FOOD AND DRUG ADMINISTRATION (FDA)

CENTER FOR DEVICES AND RADIOLOGICAL HEALTH (CDRH)

IN VITRO DIAGNOSTIC TESTING

FOR DIRECT ORAL ANTICOAGULANTS

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WELCOME AND INTRODUCTION

DR. DOLLINS: Good morning, everybody. Welcome to the CDRH's public workshop on in vitro diagnostic testing for direct oral anticoagulants, or DOACs. My name is Claudia Dollins. I'm a senior reviewer in the Hematology Branch of the Division of Immunology and Hematology. First of all, as the chair of the organizing committee for this event, I want to thank all of the attendees for coming here today and participating. And of course, I specifically want to thank the speakers for today for all the efforts.

I'm just going to go over some small administrative items before we get started. Obviously, please make sure that you either shut off or silence your cell phones. There's going to be -- there's meetings held this morning in the B and C sections of this building a well. So I want you to be mindful -- it would be nice if you could be mindful of those meetings when you're out in the hallways. As most non-FDA folks may have noticed, your access is actually restricted to this building. To make sure that you don't starve, we actually have lunch available at the kiosk out here. Tickets for that are available until 10:30 this morning, so if you haven't done so, we would encourage you to purchase a ticket for lunch. And the restrooms are located on either side of the building.

This morningthe workshop is divided into an a.m. and p.m. session. In the morning, we're going to talk about laboratory perspective and the clinical perspective, whereas in the afternoon we're going to talk about industry development efforts and our CDRH perspective. We have scheduled time for each -- after each block of speakers, rather than after the individual talks, for questions. So I would like to ask you to hold off on questions until the end of the blocks of speakers.

That's all for administrative items. So without any further ado, it's my pleasure to introduce Lea Carrington. She's the director of the Division of Immunology and Hematology, Immunology Devices in FDA's Office of In Vitro Diagnostic -- in OIR. Prior to becoming the director of DIHD, she served as the chief of the Hematology Branch, which reviews a variety of devices and assays for hematology, coagulation and other body fluids. Before joining the FDA, Ms. Carrington worked as a chief medical technologist in the special coagulation laboratory at Walter Reed Army Institute of Research. Ms. Carrington is an ASCP-certified medical technologist and holds an MBA from Johns Hopkins University and a master's of science in biomedical technology development and management from Georgetown University and Virginia Tech.

MS. CARRINGTON: Good morning. Again, thank you all for coming. I'm Lea Carrington, as Claudia just mentioned. And it is my pleasure to welcome you all, including those who are available on the webcast, and thank all of our presenters for being here with us today. I would like to say that we are very happy to get started on this particular topic. It is of great interest to us, and we are definitely looking at ways and approaches to regulating these devices.

The Division of Immunology and Hematology Devices reviews a variety of hematological devices, including submissions for direct oral anticoagulants. The organizers of the DOAC workshop committee include Claudia Dollins, who is the chair of that committee, Iwona Fijalkowska, Niquiche Guity, Marina Kondratovich and Abraham Tzou. Again, we are looking to gain feedback and engage in discussion on the laboratory and clinical perspectives of DOACs, which is our a.m. session, and also get this from the clinical perspective as well as discussion on commercial development, which we'll be getting from our manufacturers later in

the afternoon.

The review of premarket notifications and subsequent determination of substantial equivalence is performed in the context of the proposed intended use. The intended use specifies what the test measures, why the test is being performed and the testing population and setting for the testing. Listed are some of the elements to be addressed in the intended use. Typically, the intended use and the indication for use are combined for in vitro diagnostics and sets the foundation for the validation studies to be performed to support the crafted intended use.

So what are some of the studies necessary to evaluate the performance characteristics of the devices' intended use? We need to review the analytical performance characteristics and also we'd like to see method comparison utilizing clinical samples and that is compared to a predicate or a reference method, sometimes both. The performance data needed for the review is based on the device output and the interpretation of that result. So the premise of today's workshop is based on the preliminary information we have reviewed for measurement of or assessment of DOACs, some of the submissions we've seen thus far. And that is the reason that we are conducting this workshop, to get more input and feedback on those approaches.

Importantly, the DOAC drugs were approved without a requirement for monitoring. So currently, manufacturers are developing devices to assess the effect or concentration of direct oral anticoagulants. There are currently no cleared or approved devices that measure that. And the goal is we want to ensure that we enable timely access to safe, effective and high-quality medical devices. The objectives that we're going over today are designed to optimize and improve patient outcomes. That's the mission of the agency.

So during this workshop, we will identify clinical circumstances and conditions for testing of DOACs, anticoagulant activity or concentration would be relevant, [and] the clinically meaningful interpretation of coagulation testing results for patients who are receiving DOACs. And we would also like to have an overview of the regulatory requirements for clearance of IVDs intended for coagulation testing in patients who are receiving DOACs. So we'll talk a little bit now about FDA outreach to aid in shaping our regulatory process. We would like to solicit the feedback and, in particular, we have an interest in who should be tested and how the device output is to be clinically interpreted. What clinical evidence is going to be needed? The analytical performance requirements that would be acceptable and the considerations for future development of these types of devices?

So this is my final slide to conclude. And this is something that we encourage manufacturers in particular to do and that is interact with the FDA early and often. And you can do that through our pre-submission process. It is free of charge and we are happy to provide you with feedback on your clinical study design and any studies that you're proposing, including giving you feedback on your intended use. We want to make sure that your proposed study supports the claim that you plan to pursue. And we are happy to discuss any regulatory strategy and your study design.

So with that, we anticipate a productive and informative meeting today and hopefully there will be plenty of fruitful discussion so that we can ensure access to safe and effective tools for treatment and measurement of DOACs. Thank you. Enjoy the conference today.

[Applause.]

DR. DOLLINS: Okay. Now, I want to introduce our first -- our next speaker. Our

next speaker is Dr. Robert Califf. He's currently the deputy commissioner for medical products and tobacco for the Food and Drug Administration. Dr. Califf provides executive leadership to the Center for Drug Evaluation and Research, the Center for Biologics Evaluation Research, the Center for Devices and Radiological Health and the Center for Tobacco Products. He also oversees the Office of Special Medical Programs and provides direction for crosscutting clinical, scientific and regulatory initiatives, including precision medicine, combination products, orphan products, pediatric therapeutics and in an advisory committee system.

Prior to joining the FDA, Dr. Califf was a professor of medicine and vice chancellor for clinical and translational research at Duke University. He also served as the director of the Duke Translational Medicine Institute and founding director of the Duke Clinical Research Institute. A nationally and internationally recognized expert in cardiovascular medicine health outcomes and research, Dr. Califf has led many landmark clinical trials and is one of the most frequently cited authors in the biomedical sciences, with more than 1,200 publications in peer-reviewed literature.

LABORATORY AND CLINICAL PERSPECTIVES

OVERVIEW OF DOACs AND THEIR CLINICAL INDICATIONS

DR. CALIFF: Thanks so much. And it's great to be with you all this morning, but ever so transiently, I'm afraid, because of other duties. But I do want to express my excitement about this meeting. As most of you know, I've worked in this area my whole career, struggling as a young person in the days when we -- in cardiology, we thought if someone's not bleeding, they're not being treated because we really didn't have enough specificity in the treatment to know when to stop, as we were beginning with thrombolytic therapy and the implantation of balloon angioplasty devices into blood vessels. And at that time, we had, of course, the

nonspecific inhibitors, the vitamin K antagonists and heparin that are still in process, with a lot of work still ongoing.

But over the past few years, it's really been an exciting time with the direct oral anticoagulants, a number of them now in the market and setting the stage, I think, for you all to come together today to help advance the field. I have recusals for specific matters related to several of these drugs. So I won't be able to participate in the meeting and cannot participate in any decision-making related to these activities. But I do want to really point out that, at least in my view, this meeting exemplifies a critical role for the FDA in advancing the science of technology development and use, so-called regulatory science.

And if you look at the agenda today, it's really quite remarkable. The morning is science and then you get into actually applying the science to things that are useful to people in a very direct way. This is what the FDA should be doing. And I'm certain that good things will come when so many knowledgeable people come together, convened in the pre-competitive space, talk about science-related issues, apply it to public health and in this case really to what we're now calling precision medicine.

Speaking of precision medicine, we're all increasingly aware of the importance of linking tests and interventions. This, in many ways, as medicine has evolved sort of in a disjointed effort where tests were developed and then somehow magically doctors were supposed to figure out what to do with the tests when it came to the interpretations and decision-making about which interventions to use. But we now know we're in an era where these activities are going to be more tightly linked as time goes on, exemplified by the oncology field where chemotherapeutic regimens are being determined rapid-fire. This is important for the FDA in many ways. But one way that's I think also exemplified today is it means the centers

at FDA really ought to focus on working together. There are many forces in play in society that would have the centers stick within their own boundaries.

But in order to make the best of this field, like many others that are evolving, cross-center collaboration will be emphasized much more going forward. In evaluating tests, it's also important to think about both clinical validity and analytical validity. And I'm really glad to see today both of these are being discussed. I think as we look across the spectrum of laboratory tests, it's pretty clear that there's a much deeper understanding of analytical validity and it's quite reasonable that this is a major focus today. But after all, in the real world, as tests become available in a complex environment, the clinical validity issues are becoming more and more important as people make decisions about when to use a test and when to take that information and change treatment. So I'd love to join you. But the great effort across the FDA and the response of the laboratory and clinical communities give me confidence that you'll come up with something good today.

So just a few comments about the problem in general. Obviously, the target conditions here are huge and that's a word I guess we're all using now in Washington. Huge is a big term. But these are truly huge issues for our society, accounting for enormous tolls of death and disability. And for the most part, what's being talked about at this meeting is a great model for other thinking that needs to go on because it's relatively simple.

In this situation, the big tradeoff is between preventing thrombotic events and bleeding. These are things that we understand a lot about. The clinical manifestations are fairly clear. But of course, we never know about who is safe from having a stroke. What we do know about is the harm of an intracranial bleed or a fatal bleed is plainly obvious. So it's imperative that continue to work on safety is a paramount issue.

Now, one advantage of this meeting is that the molecular targets of these new drugs, thrombin and factor Xa respectively, are relatively discrete compared to the more broadly applicable previous anticoagulants. But the coagulation system, as you all know, is tricky, with many redundancies and feedback loops. And so, what seems like it should be simple and direct, as with many other drugs that we've worked with in the past, turns out to be a lot more complicated.

Currently, as has already been emphasized, routine monitoring is not required in the labels of these drugs. But in the workshop, you're going to discuss some pretty interesting issues. Are previously cleared and commonly used screening tests of any value at all? What are the right intended use populations? How do you define and describe the intended use in a way that the clinical world can understand it and the device manufacturers can develop their devices to be optimally used? What's the role if intra-patient variability?

The bleeding and thrombotic events are not simply a result of the drug effect but of many other factors related to the clinical condition of the patient, the environment. Obviously, a surgical procedure versus a free-living individual lead to very different factors. So in this multifactorial situation, how do we really understand the clinical validity across the range of possible indications?

And as I said, you know, a good example to think about is the contrast between routine monitoring in an outpatient with what to do when an acute injury or bleeding episode occurs that might necessitate determination of just the presence of NOACs [new oral anticoagulants], and the specific concentration or biological effect. And then, the importance of the output. What type of output is useful for clinicians and laboratories and how can this output be appropriately evaluated, something that we're increasingly concerned about across

the FDA. It's not enough to have a good test. If you can't explain your test in terms that clinicians can understand, you've got a real problem in terms of the value of the test.

So please remember, FDA is dedicated to serving the public and patients who are at the center. So one far out issue, not to be the focus of today, but I think you should all keep in mind, is obviously home monitoring is becoming a bigger issue and interpretability of tests not only to clinicians but to people who may be using them in their home environments is an issue that we're dealing with in many areas, including, as you know commonly used anticoagulants.

So I'll close with a personal anecdote. I'm finding in this job, it's common to quote that the FDA regulates 20 to 25 percent of the economy. I'm beginning to think the FDA regulates everything that my family is concerned about. I can take any family member and give an anecdote about the personal relevance of the FDA to them. And in fact, I was home in South Carolina this weekend and my mother, with permission, has given me liberty to talk about her situation. She's a remarkable six-year survivor of multiple myeloma thanks to several new drugs that have been developed and rapidly approved by the FDA, but of course developed thanks to great new biotechnology. But she also has atrial fibrillation.

So she was a longtime warfarin user and switched to DOAC because she found the home monitoring frequency to be troublesome. And at age 88, she felt she should be able to eat anything that she wanted and not worry about it. But she recently had a squamous cell carcinoma that required Mohs surgery and had perfuse bleeding from the site that was taken to put the skin graft on. And it raised the question, how would you measure what the right level of DOACwould be in this situation? And by the way, what is the right dose for an 88-year-old person who had multiple comorbidities? Might this be a case where monitoring would be

indicated? Well, she was pretty interested in what I knew about this. Turns out, I told her I couldn't be involved in any decisions related to these drugs. But I could refer her back to her cardiologist.

But just keep in mind as you go through this, we're interested in regulation. We're interested in science. We're interested in a vital industry in the United States. But in the end, this is really about a population that's enormous, who's very much affected by the decisions that we make together and by the ecosystem which is convened in this room. So thanks for giving me a chance to say a few words. And I'll follow you with interest from the side as you go through this.

[Applause.]

DR. DOLLINS: Well, I'll go ahead and introduce the next speaker while we get our slides up. Our next speaker is Frederick Korley. Dr. Korley is an assistant professor of emergency medicine. He joined the faculty -- he joined the faculty in 2007 after completing his chief residence year at Northwestern. He was inaugural recipient of the Johns Hopkins Robert E. Meyerhoff endowed professorship. Dr. Korley's research activities involve translation of novel diagnoses to inform clinically rational, timely and cost-effective diagnosis of cardiac and brain injury in the emergency department.

Dr. Korley is the recipient of numerous clinical and research awards including the Johns Hopkins clinical scholars award 2010-2012, department of emergency medicine teacher of the year award 2010, department of emergency medicine attending of the year award, Johns Hopkins clinician scientist award and the Harold Amos medical faculty development award, sponsored by the Robert Wood Johnson Foundation. So it's my pleasure, Dr. Korley.

CHALLENGING CASES INVOLVING EMERGENCY DEPARTMENT PATIENTS ON NOVEL ORAL

ANTICOAGULANTS

DR. KORLEY: Thank you, Claudia, for the introduction and for the invitation. I think I have the easiest job today because I was asked to talk about the emergency department's experience with these novel medications. And so, what I did was sent an email to my colleagues and said, what do you guys think? What cases have you had? And so, what you're going to see will be a series of cases, mainly from myself and my colleagues and a few from the literature just talking about the experience that everyday emergency physicians have with these medications. I don't have any conflicts of interest. But I am going to warn you that I'm going to give a very biased talk because, as an ER physician, I don't see the good, right? I don't see the strokes that were prevented. I see the bad, the worst and the very bad. And so, what I say [take] with a grain of salt. But that is my perspective.

So we're going to talk about six scenarios that would indicate testing of the levels of anticoagulation for patients on these novel medications. We're going to talk about cases involving stroke, trauma, evaluation for venous thromboembolism, spontaneous hemorrhage, emergent procedures and the need to triage those and cases of intentional -- or unintentional drug overdose. But before I talk about the cases, I just want to paint a little bit of a scenario of my perspective and, you know, the world in which I operate.

And so, the emergency department, it's pretty special. You know, it's pretty chaotic and it's very different from most medical settings. I get to meet about 40 new patients a day when I work an eight-hour shift. And typically, I have about five minutes to get to know them. So I'm very different from the primary care physician, who has the luxury of getting to know people longitudinally. Among the 40 people, it is true that, you know, probably 10 of them are not sick. But there's a bunch that are going to be super sick and I will be taking care

of them at the same time. And so, I need to be able to make very quick decisions. The good part, though, is that the biggest decision I need to make is whether someone is sick or not sick. So when someone comes in and they're talking and I'm like, yeah, yeah, yeah, are you sick or not sick. That's all I care about. So that's our perspective. And so, when people come in on the DOACs, we need to figure out, you know, what the risk profile is because the majority of my job is risk stratification.

