin response curve on the right was established with a non-responsive reagent yielding a therapeutic range of 58 to 68 seconds.

And with a non-responsive reagent large changes in the heparin level yield very little difference in the PTT theoretically making it more difficult to monitor and adjust therapy.

Title: Heparin responsiveness

To summarize, the advantage of a more heparin responsive reagent is that a wider therapeutic range is obtained and this has the potential to make dosage adjustments more easily detectable.
Representative reference and therapeutic ranges for the Stago PTT reagent are shown on slide 25. The data were obtained from 21 different labs using 11 different lots of PTT reagent. And listed are the mean lower and upper limits for the reference and heparin therapeutic ranges as well as a standard deviation and coefficient of variation.

Title: STA PTT-A factor sensitivity

The PTT is also ordered to screen for factor deficiencies. And of the factors detected by the PTT deficiencies of VIII, IX, and XI which are associated with clinical bleeding, are the most significant.

The Stago PTT reagent is manufactured for sensitivity to these factors at approximately 30%, meaning that levels below this should yield a value above the upper limit of the reference range. Results from a factor VIII sensitivity study are shown on slide 26 and with this lot of reagent a factor VIII sensitivity of 25% was obtained.
Factor IX and factor XI sensitivity studies are shown on slide 27, yielding sensitivities of 28% and 32% respectively.

What is the lupus anticoagulant and what is the relationship to the PTT? Lupus anticoagulants are a type of anti-phospholipid antibody whose protein targets are either prothrombin or beta 2 glycoprotein 1. Lupus anticoagulants may be associated with autoimmune disorders or they may arise spontaneously.

Clinically they are associated with thrombosis, recurrent fetal loss, and infertility, and therefore their detection is important. In the laboratory an LA will interfere with phospholipid dependent clotting tests such as the PTT. In fact, a prolonged PTT may be the first clue that an LA exists.
PTT reagents also vary significantly in LA sensitivity as demonstrated by the data shown on slide 29. In this study 40 known LA positive samples were tested with 12 different PTT reagents.

LA sensitivity ranged from 78% to 100%, 100% value meaning that all LA positive samples yielded a PTT value above a certain threshold level, and the Stago PTT reagent, PTTA, yielded a prolonged result for all LA positive samples.

So that’s why one of the reasons why we consider it to be a moderate to high responder to LAs.

In summary, why switch to a more responsive reagent system? Well, the main reason is that responsive reagents yield wider therapeutic ranges which may lead to more sensitive and effective monitoring of both heparin and oral anticoagulant therapy.
Once a decision has been made to switch reagent systems, how is this best accomplished? It is not the intent of this presentation to go into the minute details, but to general guidelines and references.

Transition to any new system, whether it is a more responsive reagent system or not, includes method correlation studies, precision studies, and setting up the new normal and therapeutic ranges.

And when establishing the normal range it is important to test over several days to introduce testing variability, to test an equal number of males and females, and to screen for medications.

The CLSI, which is formerly the NCCLS, their recommendations state that hospital or clinic patients should not be included in the reference range unless absolutely required. Now there is debate on this issue with some arguing that they should be included since this is the population that will be served.

As a compromise it is probably best to obtain as many employee samples as possible, commercial sets of normal plasmas, and then as a last resort clinic samples.

Usually the reference range is expressed as the mean plus or minus 2SD, but keep in mind that this includes only 95% of the normal population. And in some cases a 2.5 or 3 SD reference range may be considered.

However, this should only be done in context of the reagent factor sensitivity since it is important not to miss a factor VIII or IX deficiency. One last point regarding establishing reference ranges for coagulation studies.

The only situation where it is acceptable to adopt published reference ranges rather than perform the studies on site is for the determination of pediatric and neonatal reference ranges. And this is obviously due to the extreme difficulty in obtaining normal samples for these age groups.

Fortunately, there have been several recent studies performed establishing neonatal and pediatric reference ranges and those references are available by request. Other areas of consideration are the transfusion and biopsy cutoff values. And these values may be either expressed in PT or PTT seconds or in the INR.

It is therefore critical to communicate with the blood bank and other affected departments to alert them to the new reagent systems and how this will affect these critical values.
In closing, I can’t overemphasize the importance of communicating to all departments when a change is going to occur, what the changes will be and more importantly why it will occur.

One suggestion is to form a changeover team with a representative from pharmacy and nursing who can participate in the education and communication process.

Communication will not only ease the transition process, but meet CAP requirements which state it is the laboratory’s responsibility to make recommendations for laboratory monitoring of heparin, oral anticoagulant therapy, as well as the thrombin inhibitors.

I’d like to thank you for your attention and for additional information or to request references, please visit our Web site at www.stago-us.com. Thank you very much.