So I'm going to talk about the first case where there was a 65-year-old male who came in with acute onset altered mental status. He had weakness in the right arm and in the right leg. His symptoms started an hour prior to arrival and head CT scan revealed an ischemic stroke. Now, this patient is on Coumadin. And, however, his family says he's not always compliant. So his INR is 1.2. So should this patient be given IV thrombolytics? And I'm going to sort of, be posing questions. I don't expect anyone to answer. I'll answer my own questions. But this is the era before the introduction of these DOACs. And this is a very simple case. Patient has a stroke. CT scan shows a stroke. Is supposed to be on Coumadin, but thankfully they're not taking it. We know for sure the INR is 1.2. So should we give thrombolytics? Absolutely. Right? This is easy.

So same case, this is exactly the same thing. I just switched the medications. And this patient -- this is actually what the real case was. The patient is on Pradaxa. Now, what should I do? It's tough because, you know, it's possible that they were compliant and that they are truly anticoagulated. But there is also a chance that they're not compliant or, for whatever reason, they were not on the optimal dose or they may have other medical problems that have made them, less therapeutic. And so, this patient for sure will not be getting IV thrombolytics and could be harmed from that or at least, you know, would not derive the benefit that he

could get from IV thrombolytics. So it would be good in this instance to have some sort of objective measure of how anticoagulated this patient is.

So the next case, it's a case of a 92-year-old Army vet and the physician who sent me this case, you know, was really devastated about the outcome of this case because the patient came in with altered mental status and shortness of breath after he fell on a flower pot. He doesn't take Coumadin. But let's say he takes Coumadin and his INR is 10. And his CT scan, initial CT reveals a small hemothorax. So a little bit of bleeding in his chest. So I asked myself, what are the next steps. So knowing that his INR is 10 and he has a tiny amount of bleeding and he's 92, you know, that makes me raise my level of suspicion for what we call badness. It's a very scientific term, badness. And so, you know, I would have to make sure I give this patient a lot of attention, including immediate reversal of the anticoagulation.

If I happen not to get a trauma center, you know, this is someone I don't want to be near me for too long. You want to make sure you initially stabilize and transfer them to a trauma center because the fear is that after a couple of hours with the blood being so thin, you know, this tiny hemothorax is going to expand and, A: the patient can become hypotensive, have low blood pressures, you know, from all the bleeding and, B breathing is going to become a problem because the chest is going to be full of blood. So knowing that the INR is 10 really helps. And if, on the other hand, the INR is 1.7, then I don't really care too much. You know, I could watch him, probably get a repeat CT scan in six hours, see how he's doing, monitor his vitals, see how much pain he's in, see what his oxygenation is doing. But knowing that the INR is 10 versus 1.7 really helps me out in trying to triage how sick or not sick this patient is.

So this is what actually really did happen:the patient was on Xarelto. CT showed the same thing. And so, you know, initially it was hard to gauge where this was going to go.

And ultimately, the patient got very sick very quick. And the case was at a non-trauma center and so they were not as equipped to rapidly stabilize this patient. And so, the patient did not make it. And again, this illustrates the very importance of having that sort of number. Especially when you're really busy. You know, on the one hand, you could spend a little bit more time, talk to patients, call families, see how compliant the patients are with their medications, see if they've had any sort of bleeding history, if they have dumb bleeds and stuff like that. What we're doing is pretty time-sensitive. And we may not even have the luxury of talking to the patient because, a fair amount of the time, our really sick patients have altered mental status and are not able to give us very reliable histories.

So the next case is a patient with a history of pulmonary embolism. This is someone I saw who also has a history of atrial fibrillation and came in with chest pain and shortness of breath. Now, actually I've seen many of these patients. So I've seen people like those who have been on Coumadin and the INR says 1.2. And, you know, I've listed a bunch of vitals. But suffice it to say, you know, everything looks normal. Their vital signs look okay. Their oxygenation is good. But because of the history of having a blood clot to the lungs and their INR now is 1.2 and they're now here with chest pain and shortness of breath, my biggest concern is do they have a blood clot.

And the way I figured it out was to get a CAT scan of the chest. Now, you can approach this one of two ways. A is scan everybody. But we do know that with repeated exposure to medical radiation we give people cancer? At least I think so. Or you could also say that, look, you're supposed to be on Coumadin. Your INR was not supposed to be 1.2. So let's assume you have a PE. Let's assume you have a blood clot to your lungs and just treat you, which I think it's, you know, one feasible option as well. And that's what I do in the majority of

these cases where patients are supposed to be anticoagulated and they are not. But the good thing about knowing what the INR is in this particular situation is that if the INR was, say, 3, and they're still having shortness of breath and chest pain, then I really want to know whether they're still having blood clots to the lungs despite having a therapeutic INR because then it means that they may need something else other than Coumadin. So knowing that number is pretty important.

So let's switch this and say the patient is now on Eliquis, and we have the same scenario. It becomes really difficult to triage, what to do with this patient in terms of diagnostic testing. Obviously the easy thing would be to just scan everybody. But as we talked about, you know, it's not always the best thing for the patient. And so, again, you know, having some objective measurement and especially a number that allows some sort of binary decision-making is important because, again, especially for us, we just don't have a whole lot of time to sit down and think through all the different permutations of what non-binary decision-making could be. It's nice to know that this is sick or not sick.

So the next case, and I think I'm going to speed up a little bit, is of an 86-year-old who has a history of hypertension and atrial fibrillation, anticoagulated on Coumadin who presents with five days of dizziness, abdominal pain and flank pain. And so, a CT scan was obtained and it was notable for a 5.2-cm common iliac aneurysm with extensive bleeding in the retroperitoneum. And so, it was -- the concern was that this fairly large aneurysm ruptured and caused some bleeding. However, there was no active bleeding, which makes a difference. So this suggests that he had an aneurysm. It's pretty big. It opened up but somehow, because of his positioning, the bleeding stopped. So we have this sort of nice window of stability. But that could change, you know, at any time. And we are fortunate enough to know the INR and it's

1.2.

So you know that this patient is at risk for bad things happening. But the badness is now going to be a chance event, a chance event that the area of bleeding, which now presumably has been closed up by a blood clot, could open up again and chances are, you know, it may take some time before it opens up, especially because, you know, the INR is 1.2 and we don't have to worry too much about it. Now, these aneurysms are pretty life-threatening because most of the time, one day it involved large arteries. What happens with [an] aneurysm, and I'm not sure where everyone is in terms of medical knowledge, is that it's sort of a ballooning of the walls of an artery. So the walls become very thin. And if this ruptures, you know, it's a big hole in one of your arteries. And so, you bleed out pretty rapidly and these things can be very fatal.

So this patient was lucky. There was initial bleeding. It stopped. But it could happen again. And when it happens, you know, you're not going to have enough time because this patient could rapidly die. Now that we know the INR is 1.2, we don't have to be too worried because most things can probably be taken care of. And the next step for this guy will probably be going to interventional radiology and having a stent placed to prevent future bleeding. So this would be nice and controlled. However, if their INR was 10, you know, that changes everything because before you can get him to radiology, you need to reverse his anticoagulation because, that really is what's going to make things go bad rapidly. If he is on Xarelto, it makes it really hard to figure out what to do.

And again, you could err on the side of being aggressive. And for all of these people, you know, rapidly reverse them. But we have to weigh the benefits of that versus the cons. One thing to do would be to give everyone like this factor concentrates and make sure

that that is not -- at least that this is not a problem, which is what most of us would do now because we don't have any precise measurements of patients' anticoagulation level. But it would be nice to have precise measurements. So we don't have to give such expensive medications to everyone. And these factor concentrates, you guys probably know a whole lot of about this, that they are really expensive.

Patient came in with an acute onset headache. They called the headache the worst headache of their life. And it happened during exertion about 20 hours prior to presentation. She is on Coumadin and the clinician is worried about a subarachnoid hemorrhage. So what happens when someone comes in with the worst headache of their life and it happens suddenly and especially happens on exertion? Again, the worry is that you have an aneurysm, ballooning of one of your big arteries in the brain and that it has ruptured and that you're bleeding. If you get a CAT scan within six hours of a patient having these symptoms, the CAT scan is actually pretty good, about a hundred percent accurate that, oh, you have a hundred percent sensitivity for detecting all bleeding if you present and get a CAT scan within six hours.

If you get the CAT scan after six hours, chances are if there's a small bleeding that could break down of the blood products and so the sensitivity is not as good. It's actually still pretty good. It's about 95 percent accurate. But I'm sure no one in this room wants to be part of that 5 percent. Everyone wants to get to that hundred percent accuracy. So what we do for people who have -- or people in whom we have strong clinical suspicion for bleeding in their brain, who have a negative CAT scan, is that we do a spinal tap where we use a needle to get into the spinal column and get some fluid and see if there's any blood in there. It's invasive. But it helps to get to hundred percent accuracy that, you know, the sudden onset headache

was not from an aneurysm or bleed. Now, this is easy if the patient's INR is 1 or less. But if the INR is 3, then the fear is that when you do this procedure, you're going to introduce bleeding around the spinal cord and that can actually potentially cause paralysis down the line. And so, for procedures, it's really important to know objectively what a patient's level of anticoagulation is.

And the theme is going to be the same for the next case of a 60-year-old with a history of diabetes, HIV and pulmonary embolism who is on Coumadin and presents in septic shock likely due to a urinary tract infection. So she's hypotensive. She's in acute renal failure. She needs a central line and her INR is 2.5. And so, INR is 2.5. you know, she needs a central line. It's not the end of the world. You just have to be careful. And so, clinically in this situation, we probably would do an ultrasound-guided internal jugular vein line. Good thing about the IJ line is that, you know, it's at a site that is easily compressible. So even if there's bleeding, you can easily stop it. And if you use ultrasound guidance, your success rate goes really high up. If the INR is 8, you know, it's still doable. If the choice is, either the patient is going to be hypotensive and have a cardiac arrest ultimately versus risk some bleeding, especially if you're putting in an internal jugular vein line, you know, the benefit outweighs the risks.

So you probably would still put in a central line regardless of the INR. But it's good to know what the INR is because then you know how careful you have to be. You could say that you have to be careful all the time, which is true. But there's a certain level of alertness that you're going to have then you know the INR is 8 or 10 versus when you know it's 2.5. And again, I'm just switching. Same case, if they're on Xarelto now, you know, we're flying blind, have no idea. And amongst all the cases I've mentioned this is probably the least

worrisome because you just have to assume that they are super highly anticoagulated and you have to, you know, pay all the attention you can pay to make sure that, , the procedure goes on successfully. But again, it would be nice. It would be more precise. It would be more data-driven to have information on the exact level of anticoagulation.

And I'm going to talk about the very last case. For those of you who are not from the East Baltimore area, there is Colt 45. And this patient with a history of nonvascular atrial fibrillation on Pradaxa presents after unintentional – or after intentional overdose, the patient presents altered and the paramedics found empty bottles of Colt 45, hydrochlorothiazide and Pradaxa. The patient has no active evidence of bleeding. So most ingestions come in as poly-substance ingestions. And the paramedics help us a lot because they usually can tell us the things they found in the field where the patient was found and that can help direct our initial management because a lot of times, you know, these patients are altered and we can't get any reliable history from them.

And so, the management for these patients, at least in the beginning, it's irrespective of the particular substance they ingested. But after you've done the initial resuscitation and their vital signs are stabilized, it's important to know what they took because that helps you figure out what to expect down the line. For example, if I knew that this patient, took a ton of Coumadin, yes, depending on when he took it, you know, the INR may not change immediately. And so, it may not change immediate management. But down the line, it's going to be important to know. And it's going to be very important for planning additional salvage treatment, like dialysis, for example.

So I hope through these seven cases what I've been able to share with you is that, at least from the emergency department standpoint, there are many opportunities for a

more precise approach to these patients, a biomarker-driven decision-making in these cases. And to recap, this sort of biomarker-driven decision-making is going to be very important in risk stratification of patients with acute bleed. And we've talked about a number of different instances of acute bleed, very important for determining which stroke patients get thrombolytics or not. And also, among the stroke patients, trying to figure out or risk stratify who is going to be at risk for hemorrhagic conversion of their ischemic stroke or not.

For intentional/unintentional overdoses, it's going to be really important to know the amount of substance the patient took. And for evaluation of thromboembolism, knowing their current level of anticoagulation is also going to be important. And as we've discussed, any time you're going to do procedures, it's always important to know what to expect in terms of the risk of bleeding and so, once again, having a precise measure of patients' level of anticoagulation is going to be important. And I have a couple of references, and that's the end of my story. I'll take questions after the next lecture.

[Applause.]

DR. DOLLINS: Thank you, Dr. Korley, for outlining the clinical need for reliable and readily available tests. We're going to switch gears a little bit. We're going to talk about the basic principles and attributes of an ideal assay. Dr. Adam Cuker is from the University of Pennsylvania. He's an assistant professor of medicine and pathology and laboratory medicine at the University of Pennsylvania. He received his M.D. from Yale University and completed an internship and residency in internal medicine at Brigham & Women's Hospital and Harvard Medical School. He continued his postgraduate training at the University of Pennsylvania, where he was a fellow in hematology, oncology and completed a master's degree in translational research. He's a director of the Penn Comprehensive Hemophilia and Thrombosis

Program, clinical director of the Penn Blood Disorder Center and associate director for clinical research at the Penn-Chop Blood Center for Patient Care and Discovery.

MEASUREMENT OF THE DOACs: BASIC PRINCIPLES AND ATTRIBUTES OF AN IDEAL ASSAY

DR. CUKER: Well, thank you very much, Claudia, for the introduction and thanks to the FDA for the honor of being able to participate in this really interesting meeting today. These are my disclosures over the last 12 months. The only real disclosure of relevance is that I do receive research support from the FDA through work in a really unrelated Mini-Sentinel project. And this is the outline for my brief presentation. I'll be highlighting the variability in DOAC plasma levels, introducing the concept of something I call the on-therapy range and how it differs from the more familiar therapeutic range, briefly talking about why we might measure DOAC levels, on the heels of Dr. Korley's really nice discussion of that topic, and then conclude with what I see as the attributes of an ideal assay for DOAC measurement, which will hopefully help frame the subsequent discussion.

So first of all, variability in drug levels. This is a table that summarizes the median trough and peak levels for the four FDA-approved DOACs. These are patients taking standard doses of these drugs. And I want to highlight a couple things. First, I think many of you are familiar with the variability in drug levels within a given patient. And that's nicely seen when you compare median, peak and trough levels. And so, for the twice-a-day drugs, like dabigatran and apixaban, the peak -- median peak level is about twofold greater than the median trough level. When you look at the once daily drugs, like rivaroxaban and edoxaban, the median peak level is about tenfold greater than the median trough level. But I think what's even perhaps more remarkable is the variability in levels between different patients. And so, we can get a little more information about this by looking at the 5th to 95th percentile trough

and peak levels for patients.

And I'd just like to highlight one example and that's the trough level for rivaroxaban, patients taking 20 mg daily. The median trough level is 26 ng/mL and the 5th to 95th percentile ranges from 6 to 87. So that's a 14.5-fold difference. And so, I like to try to put that into some sort of perspective by comparing it to another biological trait with which we sort of all have an intuitive familiarity. And that's adult male height. And according to U.S. Census Bureau statistics, the 5th percentile height of a U.S. adult male is 5'4 and the 95th percentile is 6'3. Well, what if male height in the U.S. varied to the same degree that rivaroxaban trough levels varied? This is what we would look like, right? There would be enormous variation and we would need to have different sized chairs in the room. And so, this is really a lot of biological variability.

And so, we can use these ranges to define something that I like to call the on-therapy range. But before I talk about the on-therapy range, I want to say why we don't use the term therapeutic range, or at least why I prefer not to. So many of us are very familiar with the concept of therapeutic range. Dr. Korley mentioned warfarin. That's a classic example. So we know for most indications that clinical outcomes are optimized when the INR is between 2 and 3. We monitor the INR. We adjust the dose of warfarin to hit the target. Well, there are certainly emerging data linking clinical outcomes with DOAC levels. But we really haven't defined ranges where clinical outcomes are optimized, nor do we at this point have the ability to do routine monitoring and dose adjustments to hit a target range. So it's really not appropriate to think about therapeutic ranges when we're talking about DOACs.

But we can think about on-therapy ranges. And I like to define the on-therapy range as the interval delineated by the 5th percentile trough level and the 95th percentile peak

level. And so, if you think about it, the vast majority of patients in steady-state, anytime during therapy, are going to have a level somewhere in this on-therapy range. And so, drug levels below the5th percentile trough level can be considered below on-therapy and those above the 95th percentile peak as above on-therapy. And so, I think it's a useful concept, this on-therapy range to keep in mind as we go forward.

So why might we want to measure? And Dr. Korley did a really nice job of illustrating some real-life clinical scenarios. This is just going to be a brief summary of some thoughts from my clinical experience. There are times when you might want to measure DOAC level because you suspect a below on-therapy level. And here are some examples. Treatment failure, maybe the patient presents with breakthrough thrombosis. The preoperative state, the drug has been held in preparation for an elective procedure, concerns about noncompliance, obesity, renal hyperfunction, GI malabsorption or some types of drug interaction.

There are also situations where we might suspect an above on-therapy level. The patient presents with bleeding, an overdose or has a characteristic that could be associated with bioaccumulation such as renal dysfunction, low body weight, advanced age or some types of drug interactions. And then, there are other situations where we really might not have a preconceived idea about where the level's going to be. The patient presents with trauma, requires an emergent procedure or we're thinking about using a reversal agent and we'd really like to know if there are clinically relevant levels of drug in circulation that would warrant use of a reversal agent.

So that's why you might want to measure. Now I want to talk about attributes of an ideal assay. If we are going to have a test to measure DOACs, what would we like this test to look like? And so, you're going to be seeing lots and lots of figures, I think, just like this

probably throughout the day where we have the assay results on the y-axis and the plasma drug concentration as measured by the gold standard method -- liquid chromatography tandem-mass spec -- on the x-axis. And so, if we're going to be measuring drug, we would really like to see a tight agreement or tight linear correlation between the assay result and the drug level.

And so, this figure is an excellent example of that sort of agreement. And I've arbitrarily said that it would be nice to have an R-squared value greater than 0.9, but certainly some measure to tell us that we have a high degree of linearity. Well, I already told you that there are times where we might want to be able to measure below on-therapy levels and times where we might want to measure above on-therapy levels. So we want this linearity to exist across a broad range of concentrations. The assay needs to have sufficient sensitivity to detect the lowest clinically relevant concentrations. And so, something to watch out for is a linear relationship but where the slope of the line is too flat and there's barely an uptick in the assay result as we go from below on-therapy to above on-therapy levels. This is too flat of a slope. The assay is insufficiently sensitive.

Ideally, we'd also like our assay to be specific, for it to be able to detect only the drug of interest and not be influenced by other drugs and for it to not be influenced by interfering factors like a lupus anticoagulant or a clotting factor deficiency. And then, finally, there are situations where we want to be able to measure and know the DOAC levels on an emergent basis. And so, ideally this is an assay that should be available 24/7. It should be technically simple to perform. And it should have a short turnaround time so that we can have results in a clinically meaningful period of time. So I can tell you that at least right now, as a clinician, the assays that we have available to us, none of them meet all of these idealized

criteria. So we are hoping for better.

I just also want to take a moment and highlight some examples of what I see as problematic assays. Again, something to keep in mind as we go forward. So this is a pretty obvious one, right? We have a big problem if this is what the relationship between the assay and the plasma drug concentration looks like. There is very little agreement, very poor agreement here. And the assay is not going to be useful for predicting drug levels or even giving us a good sense of roughly where the drug levels are. Sometimes there is a relationship between the assay and the drug. But it's non-linear.

So one of the classic examples is a curvilinear relationship. And curvilinear relationships are problematic because you get a flattening out at higher concentrations. And as that curve flattens, it becomes difficult or impossible to quantify drug at higher concentrations. Now, there may be ways to do mathematical manipulations to turn this curvilinear relationship into a linear relationship. But we're looking for linear. And then, of course, again the problem of insufficient sensitivity where the slope of the line is too shallow. There isn't a meaningful increase in assay result as we go -- as there are meaningful increases in drug concentration. So these are problematic assays or relationships to keep in mind as we go forward and evaluate assays.

So this is my final slide, just to sort of grease the wheels for the remaining talks. There is substantial intra- and inter-individual variation in DOAC levels. Therapeutic ranges have not been defined. But we can use this concept, which I think is quite useful, of an on-therapy range, which is, again, delineated by the 5 percent trough and the 95 percentile peak level. And laboratory measurement, although not routinely indicated at this time, may be desirable in special circumstances. And ideally, the assay that we have available to us should

show good linear correlation or agreement with drug levels, as measured by the current gold standard method, tandem-mass spec, across a broad range of clinically relevant concentrations. It should be sufficiently sensitive to detect even the smallest clinically relevant concentrations. It should ideally be specific and it should be available on a stat basis with an acceptable turnaround time. Thank you for your attention and we'll look forward to the next speaker.

[Applause.]

DR. DOLLINS: After this introduction to the ideal attributes of a device, I now want to introduce Dr. Dorothy Adcock-Funk. She'll be talking about methods for measuring direct oral anticoagulants. And Dr. Adcock is the medical director of the Colorado Coagulation, a wholly owned subsidiary of Laboratory Corporation of America. Dr. Adcock has been involved in the field of hemostasis on a national and international basis for the past 20 years and is involved with a number of hemostasis-based organizations, to include ISTH [International Society of Thrombosis and Haemostasis], NASCOLA [North American Specialized Coagulation Laboratory Association], CLSI [Clinical and Laboratory Standards Institute], CAP –College of American Pathogistis] and ASCP [American Society of Clinical Pathology]. She has authored a number of CLSI guidelines, peer-reviewed papers and book chapters on hemostasis.

METHODS FOR MEASURING DOACs

DR. ADCOCK-FUNK: Thank you, Claudia, for that introduction. And thank you to the FDA for allowing me to participate in this workshop. And also, thank you to Dr. Korley and to Dr. Cuker for setting the stage for my presentation today, which is methods for measuring the direct oral anticoagulants. And I already blew it. So these are my disclosures. And so, in my discussion today, I will begin with a very brief introduction as to the way these drugs work. And then, I'll talk about their effect on routine assays and then specialty assays that may be

used to measure the drugs, including mass spectrometry, clot-based assays and chromogenic assays.

This is a cartoon of the coagulation cascade. This is the manner in which clotting occurs in the laboratory. This is not a physiologic means. But it allows us to understand the laboratory tests that we can potentially use to monitor or to evaluate these drugs. So we have the PTT, which evaluates the intrinsic system, the PT, the extrinsic system. And then, we have other activators of the clotting cascade. Snake venoms, such as Russell's viper venom, which can activate factor X or factor V but leads to activation of the common pathway and then ultimately to fibrin clot formation, which can be detected in the laboratory. And then, the very bottom of the cascade can be activated by another snake venom, ecarin, which causes activation of prothrombin, or we can add thrombin to look at the conversion of fibrinogen to fibrin. This is just an overview of the clotting cascades.

Well, what about the drugs that we're talking about today? We have our direct thrombin inhibitors, which, of course, inhibit thrombin, otherwise known as activated prothrombin, and the small" a"following the Roman number means that it's the activated form of the factor. So direct thrombin inhibitors have a great impact on ecarin-based or thrombin-based clotting times. Then, we have our direct Xa inhibitors that inhibit activated factor X. These agents, as well as the agents farther down in the cascade, can be evaluated by looking at assays that are based on Russell's viper venom and they may also impact both the PT and the PTT because of the way in which these drugs interact in the cascade.

So the first thing we're going to talk about is DOAC-treated patient samples in unmodified PTT, PT and thrombin-time assays. This was a study where patients were administered dabigatran and on-therapy levels were measured. And as you can see, we've got

the on-therapy vertical bars. And then, we took these patient samples and we measured the drug levels using mass spectrometry. And then, these samples were also tested in different PT and PTT systems. And as you can see, we have about eight different PT and PTT assays here. And there's marked variability in the response, depending on the reagent used. And you can see that that its evident with the PT assays as well as with the PTT assays.

And as Dr. Cuker mentioned, there's another demonstration here and that's with the PTT we get the curvilinear response. So we tend to get a plateauing as the drug concentration increases. Furthermore, what I'm demonstrating here is that we've got a fair number of patients that have a normal PT or a normal PTT, despite having an on-therapy level of drug. The thrombin time, as I mentioned, is exquisitely responsive to the presence of dabigatran. So even at very low doses, we tend to get an elevation of the thrombin time. And when patients are on therapeutic levels, the thrombin time tends to show no clot detected. It's exceeded its ability.

Here's another study where patients were administered rivaroxaban and it's the same thing. We determined an on-therapy range by measuring samples using a mass spectrometry assay and then we tested samples in a number of different PT and PTT systems. And you can see with the PT, the marked variability, the marked variation in slope or drug responsiveness. And in fact, in a similar study, Dr. Samama suggested that the amount of rivaroxaban needed to double the PT varied from 66 to 700 ng/mL depending on the reagent used. And this has no correlation with the international sensitivity index. And again, we have even higher concentrations of drug with patients that have normal PTTs.

These are studies we did where we spiked normal plasma with either edoxaban or apixaban to again show you the variability that we see with these reagents. But with the

apixaban, what we're demonstrating here is that apixaban tends to have almost no effect on the PTT and very little effect on the PT. So it can be very difficult using these assays to determine if a patient's on apixaban. And finally, this is another study where we took the patient samples that had been administered rivaroxaban and we had a known drug concentration and we measured these with various PT and PTT reagents. And this is an example of looking at one particular PTT reagent. And then, we also took normal plasma and we spiked it with rivaroxaban. And then finally, we took a commercial rivaroxaban calibrator and evaluated that as well.

And as you can see, these three slopes here are markedly different. And in fact, the PTT doubling time, using a calibrator for rivaroxaban, required 349 ng/mL of drug whereas the patient sample required over 1,500 ng. So there's marked variation. It's often stated in the literature that since rivaroxaban does not have metabolites that show anticoagulant activity, that spiked samples and ex vivo samples should have the same response. Well, clearly, you can't make assumptions in coagulation. We're showing here that they do not have the same response. It's also been recommended by the ISTH -- the International Society on Thrombosis and Hemostasis -- that a laboratory can determine the responsiveness of their reagent by using commercial calibrators in their PT and PTT assays.

Well, not all calibrators are appropriate for clot-based assays. And the thought is that these Hyphen Calibrators, while optimized for another assay, should clearly not be used in a clot-based assay because they're likely hypercitrated. So when we talk about laboratory detection of the DOACs using routine assays, I hope I've demonstrated that the PT and PTT showed tremendous difference in their responsiveness to different reagents. Apixaban has little effect on the PTT and the PT. The relationship of DOAC concentration to clotting time is

not predictable. Some assays clearly show a non-linear response or insufficient sensitivity and that commercial DOAC calibrators should not be used to determine reagent sensitivity unless they're purposed for that. And then, the thrombin time is just too sensitive for the direct thrombin inhibitors to be used for quantitation. So the unmodified PTT, PT and thrombin time assays are not suitable for DOAC quantitation.

So what assays can we use? In order to quantitate the drug, we have to use a drug-specific calibrator, an appropriate drug-specific calibrator. And once we have this, there are a whole variety of methodologies that can be used. Liquid chromatography, tandem-mass spectrometry is one assay that can be used that's specific to each DOAC. We can use chromogenic assays that look at the inhibition of activated factor II or activated factor X and then use clot-based assays. Clot-based assays can be based essentially on any of the reactions that I've pointed out here or they can be global assays, which look at the entire coagulation cascade.

So what I'm going to talk about are these various assays very briefly. So liquid chromatography mass spectroscopy is one assay and it's a very specific assay. It's considered the gold standard. And I'll show you that it's specific, sensitive, precise, accurate, robust over a broad range, which is what is needed. The chromogenic assays will not differentiate between the DOACs within a class, although they are sensitive, precise, accurate, robust. The clot-based assays are not specific and may not distinguish a direct thrombin inhibitor from a direct Xa inhibitor. They have the potential for interference by underlying coagulopathies, lupus anticoagulants, liver disease, and there's the potential for limited sensitivity, especially at the low end, greater imprecision and greater lot-to-lot variability.

So this is an example of the mass spectroscopy assay and there are various types

of mass spec assays that can be used. It is thought that the tandem liquid chromatography-mass spectroscopy is the gold standard. And in this process, this process ionizes the compound and measures the mass-to-charge ratio and compares this ratio to an internal standard which allows identification of the drug and then calibration with a specific calibrator allows determination of concentration. So this methods distinguishes specific drug. An analysis of one sample can determine which DOAC is present and at what concentration. Technically, this is a difficult assay to perform and this is some validation data from our laboratory. And what we're showing here is that the assay has a broad range of sensitivity from an LLOQ of 4 up to almost 500 ng/mL with excellent precision, accuracy. So it's a very robust assay.

But while this assay is considered the gold standard, for good reason, there are considerations. There's the potential that the measured concentration by mass spec does not correlate with the DOAC anticoagulant activity. And I'll give you two examples. So [for] dabigatran, is your mass spec assay measuring free or total dabigatran, which is free plus conjugated? So dabigatran glucuronide, which is the conjugated form, adds about 20 percent anticoagulant activity. And what you can do is you can have an alkaline hydrolysis prior to measuring the sample, which splits the conjugate and allows measurement of total dabigatran. Edoxaban is an example -- has a functional metabolite, M4, that circulates at a level of about 10 percent of the parent compound. With the typical edoxaban mass spec assay, you measure only the parent compound and not the metabolite. So therefore, this may cause discrepancies between the clot-based and the mass spec methods. And this also has impact on calibrator development for clot-based assays.

So how is the calibrator made for (a) mass spectroscopy? You know, we consider mass specroscopy the gold standard. But this assay is also calibrated. And where does that

calibrator come from? If you look in the literature, it appears as though some of the calibrators used for the assays, some of the drugs are obtained from chemical manufacturers. So before that assay is used to -- that drug, that manufactured drug is used to calibrate the assay as a synthesized compound, the composition and purity should be qualified. And I'm just going to throw out a thought here, is that should these synthetic compounds be fingerprinted to the compound manufactured by the pharmaceutical before they're used as calibrators. And I'm told that this can be done with product ion scans, which I'm not an expert on. But it's important to realize that there are no universal DOAC standards and there's no universal method for making DOAC calibration material. And this will potentially impact all calibrated assays moving forward.

So I'm just going to now go over the most commonly used assays to measure DOACs. This is an example of an assay to monitor the direct thrombin inhibitors. We evaluated a dilute thrombin time. And you can see that the assay has a very good range over the needed on-therapy range. It does have some limited low end sensitivity and this assay is insensitive to levels of coagulation factors because the patient sample is mixed with normal plasma, vitamin K antagonist therapy and lupus anticoagulants. Excuse me. And this is an RUO assay. The other most commonly used method to measure the direct thrombin inhibitors is an ecarin snake venom which has a metalloprotease that converts prothrombin to meizothrombin, which can be measured using a chromogenic substrate. And the OD that is measured is inversely proportional to the amount of drug present. And this assay also has a very good range. And I understand there's just been a new version of this assay that has good low end sensitivity. So it's showing the appropriate slope, as Dr. Cuker mentioned, and a broad range.

Now, if we move to the Xa inhibitors, the most common method is an anti-Xa

assay. And this is the assay that's typically used to measure heparin, a very simple assay. Patient sample containing the direct Xa inhibitor is mixed with reagent, which is factor Xa in excess. And the residual Xa is inversely proportional to the drug being measured. And so, this assay can be calibrated with the specific DOAC that you want to measure. So here, we used a rivaroxaban calibrator and you can see an excellent slope. And it's been recommended that these are used with non-AT-supplemented reagents because AT (anti-thrombin) supplementation may cause overestimation. And it's also been recommended that human Xa be used in the reagent rather than bovine. So this was a study looking at patients on apixaban that was administered therapeutically. And the apixaban was measured in our laboratory using two different anti-Xa assays, one calibrated with an apixaban calibrator and one calibrated with low molecular weight heparin. And what we're showing you here is that there's a strong linear relationship between the drug concentration and the anti-Xa activity regardless of the calibrator. So the use of a drug-specific calibrator does not make the assay specific for that drug. We've brought the apixaban and the edoxaban assays up in our laboratory with the Stago reagents. And I'm just demonstrating [that] the assays performed very well and we've got a very nice, broad range of sensitivity.

So in conclusion, there are a variety of methods available to measure the DOAC drug concentration. Some are better than others in regard to specificity, accuracy, precision, sensitivity, lot variability, reagent components. But what we really need is standardization of assay calibration. And then, finally, while these methods measure drug concentration in ng/mL, they do not provide a direct measurement of the degree of anticoagulation. And this is a paradigm shift compared to what physicians are accustomed to in monitoring vitamin K antagonist therapy. So thank you very much.

[Applause.]

DR. DOLLINS: Dr. Adcock, thank you so much for that wonderful introduction in the currently available tests and their shortcomings. Our next speaker is Mr. Robert Gosselin. He will discuss the clinical laboratory establishing and offering a DOAC measurement test. He's going to talk about what's important. Dr. Gosselin is a senior specialist for the special coagulation section of the pathology and laboratory medicine department at the University of California, Davis Medical Center in Sacramento, California. He currently serves as an officer for the North American Specialized Coagulation Laboratory Association, is a member of the board of the International Society for Laboratory Hematology [ISLH]and serves as an associate editor for the *Journal of Laboratory Hematology*. He brings a wealth of knowledge in coagulation, as he has been a licensed clinical laboratory scientist for the past 28 years. Thank you so much.

THE CLINICAL LABORATORY ESTABLISHING AND OFFERING A DOAC MEASUREMENT TEST: WHAT'S IMPORTANT?

MR. GOSSELIN: Good morning. I'd like to thank Claudia and the FDA for inviting me. I'm at the same table with doctor, doctor, doctor, and I'm just a simple lab mule. So I'm going to give you kind of my perspective of what we did at UC Davis and what I think are the problems that we have as laboratory folks with the new drugs. I, believe it or not, do have some disclosures. So the outline is some of the things that are of concern to us at UC Davis and some of the challenges that we had. We are measuring DOACs and I'm not sure if we're going to be in trouble with the FDA after today. But I'll kind of tell you what we did and how we did it and what was the reason and then kind of conclude with some of the issues that we still currently have, besides the fact we don't have any FDA-approved tests.

It kind of started for me in 2011 when I was in the emergency department,

similar to Dr. Korley, is that I was doing a study with another faculty physician about something else. And he had a patient who happened to come in with a head injury and said, oh, what is this drug dabi, dabi, dabi-something. And I said, oh, dabigatran. And he said, yes. I have somebody with a head injury and she's on this drug. And while they didn't have a CT scan, they wanted to know what does that mean and how much drug is on board and what do we do. And there's nothing more horrible than to say I don't know to somebody who needs help from the laboratory. And that was gut-wrenching, to say I can't help you. And so, that sort of precipitated that we wanted to be on the front end in the future and to kind of be more proactive for helping our clinicians with these new drugs in the market.

So, for those of you who are in the lab, for those of you who are in industry, you know what we have to do to validate a method. For you that are not in the lab, these are kind of the steps that we have to do just to make sure the methods are okay, whether to get FDA-approved or not. And some of these can be easy and some can be kind of challenging. Most of them are pretty easy for these things that are FDA-approved but a little bit challenging for those that are not.

So at UCD -- and that's UC Davis Health System -- we do measure dabigatran. We do measure rivaroxaban. We do measure apixaban. We started with dabigatran in 2011. We used a chromogenic ecarin test. And kind of the reason we picked that one is I happened to have some on the shelf because I was looking at a test for looking at bivalirudin at the time. And so, we just had some kits available and I was able to modify it on one of our instruments. So it was just a matter of convenience. For rivaroxaban, we implemented in 2013 and for apixaban, we just did that this year.

What was interesting about dabigatran is on the prescribing information on the

package insert, one of the tests that they recommended to use was the ecarin clotting test. And in 2011, there was no ecarin clotting test available in the U.S. So that again was kind of a challenge for us laboratory folks to address the issues that were in the prescribing information available in the U.S. So dabigatran, so what did we do? So again, we used the ecarin clotting time. We were -- we stood on the corner and had our hat in our hand asking Boehringer Ingelheim for some drug. They were kind enough to provide us some. We also did a collaborative study where we'd taken some of this drug and spiked plasma, sent it to labs across the U.S., one in Canada and one in the United Kingdom. And we also did a collaborative study with Dr. Moll at UNC.

So we had patient samples. We had contrived samples. We had all kinds of stuff we could work with. And then, Boehringer Ingelheim was kind enough to do mass spec on the samples we collected at UNC. So we were in a pretty lucky position to be able to do some of our studies necessary for validating a lab method. Recently, Stago has a new chromogenic method, the ECA II. So it's a different one than we initially used. And we did some comparisons between the old method and the current method. And we used samples provided by the sponsor, which was Stago.

So just to give -- I'm going to quickly go over some of these -- some of our studies we did here as far as imprecisions. So with end-run imprecision, you know, 224 percent is not great. But when you look at the numbers, it's not bad. So essentially, a zero gave us between zero and 0.40 to 1. So we are satisfied with the imprecision. So the end-run, if you ran a sample x amount of times, usually we run it 10 times concurrently, this is the kind of imprecision we get around that sample. And then, the day-to-day would be taking what kind of imprecision do we have running controlled material over several days. And then, carryover,

whenever you do automation, you make sure you're not carrying over reagent and sample to your next sample. So they were all acceptable for dabigatran for the original ECA [ecarin clotting time]. When we looked at linearity, it was good. Our LLOQ, which is lower limit of quantitation, was less than 5, which was pretty good. The accuracy, I think we met Dr. Cuker's requirement. We had a little bit of scatter. And on the far right is the bias plot.

So it's not the one that you'd be saying, oh, this isn't really very good. It's more indicating where the areas of bias are. And it was actually a pretty good test. So we said we're thumbs up. We like it. We're going to put it on and away we go. We had a little bit of discrepancy with a new kit, with the ECA II, than with the older kit. A little bit on the low end and that may have been due to a calibrator issue. But as Dr. Adcock talked about, they're actually fairly good. And so, now we're using the ECA II method for measuring dabigatran. We saw a little bit more scatter on the high end. So now we kind of dilute samples, anything over 200 ng/mL, we're going to dilute and repeat and get better results that way. So the post-analytical issues is some of the requirements that we have from CAP would be if you're doing any kind of monitoring with a drug, you have to have some sort of indication what it is. So we used this comment here for dabigatran based on the RE-LY trial. I will discuss a little bit later about how much testing we do and who we do it on.

So for the Xa DOACs test verification performance, again, it was rivaroxaban, again another collaborative study with Stephan Moll's group at UNC. The mass spec was provided by Dr. Adcock's group at LabCorp. We obtained some drug from Janssen and I did some of the other stuff a little bit later. For apixaban, we got the drug from the pharmacy, munched it up the old-fashioned way in a mortar and pestle and dissolved it in some DMSO. We had multiple manufactures with different calibrators and controls who rarely used different

material to do our kind of accuracy assessment. And edoxaban, we just recently obtained from the company and, again, mass spec was provided by LabCorp.

So looking at some rivaroxaban data, again, we see much better imprecision, whether it be with end-run or day-to-day. Minimal carryover to no carryover. Another limit of the detection was pretty good, less than 6. Pretty good linearity. If we look at the UNC study, looking at patient samples versus contrived samples with calibrators, again, we get very good correlation, a little bit, not too bad on the bias plots, if you look below versus the contrived samples up above. So again, we're quite satisfied with this test and putting it in clinical use.

Now, what we don't talk about is how fast these things are. These are really simple tests. We've been doing these for a long, long time. To do a rivaroxaban or apixaban level may take five minutes at the most. So it's not like these laborious tasks where really it takes a lot of effort. They're very, very easy and very fast. So when we looked at apixaban, we kind of did the same thing, the imprecision, carryover, linearity. They were all equivalent. We're using the same chromogenic Xa that we used for unfractionated heparin that we used for low molecular weight heparin.

The only thing different is that we're just changing the calibrator material. And in a subsequent slide, I'm going to show you that there may not be much of a difference between rivaroxaban and apixaban when you're looking at the calibrator material because the slopes on the Xas are very, very close. And it's probably because their molecular weights are very close and it's probably why we may not necessarily need a different calibrator for those two. But that's a discussion for another time.

So in our post-analytical, here's our comments for the rivaroxaban based on the EINSTEIN study. And then, we borrowed some -- a link to the Europeans about the apixaban.

So again, for every result that we report out, we had this attached commented out. So what's our availability? We're not quite that great. It's a Monday through Friday thing. So I'm in a department of two right now. So we don't do it 24/7. We're not quite comfortable with everybody doing it yet. We do recommend trough collections. We're not really comfortable with peaks. We think it's better to look at steady-states versus the highest. We do have surveillance. So we do enroll in proficiency programs. If you are doing the drug levels, it's nice to see if you're actually doing well.

So we enroll in both the NASCOLA, which is [inaudible] European group and CAP. And then, since 2011, we've done about a hundred dabigatrans and six edoxaban and a dozen apixabans. And usually the requests come from our pharmacists or from clinicians. They have patients who are bleeding. They're not really hunting or looking or saying, oh gee, what if. Once in a while, we'll do patients that have some renal insufficiency, a little bit older. But most of the time, there's a clinical presentation that warrants them wanting to know what's going on and what drug level. So we're just not, gee, I want to know what they're on. It's more there's a clinical need that's more emergent.

So now, I'm going to kind of segue into what we did and kind of some of the issues that we had in the laboratory. And I think based on our clinical experience, there usually seems to be a couple of questions when it comes to DOACs. And so, that's always when somebody asks me about DOAC measurement or assessment, what's the question you're trying to answer. So what's being asked? So there's been some things about, oh, we know they're on this, we know they're on that. If they're known medications, it usually is -- it's still there. So if they stopped and they want to go to intervention, you want to know is it essentially gone. And I think that's one question. Is this still around? Is it gone? Can we go forward? And some of

the indications would be the perioperative retinoic acid anesthesia [phonetic]. Then, you have the ones of the patients where you know they're on the drug and you want to know how much is there, which is again a different type of question that's being asked. And we can answer it a little bit differently.

And then, there's the unknown, which I think Dr. Korley alluded to, which is that you don't know sometimes what they're on and they just sort of show up. And that's kind of what I think is a bigger issue in the lab right now. So for the "is it there for dabigatran," Dr. Adcock showed you the thrombin time that's not modified is very, very sensitive. And so, if it's normal, you pretty much rule out any significant levels to almost nothing with dabigatran. And most laboratories have been doing that for decades. So it's not reinventing the wheel. You can quantify the drug. So if you want to know how much is there, it's easy enough to do. Again, it's no FDA-approved methods but it's certainly easy enough to do. For the Xa DOACs, I'm going to show you some slides about looking at even just unfractionated or low molecular weight calibrated Xa methods can rule out essentially any presence of an anti-Xa DOAC. And that to me is a pretty powerful tool. Again, we only offer these Monday through Friday day shift right now. But we might be transitioning that a little bit differently in the near future.

So how much is there? We've gone a little bit over the ecarin-based dabigatran studies. We adapted ours to the VCSXP. But it can be adapted to almost any instrument. We used calibrators and controls. There's several manufacturers. I think there's still only one for dabigatran. There may be a few more coming out. I'm not sure. For the factor Xa DOACs, you have a lot more options for calibrators, kits, et cetera, et cetera. Again, if you've been doing heparin testing, whether it be low molecular weight or unfractionated, all you need is a different calibrator and you're good to go. So the concerns I think that we have in the

laboratory are just -- and in talking with colleagues is I think that if you look at in the U.S., if you look at how many labs actually do PT and PTT testing, thousands, a lot. If you look at ones doing Xa measurements about 400, so roughly 10 percent. But looking at the CAP surveys actually doing drug measurements, it's 20, essentially 20 in the whole U.S. that are doing these tests.

So I'm not quite sure what's going on outside these 20 labs who are monitoring or assessing these. Again, I don't like to use monitoring either because that usually means you need a dose adjustment. But assessing these drug levels. So I think that there's a lot of general assumptions when it comes to DOACs in the U.S. And part of it, I think, has been driven by a lot of early papers and even some of the recent guidelines. So the general assumption about reagents is that the PTT is more sensitive. The dabigatran affects the PTT more than it does the PT and the Xas affects the PT more than the PTTs. I think those general comments, clinicians cling to those and so they sort of just across the board make that general assumption, which is of course not true. The general assumption about reagent sensitivity and some of the recommendations that have been coming out are based on contrived or in vitro or unpublished or even unverified assumptions that we think it's going to work because, well, it should work as opposed to any kind of evidence saying that it actually does work. I think that's been a little disappointing because it turns out that that may or may not be true. Or nothing's required, and I think that's sort of what's the driver of the manufacturers, well, we don't have to monitor at all.

And then, the last one is the biggest in the U.S., the labs. There's a general reluctance about performing any test that's not FDA-approved in the United States. Again, we have the capacity. We have the toys. We have the sauces. We have everything. But we don't

have the anointment from the FDA. And that becomes a little bit challenging, especially now we're in 2015. It's been five years since dabigatran's been out and we don't have anything and that's a little concerning. And the hope, and this is a platform -- I probably won't be asked back -- but I hope that this is a platform to kind of move forward and get this fast-tracked, even though its' been out for many, many, many years.

So Dr. Adcock also talked about some things that I'm going to go a little bit over, but some of our data that we have about, you know, looking at some of the general assumptions that we have with early publications. So the in vitro enrichment -- you take the drug and you add it to normal plasma or you take these drug calibrators that you can get commercially versus patient samples. Now, she showed a little bit different -- sometimes their slopes aren't as bad. So sometimes they work. Sometimes they don't. But most clinicians don't even know what reagents you have in the laboratory. So they're going to look at the first picture they see and say, ooh, yes or no. And so, I think that we have to be better edifiers to our clinicians about, in their institutions, what's good and what's not so good. So sometimes this in vitro stuff works. Sometimes it doesn't.

So here's one, again, looking at Innovin, which is notoriously -- and if you look at this, you think, ooh, that's not too bad, until you look to the left and you say, well, looks at the axis, where we're really not seeing much of a change. So I think sometimes we need to make sure these slopes are really representative of what we're actually seeing. But the patient samples, most patients don't have a hundred percent of factor whatever. So I think we're really doing a disservice when we're doing in vitro enrichment. I think that has the ability for us to give relative sensitivities. It helps us look at different tests and say, well, this drug affects this test and that test. But we shouldn't be hanging our hat when it comes to looking at clinical

responsiveness of patients. In real-world patients, I think drug enrichment helps. But it's not a silver bullet for us for assessing sensitivity.

So these are the different responses of PT reagents to rivaroxaban. Again, this is published data. This is in vitro stuff. Again, I think this is good, when you do in vitro drug enrichment, because it tells you, well, one's better than the other. That's fine. But again, I wouldn't say this is the kind of yardstick we should be using for clinical samples. And we talk about the same reagent. This again, using in vitro enrichment, is good information for a laboratory that in the given reagent system, one's better than the other as far as more sensitive to rivaroxaban versus apixaban. But I think, again, it could be very, very misleading when you're talking about clinical samples.

Now, lastly, I think both Dr. Cuker and I, we talked about specificity of these tests. This is looking at a Coamatic anti-Xa chromogenic Xa, that if you were to take -- what I did here was looking at you get a number when you're doing the test. You get sort of like an optical reading. You get data, a raw data number. If you plug that into different calibration curves, you're going to get different results so that the sensitivity of this test tells you that at the lower limit of quantitation, the LLOQ of 0.3 for low molecular weight heparin, you can measure pretty much really, really low amounts of these new drugs, the anti-Xa DOACs.

Now, what the little circles are is saying that because there's no specificity here, you could have low molecular weight heparin. You could have unfractionated heparin. You could have apixaban. You could have rivaroxaban. It's not specific to that particular drug. It's just telling you that you have some anti-Xa drug on board. But because it's very sensitive, I think it's a pretty powerful tool, that it would be equivalent to the thrombin time for dabigatran in that if you have essentially something less than the LLOQ for a low molecular weight anti-Xa

result, you can exclude the presence of a drug.

So when is that important? And I think that Dr. Korley also brought up that, for us, we're a trauma center. We're a level one trauma center. We're also a stroke center. We also have visitors, not that they come to Sacramento, for God's sakes. They go to San Francisco. They go to Tahoe. And we're in between those two. So if they happen to stop at our place, they're visitors. We don't know anything about them. So I think that what we need to consider doing is that we are still riding the PT and PTT horse that we've been doing for 60, 70 years, thinking that these tests are sufficiently sensitive to screen anybody for anything and the answer is no.

So what we're trying to say is maybe we should start changing our testing algorithm in these patients where we don't know their history. We don't know anything about them. And so, we're considering looking at a different testing algorithm where we start, not with the PT and PTT, but we start with the thrombin time. We start with an anti-Xa screening and say yes or no. Is it abnormal or is it normal? And you can rule out stuff and then segue into our PTs and PTTs that kind of go a little bit further. Now, we're looking at this.

The problem we have is the cost issue associated with just having the Xa test on board because stability is not that great. And we're not doing this for everybody, just for ones that we may not know a good medication history or we can't -- you know, we have a patient that not responsive or something like that and we're going to do some interventions. So I think that this is one thing that is now our new crusade in the lab as far as trying to help out the clinicians in the ED and changing our screening methods for looking at these new drugs because clearly the PT and the PTT are insufficient.

So our challenges -- this is the last slide here -- **is that we don't have any FDA-**

approved methods, controls, calibrators. There's general reluctance in the U.S. marketplace. I know there's been talk about, well, if you modify it, you can do it -- call it an LDT. I'm not sure how you can do that for some of it. The compliance with the clear requirements, meaning the imprecision. That's pretty simple. The carryover, that's really easy. The accuracy is difficult. It's how do we get samples of these drugs and how do we compare and who do we compare it to. Well, that becomes the real challenge for the laboratory. And then, the guidelines and publications, again, I think the early indications were based on some in vitro data, not patient data. They were helpful. But I think you're going to start seeing a change in those as far as laboratory testing and sensitivities. And we need to kind of get more publications out about using contrived samples. That may not be the best for monitoring or assessing reagent sensitivities. Thank you.

[Applause.]

QUESTIONS FOR SPEAKERS

DR. DOLLINS: Now, we're going to take a couple of questions before we escort Bob out of the building. So could I start maybe with one question? Bob, in your presentation, you indicated that there's the testing frequency is really low for these drugs. So in terms of validation, how difficult is it to obtain samples across a measurement of a test?

MR. GOSSELIN: Yeah, we were very, very lucky that -- [off mic].

MALE: Could you use the mic?

MR. GOSSELIN: Can you hear me now? Okay. So, thanks Dr. Moll for playing with us. But I think that we have received calls asking about getting samples from us and would we be willing to send some samples, because I do packrat stuff. But didn't we run into the issues about delinking honest brokers and all that other stuff? That's the biggest challenge that

we have for verifying the performance of a test with the DOACs is accuracy assessment. What's a sufficient number? How do we get them? That's tough. I mean, there are some commercial places that are selling this material, like Clinisys. But I don't know how cheap or expensive they are. I'm sure it's the latter, not the former. And then, you know, how many do you need? I think what would be really nice, since I have the soapbox and I will get kicked out, is that if you would provide laboratory with guidance as far as what would be a decent correlation or verification of performance study, at the very minimum, for these unusual tests. That would be very helpful.

DR. DOLLINS: Good.

DR. HOFFMAN: My name is Maureane Hoffman. I'm at Duke University and the affiliated Durham VA Medical Center. And I just had like sort of a couple of comments and questions about using the anti-Xa activity assays. We run the Stago instruments and their liquid anti-Xa reagent in our laboratory. And it's a nice reagent. We run it in a general laboratory. But we got calibrators from Stago and we sign an agreement saying they're for research use only. And so, I don't know even if I -- I mean, I guess I'd have to get calibrators from somewhere else because I signed something that says I'd do this for research use only, first of all. And second of all, it is at least our region VISN's interpretation that the VA directs that you'll only use FDA-approved tests in your laboratory. Maybe that's a misunderstanding. But we have those two issues. And I wondered how other people had dealt with that at the current time.

MR. GOSSELIN: Guess nobody's going to take it, huh? [Off mic.] Now I'm on. Thanks, Russ. The research use only. I don't think, and I'm not a clinician and I'm sure these gentlemen here, these clinicians here can answer that. I don't think they really care if it's RUO.

And we append that in a little commentary, research use only. That's a cursory glance. They need help. They want help. They need information. And for us to roadblock by saying, well, you know, we don't have it because, wow, that's just not very good. If it's my mom, if it's my son, if it's something that says, gee, you can't get something because it's not FDA-approved, when we have the capacity to do it, that's challenging.

I know there's rules and regulations and all that kind of stuff and it's well-intended. But if we have no choice, I mean, we absolutely have no choice, and we have to do something, it's very tough. And I know we've had a lot of letters come in saying you can't do this, you can't do this, you can't do this. And at some point, I think we have to draw the line in the sand and say, well, we've got to do this because clinicians need our help. Am I still in the room?

DR. DOLLINS: Yes.

[Laughter.]

MR. GOSSELIN: I'm just hoping it gets a little bit better for us because it's not just DOACs. There's a lot of tests out there that we really need for clinical utility that needs to somehow move through the line a little bit faster so we can help our clinicians. We're just conduits of information. But I don't think any clinician would accept the fact that, well, we can't do it because there's no approved method is a good answer.

DR. HOFFMAN: [Off mic.]

MR. GOSSELIN: The caveats associated with the test, right, or billing or reimbursement or getting in trouble when somebody inspects you, that kind of stuff, yeah.

DR. ROSE: Hi. My name's Marty Rose. I'm from CDER, the Center for Drug Evaluation and Research, where I'm in the Division of Cardiovascular and Renal Products. And

I've been involved in the review of the various DOACs. So most of the discussion so far has been around use of assays to determine what to do when there's a problem with the patient who's taking a DOAC. Is there a DOAC on board? Which DOAC is it? There hasn't been much discussion of what to do to guide dosing of DOACs when you know which DOAC the patient is taking and you know his or her renal function and hepatic function. And this is a question for anyone on this panel. Has there been any activity in that area in your institutions?

DR. CUKER: I'll try that one. In my institution, there is no routine monitoring. But there are special patient populations where there's essentially monitoring being done, at least a single test to make sure that the patient who's had, you know, 12 cm of their bowel resected is adequately absorbing the drug. And so, we might measure -- we might have a request to measure a trough level in a patient like that just to make sure that it's in the range that we would expect it to be in. So there are situations where -- special situations where we do monitoring.

Now, I think -- I know Dr. Reilly from Boehringer Ingelheim is in the room and published a very important paper a couple of years ago linking dabigatran trough levels at steady-state and clinical outcomes. And, you know, one at least wonders whether there might be a role for more routine monitoring to optimize clinical outcomes. I think we would need trials to figure out whether that was appropriate or not. Right now, nobody would know how to do that or exactly what target they're shooting for. And of course, we don't have lots of different doses available to us to adjust. But I think it's a fascinating question and one that's, you know, certainly worth posing to the FDA, people like you.

DR. ROSE: Okay. You'll be seeing some of Dr. Reilly's data later. Thank you.

DR. MOLL: Stephan Moll, from Chapel Hill Hematology. Bob and Dot, could you

teach me -- are you able to bill for these tests that are not FDA-approved?

DR. ADCOCK-FUNK: You can, but they're not always reimbursed.

DR. MOLL: And Bob, your 166 tests?

MR. GOSSELIN: I don't know. I'm the wizard behind the curtain. I don't care about billing because we don't do enough of it that it's going to make or break the bank. So I think that we're offering the service. And if we take a loss, we take a loss. I have no idea about billing.

DR. MOLL: And Adam, you run the coagulation lab. Do you get reimbursed for it?

DR. CUKER: I don't run the coagulation lab. And I don't know.

DR. MOLL: And I order these tests as a clinician every so often. And I don't know whether at my institution it's reimbursed or not either.

DR. DOLLINS: Do we have any questions online or --

MS. SHRESTHA: Yes, we do. The first question that we have is for Dr. Adcock. It says how do you obtain the DOAC calibrator? What are the vendors that supply the calibrators and will FDA or ISTH develop traceable international standards?

DR. ADCOCK-FUNK: So I think Mr. Gosselin's presentation listed the various resources available to obtain the calibrators. I can't speak to any efforts that I know of through the ISTH to develop some standards as to how calibrators should be manufactured. And I hope that I've made the point that that's very important moving forward.

DR. DOLLINS: All right. I think we're out of time for questions at this point. We're going to take a 20-minute break and we're going to rejoin at 11:10.

[WHEREUPON, the foregoing went off the record at 10:48 a.m., and went back

on the record at 11:12 a.m.]

DR. DOLLINS: All right. We're going to continue the discussion with our next speaker is Stephan Moll. He's going to talk about when are tests for DOAC measurement clinically needed and how to interpret and use the results. Dr. Moll received his medical degree from Freiburg University in Germany. He completed an internship and residency in internal medicine and hematology oncology fellowship program at Duke University Medical Center in North Carolina and a one-year clinical coagulation fellowship at the University of North Carolina, Chapel Hill.

Dr. Moll has been a faculty member at the University of North Carolina in the Department of Medicine and Division of Hematology Oncology for the last 15 years, now at the rank of a full professor. His clinical interest is coagulation and classical hematology with a particular focus on thrombosis and anticoagulation. Dr. Moll's research interests include clinical trials on new anticoagulants, better uses of established anticoagulants, anti-phospholipid antibody syndrome and post-thrombotic syndrome. He takes a special interest in clinical medical education of patients, the public and healthcare professionals. Thank you so much.

WHEN ARE TESTS FOR DOAC MEASUREMENT CLINICALLY NEEDED AND HOW TO INTERPRET AND USE THE RESULTS

DR. MOLL: Well, thank you very much, Claudia and the FDA as well. This is incredible, clinicians coming together with regulatory agencies and laboratory folks. I'm a clinician. I'm a clinical researcher. And I'm a coagulationist. And I've been a consultant to Portola, that makes a reversal agent for the anti-Xa agents. But I'm not on the speaker bureau of any company and these are my own slides.

First question may be why would we want to test -- and some of this has been addressed earlier. Number one is to detect whether patients on an anticoagulant drug, where their level is really above or below the therapeutic range, we'll call it the expected range, putting him or her at risk for bleeding, or for thrombosis if the level is too low. Another issue is, is there any residual drug effect prior to any major surgery or some interventions. Number third would be to check compliance. Question is what is the therapeutic range. And I do agree with the previous speakers. We do not know what the therapeutic range is. We have an on-therapy or an expected range, which we know from the mostly phase III clinical trials. But we don't really know what is a therapeutic range defined as the optimal range where bleeding and clotting is optimally balanced, a range that we can use for every individual.

Now, the timing of testing, obviously as a clinician, when we interpret these data and when we use them, the timing of the testing is important because the drugs, as you know, reach their peak within roughly one-and-a-half to three hours. That's a very brief peak. And then, the fall-off comes in. So it needs to be relatively accurately measured to get a peak. And there is variability between individual patients when the peak is reached so that the peak level can be difficult to interpret if you don't know exactly when to obtain it and it may not mean the same patient to patient. Then, we can certainly also obtain a trough level, which may be a little more reliable.

Now, once daily versus twice daily drugs, the same issues apply and that's documented here. But sometimes, and I'm referred to the ED colleague who spoke earlier, we don't know when the patient took the drug. And we may obtain a random level. And then, the interpretation may be quite difficult, certainly if someone has a new clot or a bleed on one of these anticoagulants and we get a level. We don't know how to interpret it because we don't

know when did the clot happen, when did the bleed happen and the level that we obtain at that particular time point, does that really predict anything. So we need to keep that one in mind.

So what results do we expect? What are the on-therapy ranges? And you're not able to read this, and this is fine. A junior colleague has put together the publications of the different drugs and I've listed them here, and what has been published as peak and trough levels, often from human volunteer studies of either the phase I, phase II clinical trials, less so from phase III clinical trials or post-marketing experience, the phase IV clinical trials. So there's a variety of different therapeutic or on-therapy ranges that fluctuate out there. The coagulation labs that do do the testing and report levels attempt to look at what patient population do they really refer to when they give you a therapeutic range.

Now, I've tried to maybe just put a few of these studies up here in a more readable form and these are the various drugs with the various doses. And I've listed here the trough and the peak levels. Let me just step around here, that I can see this same slide that you see -- with the peak and the trough values and then I've listed here the references. Now, there are -- and that point has been clearly made -- huge inter-individual ranges of variability that patients on the same dose may have almost a tenfold different level at peak or at trough. That's a well-known fact. And that's documented here, for example, for rivaroxaban, 20 mg once daily. The peak value is here listed as 103 to 660. The trough level's at 8.9 to 92 ng/mL.

Then, how does this compare to the studies -- let me just see here. That will be a little difficult since I don't have a clip-on microphone. Can you hear me in the last row? This is preferable for me. This is the lab report of the patient [off mic] and it's on rivaroxaban, 20 mg once daily. This is what the lab reports and this is LabCorp of America. And they have a range

given here from a publication. They have a peak and trough level. And this patient obtained the peak level which is listed at 460. And we'll get back to that in a little while. But I want to just show to you that the peak level corresponds relatively well with what's been published from real patient population at my institution and the trough level also corresponds relatively well, even though you see that, for example, the upper limit here of 660 is quite different to this 360.

So we need to keep in mind that the on-therapy ranges are not really well-defined like an INR 2.23 but rather have feathering of edges. Now, it's also interesting that there's not just inter-individual variability but also, in the same individual, if he or he gets tested at peak or at trough over several time points, that there's quite a bit of fluctuation in an individual patient. And Jeff White and his team showed that in this publication -- and I'm not showing the data per se, but he showed that people at one time point may have an on-therapy or expected range and then two months later at peak or trough have a subtherapeutic or over-therapeutic level. And the point there really is if we rely on one time point to determine, for example, in our obese patient, is the DOAC at the right level, a one time point test may not really predict what level the patient has in two months.

This has been one of the most striking publications and I'm really thrilled that Dr. Reilly is in the audience here. And I'm actually a little embarrassed to talk about these data here with you in the audience. But it's been an impressive paper because we all know these kind of graphs from the warfarin history. Down here, listed with warfarin, typically the INR, listed up here the events of either bleeding or clotting. And in this case right here, it's dabigatran trough concentrations from the phase III a-fib trial. And as you see with this graph right here, as the levels increase, the risk for thrombosis goes down, as expected. And then,

what you see is with increasing dabigatran levels, the risk for bleeding goes up, as expected. And it continues to go up quite significantly here, whereas the protective effect against thrombosis almost levels out.

And the point has been appropriately made that with increasing our high levels right here, that you don't gain much benefit but you lose a lot with increased bleeding risk. So the point has been made, and one of the audience members asked that earlier, what about routine monitoring in everybody at some point, just once to determine is this patient right up here in the very high range, where maybe the drug is way too high and poses a risk for bleeding? And maybe that would be appropriate. I think we are lacking the data as to what do you then really do. Do you dose reduce? I wouldn't quite because we don't know how the patient would behave. But maybe there would be a patient to say, look, the dabigatran or the DOAC is not the right drug in this situation. Let's maybe use warfarin.

But I think, Dr. Reilly -- and the FDA has your data too -- if you could do another analysis, and the FDA as well, just excluding, for example, the very high and the very low level patients, just looking at the ones in the mid-range to see how does that group compare to the warfarin patients and how about just the ones with the very high levels compared to the warfarin and how about these to get more details on how these patients behave would be very helpful for me because then I could say the patient with the very high level, I'd rather not treat with dabigatran. Now, the expected range here or the 10 to 90th percentile is this right here with a mean value right here. But you see there are a number of patients out here.

And really, as a clinician, I would say, well, the optimal range -- the therapeutic range seems to be kind of like here, where there is a good balance between risk of bleeding and thrombosis. But that needs to be defined further, what is really the therapeutic range. Now,

there are some patients -- and I don't -- won't go further into detail. But Dr. Reilly's paper has it listed, who are these people who are mostly up here and those are the elderly, women, of low body weight, like my mother, age 87 who weighs only 65 kilos who has a-fib. Maybe she would not be appropriate on this drug.

Or maybe in her, a drug level would be good to determine is she at this high level where she shouldn't be on the drug. Now, the good thing is that the edoxaban folks have also published very similar data. And these are the trough edoxaban levels from their a-fib trial. And there's a similar graph, even though it's a little more difficult, I must say, to draw a therapeutic range. Higher levels, more bleeding. Lower levels, better -- or higher levels, better thrombotic event -- thrombotic protection. And this is the intracranial bleeding risk. And it would be nice to be able to draw a box here where the therapeutic range is. But I find that a little more difficult.

These are the data on the rivaroxaban phase III clinical trial. So if there's any rivaroxaban representative, I would love to see -- and I think the scientific and clinical community has a right to get these data from the clinical trials about the drug levels correlating with efficacy and safety. And Dr. Reilly, that's why really I have the highest respect that you got your data published. This helps a lot in understanding this whole issue. I will show you the data on apixaban. And you may just study them for a second. Again, it would be nice to see additional data.

So my summary of the expected drug levels is the big differences between -- excuse me -- patients. There are also day-to-day or week-to-week, month-to-month fluctuations in a given patient. There's really limited knowledge of correlation of drug levels with efficacy and safety and there's a lot we need to learn. So what does the level really tell

you and what do you do with the level? I will answer that. But I want to make a point whom to test. The way I think about that is there are 10 patient groups.

Number one, the obese patient, body mass index [BMI] above 40, weight maybe more than 120 or 140 kilos. These patients have been enrolled into the clinical trials but often not the very extreme body weights. And the data, how they have been presented are typically body weight more than 120, patients on the DOACs did as well as warfarin and as safe. But I wonder how about the real extreme body weights, BMI above 40, 45, the ones we see. I think further sub-analyses are really needed on that. There's some suggestion in obesity their half-life gets shorter. The drug peak and trough levels get lower and maybe the DOACs are not the appropriate one for the extreme body weights.

Similarly, the underweight patients, more data are needed. Interfering medications, now, many are metabolized through the same pathways in the liver and there's often a comment, well, there's a potential small expectation, large expectation. We typically list the anti-seizure medications, HIV medications, antifungal medications. But what do these interferences really mean for clinical outcomes. And we often don't know that.

The fragile elderly, the dabigatran study showed that the fragile elderly, the low body weight, elderly women have significantly higher levels. So maybe in those people, testing is appropriate. Also, apixaban dosing has to do with age and there's a dose reduction if there's some renal impairment and elderly age. Sometimes borderline renal function may be a reason to test. Typically, we should avoid the DOACs in these patients. But sometimes you don't have a different choice. I clearly want to know the patient who clots in spite of being on a DOAC. What is their drug level and why did they clot? Were they subtherapeutic? Or the patient has a major bleeding on DOAC. Were they for some reason completely overdosed?

And then, that point has been very well made by our ED colleague, assessing the disappearance of a DOAC prior to surgery. Is it safe? Is it gone? Is most of it gone? Dosing of reversal agents. We now have a reversal agent for dabigatran available. The one for the anti-Xa agents is in phase III for its fourth clinical trial, will possibly probably likely get FDA approval in the next few months. Now, in these studies, these drugs are not dosed based on coagulation tests. But you wonder, a number of patients -- and Adam, you knew -- I think it's about 10 percent of the patients in the dabigatran reversal study, *New England Journal*. They didn't have significant circulating drug levels. Yet, they got the reversal agent, at the cost of it, the complexity and potential side effects down the line. So maybe you could dose those more appropriately if you had a clotting test. And then, checking for compliance may be an issue for some people, just to have a test, yes, these people are indeed taking the drug, not necessarily quantitatively.

So the drug interactions, I'm not going to say too much except I talk a lot to our oncology colleagues. In DVT and PE associated with cancer, low molecular weight heparin is the gold standard, based on our guidelines. But they are cumbersome and expensive. So the DOACs are very attractive. But I'm concerned about them in some degree because there are many chemotherapeutic drugs that get metabolized through similar pathways and either the DOAC increases the chemotherapeutic side effect profile or the chemotherapeutic drug decreases the efficacy of the DOAC. So it could go either way. And I don't know how to weigh that clinically.

Patient examples. So this is a patient I tested and I see patients with DVT/PE. I do not like to use the DOACs in obese patients above a body weight of 40 based on the limited data available, even though the package insert says for all of them can be used in obese

patients. But again, limited data in the phase III clinical trials. And this was a very obese patient with a BMI above 40. And I got a peak and a trough level. This patient was on rivaroxaban and I did not want to treat him. I recommended warfarin. He insisted on a DOAC.

I said we are only going to use that if we do a peak and trough level. And I wanted to make sure that he is in the expected range. He had a peak level of 460 and that's within or actually above the expected range. So I said, I'm okay with it. In you, it's not, as I expected it might have been, low. And I obtained similarly a trough level in him which was easy. He came in the morning, blood draw trough, took the pill, stuck around for two-and-a-half hours and the peak level. And that one was 37.8, within the expected range. And I told the patient, I'm okay with you staying on the DOAC and not being on warfarin. That was reassuring.

This is a patient I did not see in person, but I got a call about from the community. Elderly patient, 86, medically fragile, low body weight, 86-year-old. Somebody had put the patient on apixaban and it was 5 mg b.i.d. for a-fib. And the physician said, I'm concerned about her. She's so fragile. Is that really appropriate or should I put her on a lower dose or warfarin? I said, why don't we obtain the level. And the level was 820, with the expected range being up to 130. And that's awfully high. And I said to him, I'm also concerned about the patient. So I thought in that situation helped and I'm not sure what drug the patient was put on, but I think warfarin.

This is a third example. Patient has triple antiphospholipid antibody positivity. Those people really like to clot. Now, the patient's on long-term anticoagulation with apixaban. I'm following the patient in my clinic. And in spite of apixaban 5 b.i.d., he developed new thrombotic skin ulcers, which happen in APLA [antiphopholipid lupus anticoagulant] syndrome. I wondered: why does he clot in spite of this being on an anticoagulant? So I obtained the level

and this was four-and-a-half hours after the last dose. So it's not a peak. It's not a real trough. And it was 159. And here the therapeutic range is not provided. But that's -- it's not provided because maybe, that, you didn't have it. But that is a relatively expected range, not at peak, but beyond peak. So I thought he clotted in spite of appropriate anticoagulation. So at that point, I switched him over and added aspirin to his management. So there was a change in management.

This is example number four. A patient on rivaroxaban needs antifungal therapy for several months to be given for toenail infection. In my mind, interferences with rivaroxaban, antifungals, anti-seizure, HIV. So I did a level before the antifungal therapy. And that's a peak level, looked okay. He started the antifungal. Peak level again, 210. So not that much difference. I said, I'm okay with you being on both drugs. That was reassuring.

Number five, patient is on rivaroxaban and fish oil, which I did not prescribe. And now, she presents with mouth and tongue bleeding and had a big bruise and nothing else really was different. And she wondered what's going on and it was pretty significant bleeding. And I wondered what was going on. So I asked her to come back. We stopped rivaroxaban for a few days. Then we restarted at the 20 once a day. She came back for a peak level. It was 39.1 at peak. Whoa, that's pretty low and I'm not sure I believe it. Maybe she -- I don't know what she does. But it was not extremely high. So it's not that, now I'm thinking that the rivaroxaban caused her bleeding.

But I must say I did not understand this level, which is a point I also want to make. Sometimes we get these levels and we don't quite know what to do with them. So our experience at UNC and we had a junior hematology fellow review the tests that have been ordered at my institution. And this is since -- this is all tests that were ordered on dabigatran,

rivaroxaban and apixaban levels between -- these are three years. There were only 28 patients that were tested and only 48 levels were sent. So it's a really low volume. And most of these tests were ordered by me and a few were ordered longitudinally on a few patients who had been on dabigatran waiting on surgery.

But the point is the volume at my institution, academic institution, is not high. Now, we do use DOACs quite a bit in our community, in the UNC Healthcare System, with all three of them. But testing is just not done very often, even though it's available. We've established the ecarin clotting time in our lab. The pathologists have complained that nobody orders it, that it's not worth having it. We send our anti-Xa levels out and we get results back typically within a few days. That's sufficient for me with my indications, the antifungal, the obesity. It will not be sufficient for our ED physician, who needs it -- who may need it immediately.

So the clinical questions or the clinicians' questions that really light up for me is should I dose adjust the DOAC if the level is too high or too low. And our FDA colleague asked me that earlier and my answer was no, I would not dose reduce or dose increase based on the level because we're lacking clinical data as to what that really means. For me, the consequence of a low or a high level is this drug is not appropriate. I would use warfarin or this patient may have bled because the level is very high or this, the DOAC, can be used in spite of the obesity because the levels are in the expected range. But I would not go from a rivaroxaban 20 down to 15 or 10 based on the level or increase the apixaban from whatever the patient is on to the next higher or lower dose.

And then, in whom should I consider test. Well, I wouldn't say should. In whom do I test? I showed you the list of 10 patients where I would consider testing every so often.

From the lab point of view, the request I would have from the referral labs, the people who do the test is to provide the name of the type of assay done. We get these reports back with an anti-Xa level and we don't quite know what it was. We don't know if was it a mass spectrometry test? Was it a true anti-Xa assay? It would be nice as a coagulation-interested person to have more details. Providing reference ranges is good because many clinicians don't know what the ranges are. But I would like to see reference ranges from patient populations and not from human volunteer, phase I or II studies.

And then, from medical societies, I would ask for some guidance on who should be considered for testing, what levels to look for and what to do with the results. And I realize there are relatively few data. But people out there are wondering should we test and what do we do. They get results maybe from outside labs and I think some guidance is needed. And then, well, I didn't put that in here because I was afraid they would kick me out together with Bob Gosselin. From the FDA, I would like, since there is discussion about the testing, to speed up the discussion. I think this is a great event. I'm thrilled to be here and I look forward to the discussion the whole day. Thank you very much.

[Applause.]

DR. DOLLINS: So I'm not going to introduce our next speaker again because I've already done so this morning. Our next speaker is Adam Cuker. He's going to be talking about the measurement of DOACs, suggestions and guidance statements.

MEASUREMENT OF THE DOACs: SUGGESTIONS AND GUIDANCE STATEMENTS

DR. CUKER: All right. Thanks again, Claudia. You have to hear from me one more time and this will hopefully be sort of a summary of the discussion up until now. You've seen these disclosures already. I haven't acquired any new ones in the last hour or two. Here's

the outline for the next 10 minutes. I want to briefly review with you professional society recommendations, especially because Dr. Moll just told us that it would be useful to have guidance, talk about where I think there is potential disagreement with those recommendations and then conclude with my suggestions.

So this is a busy table. You can see that there's really an alphabet soup of professional societies that have provided guidance on measurement of dabigatran and the factor Xa inhibitors. And the guidance that they provided can really be put into two categories. They provide guidance on screening, and I take screening to mean does this patient have levels of drug in their circulation that put them at increased risk for bleeding or are they okay to go to surgery. And then, they also make recommendations for quantification. And so, I have separated these, the guidance from the professional societies into green and red. Hopefully there aren't too many color blind people in the audience. The green means that I agree with the suggestions. The red means that I think the suggestions may be misguided.

And so, the disagreement really focuses on screening for dabigatran. There are a lot of societies that recommend using the APTT for screening and there are a lot of societies that recommend the PT for screening of the factor Xa inhibitors. Dr. Adcock did a very nice job, I think, summarizing why perhaps those are not appropriate suggestions. But I just want to kind of highlight some of the data. Some of this you've already seen before. So this is a figure that shows using a number of different PTT reagents, that curvilinear relationship between the PTT and dabigatran levels. I'll remind you that the median dabigatran trough is around 90 ng/mL.

And so, if you sort of draw a line at 90, you see that brings you to a ratio, a PTT ratio of about 1.5. But there are plenty of patients that trough, depending on the reagent you use an, and Dr. Adcock showed us this, that will have normal PTT. So they're in the on-therapy

range. They've taken their dabigatran 12 hours before. And nevertheless, they have a normal PTT. And in fact, in the study of ex vivo samples that Dr. Adcock and Gosselin and Moll conducted with their colleagues, they found that 18 percent of the patients they studied on dabigatran had a normal PTT at trough. So I do not think that a normal PTT can be assumed to exclude on-therapy levels of dabigatran.

What about the PT and factor Xa inhibitors? So here we have a figure showing the relationship between the INR and rivaroxaban levels. You can see the very different sensitivity to rivaroxaban among the different thromboplastin reagents. But again, a reminder that the median trough for rivaroxaban level is around 26 ng/mL. If you draw your line at 26, you see that the PTT or the INR is about normal or just a little bit above normal. And so, the lesson here -- and by the way, there are very similar levels available for edoxaban -- is that a normal PTT does not exclude on-therapy levels of rivaroxaban or edoxaban.

The same authors performed an ex vivo study of patients taking rivaroxaban 20 mg a day and 59 percent of those patients had a normal PT at trough. So clearly a normal PT cannot be relied on to exclude on-therapy levels. I think we've heard the situation is even worse with apixaban for the PT, and the PTT for that matter, are much less sensitive to apixaban than they are even to rivaroxaban and edoxaban. You can see the slope of this curve is very flat. And so, if you are looking for trough apixaban levels, the median there is around 100 ng/mL. That's going to give you a normal INR. And in fact, even if you look at very high levels, well above the median peak, you're talking about only marginally elevated INRs. And so, I think that depending on the thromboplastin reagent that the lab uses, I think it's fair to say that a normal PT may not exclude not only on-therapy but even above on-therapy levels of apixaban. And so, clearly it's not an appropriate screening test, as some of the professional

societies recommended.

And so, now I want to show you a table summarizing my suggestions for measurement of the DOACs. And I think that this really comes down to three questions:

A. What DOAC are you trying to measure;

B. what's the indication for testing; and

C. what assays do you have available to you,

because I think you've probably all recognized, if you weren't aware already, that one of the big problems here is that the best tests for measuring DOACs are the least widely available tests and that the tests that we all have access to, the PT and the PTT, have important limitations.

So if you are lucky enough to have specialized assays available to you, and your goal is to exclude on-therapy drug levels because the patient needs to go to the OR, the thrombin time can be very helpful. If the patient is on dabigatran, a normal thrombin time, as we heard from Bob Gosselin, excludes clinically relevant or on-therapy levels of dabigatran. Similarly, the anti-Xa assay, absent anti-Xa activity will exclude clinically relevant levels of a factor Xa inhibitor. If your goal is to measure levels, then for the dabigatran, you can use the dilute thrombin time, the ecarin chromogenic assay or the ecarin clotting time, for factor Xa inhibitors, the anti-Xa assay. And if your goal is to determine whether above on-therapy levels are present, I would suggest the same assays.

So what if you don't have specialized assays available to you? And that is the case for the vast majority of clinicians the vast majority of the time. What can you do? So if the patient is on dabigatran and your goal is to exclude on-therapy drug levels, hopefully you do have a thrombin time available to you. If your goal is to determine whether above on-therapy levels are present, you can perform a PTT. And I think that, generally speaking, a normal PTT

will exclude above on-therapy levels. But it may, as I showed you, not necessarily exclude on-therapy levels of dabigatran.

What about if you don't have specialized assays available to you and you -- and the patient is on a factor Xa inhibitor? Well, if your goal is to exclude on-therapy drug levels, you are out of luck because, as we discussed, neither the PT nor the PTT, when they are normal, exclude the possibility of on-therapy levels. If your goal is to determine whether above on-therapy levels are present, the PT can be useful, depending on the sensitivity of the reagent you use for patients on rivaroxaban and edoxaban, a prolonged PT suggests that on-therapy or above on-therapy levels are present. A normal PT probably excludes above on-therapy levels but not necessarily on-therapy levels. With apixaban, you can use the PTT. But there are important limitations. If it's prolonged, that probably means that there are on-therapy or above on-therapy levels present. But if it is normal, depending on the sensitivity of the lab's reagent, it may not exclude on-therapy or even above on-therapy levels.

So my final slide. Selection of the optimal assay depends on the drug, the indication for measurement and what you have available to you. If the patient is on dabigatran, a normal thrombin time excludes clinically relevant levels of drug. A dilute thrombin time or ecarin clotting time or ecarin chromogenic assay can be used for quantification across a broad range of levels. And a normal PTT excludes excess levels but probably not on-therapy levels. If you are measuring a patient on factor Xa inhibitors, a normal -- or absent anti-Xa activity excludes clinically relevant levels. The anti-Xa can be used for quantitation across a broad range of levels. And a normal PT probably excludes excess levels of rivaroxaban and edoxaban but not apixaban nor does it exclude on-therapy levels of any of these drugs. Thank you.

[Applause.]

DR. DOLLINS: It's my pleasure to introduce our CDER colleagues. We're going to have two speakers in the next session. So I'm just going to go ahead and introduce both of them. The first speaker is going to be Dr. Jeff Florian. He is a pharmacometrics team leader in the U.S. Food and Drug Administration, in the Office of Clinical Pharmacology, Division of Pharmacometrics. Dr. Florian received his Ph.D. in chemical engineering from the University of Pittsburgh, then accepted a postdoctoral fellowship with the FDA where he worked on their QT [interval]trial design and viral dynamic modeling for hepatitis C. He joined the Division of Pharmacometrics in 2010, where he has worked for the last five years. His primary responsibilities include assessing benefit and risk relationships to aid regulatory decisions-making for anti-viral, anti-infective, cardiovascular, renal and dermatology products.

So his talk is going to be followed by Martin Rose. Dr. Rose is currently a medical officer and clinical team leader in the Division of Cardiovascular and Renal Products in FDA's Center for Drug Evaluation and Research, where he has worked for 10 years. He received an M.D. degree from the University of California, San Francisco and a law degree from the University of California, Berkeley. He is trained in internal medicine and endocrinology. He has been involved with pharmaceutical regulation and development since 1979.

NEW ORAL ANTICOAGULANTS PHARMACOKINETICS, PHARMACODYNAMICS AND EXPOSURE-RESPONSE

DR. FLORIAN: Okay. Thank you for that introduction. And we're not switching products. We just didn't get the correct memo on the acronym to use [NOAG versus DOAG]. We can adjust this going forward. So an outline of what I'll cover, overview of some of the more recently approved oral anticoagulants, pharmacokinetics and pharmacodynamic properties -- or really, just pharmacokinetic properties of the drugs, sort of mirror what's

already been covered by Dr. Moll, as well as looking at some of the phase III trials for atrial fibrillation, including design, data collection, dose response, observations. And after that, I'll turn the talk over to Dr. Rose who will say what can be done with this information. So he gets the easy job.

Four oral anticoagulantshave recently been approved for the prevention of stroke, systemic embolism in patients with non-valvular atrial fibrillation. Just looking back, vitamin K antagonist approved warfarin, some of the difficulties with this, was monitoring highly variable PK/PD. And when you take a close look at the data compared to these other drugs, an increased risk of hemorrhagic stroke is seen. So two classes of oral anticoagulants that have been recently approved, one direct thrombin inhibitor, dabigatran, and then three direct factor Xa inhibitors, apixaban, rivaroxaban and edoxaban. And just one point to keep in mind, and this has been covered by a number of speakers already today, all drugs in this class have an on-target balance between efficacy and safety. Drugs administered -- and what we're going to focus on -- prevent stroke. But if too high levels are achieved, this will increase a risk of bleeding.

So this table summarizes some of the PK characteristics of the four drugs. There are a few items on this table that I'd like to highlight. First and foremost, dabigatran's bioavailability, somewhere between 3 to 7 percent. This is likely one of the factors that contributes to the high subject variability that is observed with this drug. As has already been mentioned by a number of speakers, Tmax for these drugs is somewhere between one and four hours. If one is looking for a peak, somewhere within that time window is where peak drug exposures are going to be observed. One item here, looking at a predominate route of elimination, there are two drugs where renal is 50 percent or more the contribution, dabigatran

and edoxaban. If we take a look at what we see from -- so these, between subject variability and within subject variability numbers are pulled from dedicated studies in healthy volunteers.

What we saw from these results, dabigatran has a fairly substantial variability, 40 to 60 percent between subject, 40 percent within subject variability. Though after some of the discussions and slides that have been presented, we'll take a closer look at the rivaroxaban numbers. One last item to look at is accumulation. So this isn't highlighted above, but elimination, half-life for all of these drugs is somewhere between 12 to 24 hours. It's a half-life that would typically represent b.i.d. dosing. Of the four drugs, only two of them are administered b.i.d., dabigatran and apixaban. Those also have about doubling exposure at steady-state, whereas the other two have very minimum accumulation at steady-state.

Briefly now, I'll go over some of the characteristics of phase III studies with these drugs in the atrial fibrillation population. So just in general, all of these trials have a large number of subjects, 10,000-plus patients with an on-treatment time approaching three years. Active control arm in all of the trials is warfarin. A subset of the trials had prospective dose adjustments based on intrinsic/extrinsic factors, what might be renal or creatinine clearance, combination of multiple factors, some drug-drug interactions. In all of these trials, there is one or two active treatment arms.

Of note, all the sponsors were advised to take forward multiple doses into phase III based on phase II study results. And in the trials' PK/PD sampling was highly [variable]-- the percentage of subjects' samples collected was highly variable, ranging from 0 to 90 percent of the population. Again, all subjects or all sponsors requested sampling in a majority of the population. And one thing to note, and we'll see this as we're going through some of the slides, data collection from these trials limits interpretation of the study results, one's ability to

identify exposure response analyses from the patient populations and then in turn derive assessments of benefit-risk.

So briefly, the dabigatran study design was a non-inferiority study, open label with respect to warfarin and blinded with respect to the two doses, 110 and 150 mg b.i.d. This trial had approximately 18,000 subjects randomized one to one to one. So you have 6,000 subjects per treatment arm. Primary endpoint was stroke and systemic embolic events. Population, non-valvular a-fib at high risk of stroke, including both warfarin naïve and non-naïve patients. Now, in this study, steady-state [inaudible] concentrations were obtained in 8,400 patients. So about 60 to70 percent of the patients on dabigatran. And these were collected at the month one visit. Shown here are hazard ratio plots for the two dose. These are hazard ratios relative to warfarin and showing dabigatran 110, dabigatran 150, stroke/FCE on the left, major bleed on the right.

One thing that was noteworthy from this is the two doses that were studied are very similar in terms of exposure, only 35 percent difference. But there ended up being enough difference in exposure to see a difference in the effect between the doses. So in dabigatran 110 non-inferiority, efficacy fewer bleeds. Dabigatran 150, it had superior efficacy but this came with the cost of what was then similar bleeds. This gives us confidence, the dose response relationship, that we can take a look at the exposure data to try and derive more information about the relationship.

So this is a slightly different figure than Reilly, whose work is excellent and will get a number of shout-outs from the podium today. But this is using the same information but trying to then just plot ischemic stokes and life-threatening bleeds. Ischemic strokes are shown here on the blue and life-threatening bleeds shown in red. The top of the plot hase the two

doses in what is the -- I believe this is the 5th to 95th percentiles for those two doses. As one can see, for those two treatment arms studied, substantial span in terms of the dabigatran exposures. One thing to note, it's very tempting to take a look at these figures and you're drawn to where the lines intersect. But where they intersect might not necessarily be where the ideal place for a therapeutic window would be. It will ultimately come down to what is a tradeoff between one event and another, maybe a whole clustering of events beyond just these two. I know from experience, getting two or more people in the room, it's very difficult to get any individuals to agree on just what is the best balance between these events. This is a similar relationship based on warfarin. And this is just highlighting dabigatran. They collected enough information where identification of these relationships was feasible. With that, I want to go to another example, edoxaban. It was also a non-inferiority trial, blinded with respect to warfarin and blinded with respect to the two doses, 30 and 60 mg. This was also a very large trial, 21,000 subjects, about 7,000 per arm with the same endpoint in population. Steady-state and post-dose edoxaban concentrations were obtained in 13,000 subjects. So they had PK sampling in about 90 percent of the population. This represented the most PK data collected of any of the four trials. And this, they collected samples at month three and the month nine visit. These are hazard ratio plots for stroke/FCE, ischemic stroke and major bleed for the two doses. This is now a twofold difference in dose, but again, similar to what was discussed with dabigatran.

With these two doses, we're able to see a dose response relationship for stroke where the lower dose has a higher hazard ratio compared to warfarin than what is seen for the high dose. Likewise, if one goes down to bleeding, hazard ratio for the high dose relative to warfarin is higher than what is observed for the low dose. So we're seeing the same tradeoff between doses. But we have a dose response relationship, and this gives us confidence to

move forward with the exposure response relationship analysis. Before getting to that, one item we'd like to highlight from edoxaban is that patients with normal renal function, it was observed that there was a higher risk for stroke relative to warfarin.

So shown here, stroke, FCE and major bleeds for the three renal function groups. In this trial, moderate, unlike had a dose reduction from 60 mg to 30 mg. This differed from dabigatran, where moderates did not receive any dose reduction. And what we see in terms of a trend, as one goes from normal -- a patient's normal renal function, who would have the lowest exposures, to mild, is a decrease. And then, the V comesback, moderates. They have exposure relatively similar to normal. This V-shaped relationship is then inverted when we take a look at major bleeds. It's the same concept that we've covered in previous slides where, as one goes down in exposure, an increase -- or one increases in exposure an increase in bleeding rate.

All of this information was then put together for an exposure response analysis similar to what was done for dabigatran. Same two events are plotted here, ischemic stroke and life-threatening bleeds. The two doses, 5th to 95th ranges, are plotted in black lines at the top of the figure. One can see that there was not as much variability with edoxaban as was seen with dabigatran. But there was an ability with the information available to put together relationships for the endpoints. Also able to put together relationships for the overall primary endpoint, stroke FCE or different bleeding such as major bleed.

Dr. Moll already covered the rivaroxaban and apixaban results. So I'll just touch on them briefly. They were sizable trials that evaluated only one dose level. Limited PK sampling ranging somewhere between 0 to 25, 30 percent. Now, with the information that was collected, it is possible to identify relationships between drug exposure or coagulation markers

and major bleeding rate. But there was insufficient information collected during these trials to characterize the relationships between drug levels and efficacy.

So just a few summarizing thoughts before I pass it off to Dr. Rose. Multiple drugs have been evaluated -- multiple DOACs have been evaluated and approved over the past five years. The major driver for development was for therapeutics without a need for routine monitoring. All of these drugs though continue to have the same balance between efficacy and safety events that were seen from warfarin. But our ability to understand those relationships are impacted by dose ranging from phase II to phase III, the populations evaluated, metrics used for the dose selection, number of doses carried forward in the phase III. This might be one of the most important elements. An then, certainly also the sampling, which handcuffs So as one final note, all these trials have passed non-inferiority. But does the information from them really reflect how to optimally use them? With that, I'd like to turn the talk over to Dr. Rose.

[Applause.]

DR. ROSE: Thank you. It's an honor to be invited here and serve on this distinguished panel. I often follow Jeff. His group is responsible for creating those very nice concentration response curves. And we use them a lot in our reviews of these drugs. I'd like to start with talking about warfarin, which is the prototype oral anticoagulant that's been used in atrial fibrillation and for other purposes. Many of you have seen this plot. It's from the Consensus Practice Guidelines for the management of patients with atrial fibrillation from 2006. The left-hand axis is the odds ratio, a measure of risk and the plot shows the relationship between the risk of ischemic stroke in the solid line and intracranial bleeding in the dotted line. And what you can see is that the -- that ischemic stroke is heavily dependent on the INR. The risk of ischemic stroke drops dramatically between an INR of 1 and 2 and then stays pretty

constant as INR goes up. The risk of intracranial bleeding starts out low and goes up. It's not -- it doesn't seem to be markedly elevated at 3. But by an INR of 4, it is definitely higher and goes -- and continues to increase as INR goes out to 7.

This is for patients with atrial fibrillation. This doesn't include patients with mechanical heart valves. But it shows you how we got to the therapeutic range that we use. It seems pretty clear that going beyond 3 buys you nothing in terms of preventing strokes but costs you in terms of intracranial bleeding. Ischemic strokes in patients with atrial fibrillation are often very severe. They're devastating to the patient, often leave them with residual disability, sometimes fatal. Intracranial bleeds are likewise terrible events. So going beyond 3 seems pointless for these patients.

Well, let's move on to the NOACs. What's so unusual? Well, they're unusual because they're not like warfarin. There are steep dose response relationships and there are serious consequences of doses that are either too high or too low. And these consequences may occur without warning and they may occur after some time on treatment. So you can't really titrate patients. You have to know where you are in terms of your concentration or your effect on the coagulation system. Another problem is that blood levels are variable. They vary by renal function, especially for the two drugs we'll be talking about in a minute. There's drug-drug interactions. And there is unexplained intra-patient variability that's somewhat worse with dabigatran than the other drugs. And all of these create problems when dose response relationships are steep and there's a real price to pay if you're out of range.

So where do -- how do we use this information? Well, we do have a lot of data from the phase III studies of dabigatran and edoxaban. And those data can be used to inform next steps. Each study included two doses and created a wide range of blood concentrations

and anticoagulant effects. And we were able to construct -- we and the sponsor were able to construct concentration response curves for both stroke and bleeding.

Before we go on to look at those, I'd like to bring in the concept of variability. There are two kinds of variability that we need to think about. One is between subject variability, the kind that you expect. And the other is within subject variability, which sometimes is unexplained. As I said, this is more of a problem with dabigatran than the other drugs. So within subject variability is low and you have low between subject variability, you can probably get away with a fixed dose for most patients, maybe all patients, at least all adults. If intra-patient -- excuse me, if inter-patient, between patient variability is higher, you'll probably need to adjust the dose for some patients. If you know what factors, such as renal function or hepatic function or age, affect concentrations, you can use those factors. If you can't really predict it using those -- predict concentrations using those factors, you can take a measurement, if intra-subject variability is low, you only need one measurement and that could be a pharmacodynamic measurement like a clotting test or it could be a pharmacokinetic membership.

Well, things change if intra-subject variability is high. The scenario of high intra-patient variability and low between subject variability probably doesn't exist. But if between subject variability is high, you will probably need to adjust doses. You could again do that on the basis of factors such as renal function. Or you can do therapeutic drug monitoring using either pharmacodynamic or pharmacokinetic testing. Of course, that's what we do with warfarin. We use pharmacodynamic testing. However, variability may be so high that it's not practical to do this. And I would say if the major selling point of your drug is that you don't need to monitor, then there is a serious problem in taking multiple drug measurements just to

know where -- excuse me, just to know what your concentration or your range of concentrations really is.

Okay. So this is the plot from Reilly that you've already seen. The red line represents the rate of ischemic stroke. Excuse me. The red line represents the rate of ISTH major bleeding. I'll come back to that in a second. The blue line represents the risk if ischemic stroke. And systemic embolism. But about 90 percent of those events are ischemic stroke. ISTH major bleeding is often used in these studies. It is not -- it is not necessarily fatal bleeding. It could be. It is not a hemorrhagic stroke. But it could be. It's basically bleeding of two units that requires a two-unit transfusion or more or it's bleeding into a critical space such as the eye or the brain. So it would include all intra-cranial bleeds, bleeds into the eye, bleeds into joints and a lot of two-unit bleeds. So it's critical to remember that.

So what you see is as dabigatran concentration increases, the rate of ischemic stroke decreases, more or less flattens out. It never entirely flattens out. And of course you see just the opposite for bleeding. Crosses at around 60 or 70. So at this point, one is getting one bleed for every ischemic stroke. That seems like a pretty -- that seems fair, except that these are two-unit bleeds. These are not life-threatening bleeds. Most of these are bleeds that results in a hospitalization where the patient leaves alive. That's not the same as having a devastating stroke.

And this room full of people could come up with multiple ways of weighting those. But within cardiorenal drugs, we think that an ischemic stroke is much more serious than an ISTH major bleed or most ISTH major bleeds and that most people would trade several ISTH major bleeds for one of these strokes, which means that you probably want to be out here rather than back here. You certainly don't want to be back here. That would be silly. But you

might -- you probably want to be to the right to this point, if you know where that -- if you feel confident you know where that point is. Obviously there's a confidence interval around both of these curves. So we're not saying we know where the right target range is. But it's somewhere in this area where the arrows are.

So how do we keep patients in the target range of exposure? One can use dose adjustment for patient factors. You've already heard this. You can use dose adjustments based on pharmacodynamics or pharmacokinetic measurements or you can combine the two approaches if there's a class of patients where the use of factors such as renal function won't work very well. You might want to take a measurement. So this chart is for dabigatran and this is in patients with creatinine clearance of 50 to 80 mL/min. So that's mildly impaired renal function.

And as you can see, most of -- and there is a putative therapeutic range here of 50 to 150 ng/mL. I'm not saying that's what we think the right therapeutic range is. But this is just for the sake of this slide. So you can see that most of the measurements fall into this therapeutic range. There's a small tail at the low end and a larger tail at this end. If you had no way of knowing who these patients were, you might want to get a measurement in patients with creatinine clearance of 50 to 80, get a blood sample and test it for either pharmacokinetics or pharmacodynamics, if you could be confident you know what the result means. I'm not sure that you can. You might need multiple measurements.

So in summary, getting the dose of anticoagulants right is important but often not easy. It's easier with the NOACs or DOACs than it is with warfarin. But it's still sometimes not easy. Available concentration response data for dabigatran and edoxaban could be used as a basis for dose adjustment schemes based on either renal function or drug concentrations.

And you could also do pharmacodynamic-based dosing if you have the right test of clotting. But it's important to consider the patient factors that contribute to drug variability and what could be the practical limitations based on within patient variability. Thank you.

[Applause.]

QUESTIONS FOR SPEAKERS

DR. DOLLINS: Thank you. We're running behind time. We have time for maybe one question before we adjourn to lunch.

DR. MOLL: Can I ask Dr. Adcock, how widely available is the thrombin clot time in the United States laboratory? Dr. Cuker mentioned it's probably widely available. What does NASCOLA and the CAP service show?

DR. ADCOCK-FUNK: [Off mic.]

DR. MOLL: So what would be your guess? Is it in 5 percent of labs?

DR. ADCOCK-FUNK: [Off mic.}

DR. DOLLINS: All right. Just in the interest of time, I guess we're going to adjourn until 1 pm. So I'll see you then.

[WHEREUPON, the foregoing went off the record at 12:19 p.m., and went back on the record at p.m.]

MS. GUITY: We're going to get started with our p.m. session. So our p.m. session will cover the commercial development and the FDA perspective. My name is Niquiche Guity. I am a scientific reviewer in the Division of Immunology and Hematology Devices. I was also one of the organizers for this workshop. And our first talk, to start our commercial development session will be done by Bryan Laulicht. Bryan Laulicht is a founder and senior executive vice president of research at Perosphere, located in Danbury, Connecticut. At

Perosphere, Dr. Laulicht and his colleagues are working on the investigational DOAC and low molecular weight heparin antidote Aripazine, also known as PER977, and a point-of-care coagulometer for monitoring the DOACs, heparin and warfarin. Dr. Laulicht received a B.A. in biophysics from Columbia University, a Ph.D. in medical science from Brown University and was a postdoctoral fellow in Harvard and MIT Health Sciences and Technology program before joining Perosphere. His talk will be in vitro diagnostic testing for direct oral anticoagulants. Please welcome Dr. Laulicht.

COMMERCIAL DEVELOPMENT

IN VITRO DIAGNOSTIC TESTING FOR DOACs

DR. LAULICHT: Thank you, Niquiche, for the introduction that contained my disclosure, which is that I'm an employee of Perosphere. And thank you to the organizers for inviting us to this really very informative workshop and for giving Perosphere a platform to discuss some of our newer developments on testing for the DOACs.

So Perosphere's interest in coagulation testing really stemmed from our drug development program. We have an investigational compound, ciraparantag, currently in phase II, which is a reversal agent for the Xa and IIa DOACs, low molecular weight heparins, unfractionated heparins and fondaparinux, but not warfarin. Our initial clinical data, our first in-human trial was run looking at reversal of single 60 mg oral dose edoxaban. We looked at safety of ciraparantag alone and efficacy. And what we saw was a complete and sustained reversal of edoxaban out through 24 hours that we monitored. In our phase II trials, we've looked at steady-state edoxaban reversal where, once again, we saw complete and sustained reversal at steady-state. And then, we re-anticoagulated at the next scheduled dose and re-reversed, showing no tachyphylaxis or any interference with re-anticoagulation at the next

scheduled time point.

We've also looked at reversal of 1.5 mg/kg subq low molecular weight heparin, enoxaparin specifically, and one again showed complete and sustained reversal. In all of these trials, we were always very vigilant to look for procoagulant signals. So we tested for D-dimer and FI/II as well as TFPI [tissue factor pathway inhibitor] and in all cases we saw no differences from the placebo group, so no procoagulant signals. But our real regulatory and technical challenge came in on the biomarker side, which is that ciraparantag, as pictured, has cationic positive charge. And as a result, it binds to and interferes with the standard point-of-care PT and APTT activators, celite and kaolin. As well, it also binds to and interferes with citrate, EDTA, oxalate and heparin. And so, when making plasma, you can no longer measure the effects of ciraparantag. Therefore, all plasma-based assays are also a challenge for ciraparantag.

So the situation that Perosphere was particularly focused on was really the emergency bleed situation. A patient comes in with an emergency bleed, maybe with altered mental status or possibly even nonresponsive. And you may not know which DOAC they're on. And you'd really like to be able to know, at the point of care, whether or not this patient is anticoagulated and then, hopefully, if you could use ciraparantag and administer it intravenously, you might be able to see them return back to baseline and you'd really like to have, again, a point-of-care measurement for making that determination before, for example, you sent them for a procedure.

So our major considerations were time. We were concerned that laboratory turnaround time may exceed the clinical decision-making window. So we wanted at least the availability to use our test at the point of care. We were also concerned about the typically narrow spectrum associated with tests. We really wanted a broad spectrum solution,

something that was sensitive to all of the Xa and IIa DOACs at the same time, as well as ideally low molecular weight heparin, which has been a challenge for assays in the past and the other heparins. So we really wanted to address the situation where you don't know which DOAC the patient is on. But you still want to know if they're anticoagulated.

So our goal was to develop a broad spectrum, point-of-care, chemical reagent-free -- and that's really from the ciraparantag perspective to avoid interference -- as well as biological reagent-free, and that's to avoid batch-to-batch reproducibility issues. And we wanted this assay to be a clotting assay sensitive to anticoagulation by all of the DOACs and heparins and that it wouldn't interfere with measuring the activity of our reversal agent, ciraparantag. So our solution is Perosphere's broad spectrum point-of-care coagulometer. Our commercial prototype is pictured here. This point-of-care device is a broad spectrum measure of anticoagulants, as well as their reversal, for both IIa and Xa DOACs, low molecular weight heparin, unfractionated heparin, fondaparinux, as well as warfarin.

Because it's broad spectrum, knowledge of the anticoagulant used by the patient is not required for use. As well, there are no anticoagulant-specific reagents required. And only 10 µL, because this is a microfluidic assay, are required of either fresh blood at the point of care or, for use in the laboratory, you can send citrated samples, provided you replace the calcium prior to testing. And you can obtain these blood samples either from a venous draw or finger stick due to the volume that's required. Perhaps most importantly, from an emergency setting, the device generates results within minutes. It's assaying multiple times per second and it actually reports the clotting time, as you can see, to the nearest second. But the total time is in minutes.

So our assay broadly assesses coagulation status and I'm going to talk a little bit

about why that is. It's sensitive to interfering substances that inhibit coagulation anywhere between factor XII activation and fibrin assembly. And that's because we're optically measuring clotting due to factor XII activation by glass and looking at fibrin assembly spectroscopically. So the activation by glass turns XII to XIIa and then you proceed down the clotting cascade. And we're really interrogating at the point of fibrin formation. That is actually what causes the peak that is reported by the assay.

Now, the ability to assess clots at the fibrin assembly standpoint is what gives us this broad applicability because activation is so far upstream. And the activation is by glass, which eliminates batch-to-batch variation issues typically associated with things like tissue thromboplastin. So one of the first things we did once we had our commercial prototype was to look at both ends, the activation end as well as the clotting end, to see if known agents would prolong clotting time. And what we saw was that with high-dose aprotinin, which inhibits kallikrein activation of factor XII to factor XIIa, that with increasing dose of aprotinin, and this is spiked ex vivo into human volunteer blood, you see a prolongation of clotting time. As well, we looked on the fibrin assembly part. We looked at the tetrapeptide that inhibits fibrin assembly. And once again, increasing tetrapeptide concentration also prolongs coagulation as measured by our point-of-care coagulometer. So we really looked at both ends of the spectrum and we see a good sensitivity to both interfering substances.

We also wanted to ask the question does our coagulometer track the PK? So here we looked into live, anesthetized rats. And we used anti-factor Xa to monitor the PK levels of, on the top panel, edoxaban and, on the bottom panel, enoxaparin, the low molecular weight heparin. And then, we, in the same rats, used at the same time blood samples, on whole blood, to look at our coagulation assay. And we saw that our coagulometer tracks the rise and return

to baseline following IV edoxaban in rats and subcutaneous enoxaparin administration. We also looked at the pharmacodynamics of PT for edoxaban, which in rats showed some good sensitivity here. And once again, much like PK, we follow the PD measure of PT with edoxaban.

We also wanted to test our coagulometer's ability to measure across anticoagulant classes. So this is all done in human volunteer blood, ex vivo spiking. So these are whole blood spiking concentrations on the x, and on the y, you see our point-of-care coagulometer's response to each of these concentrations. And we tried to measure through both the therapeutic range and the overdose range to look at linearity. And this is a limited sample size of only three volunteers, since this is a very new assay for us.

But what you can clearly see is that linearity is present both in the therapeutic and overdose range, here shown with edoxaban, the Xa inhibitor. We've also looked at the IIa inhibitor, dabigatran. And once again, both in the therapeutic and overdose range, we're seeing good linearity. And one of the exciting developments is also with low molecular weight heparin, which classically in the point-of-care coagulation format has been difficult to monitor, we once again see good linearity and good response by our point-of-care coagulometer. And we've highlighted the 1.5 mg/kg subq range. But essentially, across the range that we've tested.

So our assay is sensitive to Savaysa, Lixiana, or edoxaban, Pradaxa (dabigatran), Xarelto (rivaroxaban), Eliquis (apixaban), as well as the heparins, low molecular weight, unfractionated and the pentasaccharide fondaparinux, as well as warfarin.

And so, our near-term objectives are really twofold. We have our drug in development, ciraparantag. And we'd really like to validate our assay for use in clinical trials to evaluate ciraparantag's activity as a reversal agent for the DOACs and low molecular weight

heparin. And to do this, we'd like to assess changes in clotting time resulting from DOAC and low molecular weight heparin anticoagulation alone and then to go ahead and look at measuring the return of anticoagulation to baseline following ciraparantag administration as a measure of efficacy. For the broader purpose of looking at DOAC monitoring of any sort, we're very interested in also correlating Perosphere's coagulometer to cleared or approved coagulation and activity assays and this would really be for broad purpose of DOAC, heparin or warfarin monitoring. Thank you.

[Applause.]

MS. GUITY: Our next speaker will be Mark Triscott. Mark Triscott received his undergrad and graduate degrees in microbiology at the University of Queensland in Australia. He completed his postdoctoral work at Wake Forest University, where he went on to become an adjunct research assistant professor in biochemistry. Dr. Triscott has been involved in the field of in vitro diagnostics for the last 30 years, working for both small and well-established biotech companies. He has been with Instrumentation Labs for the last 12 years and currently serves as the vice president of R&D reagents and sensors. Dr. Triscott and his teams have successfully completed premarket notification process on over 40 products, many of which are in the area of hemostasis. Please welcome Dr. Triscott.

DEVELOPMENT OF ASSAYS FOR THE TESTING OF DOACs

DR. TRISCOTT: Thank you. And I appreciate the opportunity to come and speak to you today. It's been a great learning experience for us. What I'd like to talk to you about today is an overview of our entrée into direct oral anticoagulant monitoring -- measuring. We'd like to look at customer needs, our European customer experience, a little of the IL [International Laboratory]product development process, how we got our CE mark, some

comparative data studies and also a look at what the DOAC effect on coagulation assays in our particular systems are and then just wrap up.

So we've heard today that monitoring DOACs is not required. However, it appears that there are instances where testing may be useful and we've heard that this would be in, you know, possible cases of bleeding, around surgery, perioperative management, suspicion of an overdose. We've also heard about high and low weight, renal function. And I think it's interesting to mention that with the recent clearance of Praxbind, there may be a need to measure the level of a DOAC before and after the administration of an inhibitor. And we also find that our customers need to understand the influence of DOACs on their coagulation assays.

So the customers have sort of expressed to us that they need products that they can use on specific test systems, sort of plug-and-play. They look at broad linear range, low level of detection, as we've heard today. They'd prefer it not to be RUO, as we've also heard. And the availability of a 24/7 solution for emergency situations. And the information on the DOACs on existing coagulation assays, they'd like to know what happens with the specific assay on the specific instrument.

So as I said, we have released dabigatran and rivaroxaban in Europe. We expect to release apixaban in the next month or so, next couple of months. When testing is required, we've found that it's normally -- it's pretty urgent. This happens in trauma patients and unexplained bleeding for the most part. As we've also heard today, testing volumes are quite low and there's limited access to patient samples. We've heard that from UC Davis and UNC. And one of the experiences we also had was in a recent NEQASsurvey. We found that if an assay was used on our system, the outcome may not be exactly as expected. For example, in

normal plasma, we found a dabigatran level of 22 ng/mL. However, when the system specific direct thrombin assay was used, it was correctly reported as zero. And we'll go into that in a little bit as to why the applications for these systems are very important. So you know, by in large, the DOAC assays have been successfully utilized in Europe. As we say, they're relatively low volume. But everybody wants it. Everybody wants to have it available.

So a little bit of insight into, you know, the IL product development process and, you know, the quality system described as design control, which is what needs to be performed to release a product in the U.S. It begins with a clear understanding of the customer needs, the so-called design inputs where we actually go out and do market research. We listen to customers. We talk to key opinion leaders and, you know, we have scientific advisory boards, et cetera. We then start to build a product within R&D. This is our feasibility stage where we go through iterative development. We look at chromogenic assays. We look at clotting assays and decide which one is going to be the more robust.

We go through multiple test cycles of R&D-level reagents. We'll take it outside. We took our dabigatran assay outside and we had a very low level of detection. But it was a 10-minute assay and the customers did not want that. And so, we were able to reduce the time and still maintain a very good limit of detection. We obviously challenge our materials so that we can continue to make it. We perform guard band studies on our manufacturing processes. We do design of experiments to make sure that we know what impacts our systems. And then, we get to a point we call analytical lock. At this stage, we've locked down our formulation and we put it into our development process. The development process, you know, follows FDA 21 CFR 20.30. And we follow CLSI guidelines where appropriate. And we also perform validation through external testing using good clinical practices. And we work in an ISO 13485-certified

facility. And below, you can see the phases of our design control process, going through the design inputs to make sure that you've got the product the customer wants, the design outputs to make sure that you've built that product, design verification, once again to show you've built the product, design transfer to put it into manufacturing to show that you can make it over and over and over, design validation to make sure that it works the same way in the customer's hands and then product launch.

So we've obviously had collaboration throughout this. And I think one of the other points that was brought up was that, you know, how do you know that your standards relate back to what the pharmaceutical manufacturers are actually building. And we've worked with Bayer, Bristol-Meyers Squibb and Boehringer Ingelheim on material transfer agreements so that we can continue to get traceable materials from the manufacturers and be able to incorporate these into our calibrators and controls to make sure that you have traceability. The pharma companies were engaged throughout the entire development process. And, you know, they want their products to be safe. And if they need to be measured, they would like to have a product in place to allow them to do that -- to allow the customer to do that.

So in terms of the CE mark, you know, in order for us to be able to sell a product in Europe and other countries that accept the CE mark, for dabigatran and rivaroxaban, we completed the -- we have kits consisting of calibrators and controls, the reagents and then we have these validated test methods specific for our instruments. The CE mark process -- and this is not an inclusive list -- but we obviously perform analytical testing, similar to the testing that we do under design control. We look at precision, limit of quantitation, limit of detection, limit of repeatability, et cetera. We take a close look at carryover. We perform field testing in a clinical setting with intended use population. We fully verify and facilitate the stability. We

perform shipping studies. And as I said, we use current CLSI guidelines for our analytical testing where they're applicable.

So to look at some of the products that we have in the European market, we've got a dilute thrombin-based assay for the measurement of dabigatran. We normalize for possible patient abnormalities in factor IIa and fibrinogen by diluting the sample one to two in normal plasma. We have our calibrators and controls traceable to the Boehringer Ingelheim product through LC-MS/MS. We've got a validated test method on the ACL TOP, which I'll show you in a minute. We get very good linearity up to 2,000 ng/mL on retest. Limit of detection is excellent at 2 ng/mL. Good total and with end-run precision. And we have onboard stability claims including extended refrigerator claims since this is not a daily assay.

This is what our calibration curves look like. This is a curve which is taking into account multiple lots, multiple databases, multiple occasions. And we've looked at the curve fit through a program that we have, which provides the best curve fit in terms of the high end and the low end. And you can see at the red line, we actually have a splice in the curve. So you know, we're not going to give away accuracy in the high end or the low end because we stick to a single curve. We can actually splice curves. So you know, this is something which is not available to, you know, every lab, to be able to do this. And so, we're utilizing the full functionality of our systems using this process.

A little bit of data. The comparison of our product versus an on-market assay in Europe. The two plots on the right are paired relative differences. And this will show you the difference in sample-to-sample measurement. And you can see that the hemolysis assay on the top has a somewhat tighter distribution than the predicate assay. You can see that the method comparison with clinical samples is decent, with an R of 0.98 and a slope of 1.05.

For our rivaroxaban testing solution, we use the chromogenic anti-Xa assay, a liquid ready-to-use which has been adapted to rivaroxaban and when we say adapted, it's actually a different application, as you'll see. Once again, we have calibrators and controls traceable to liquid chromatography tandem-mass spec. We've got the method -- the locked method on the top family. Linearity from 20 to a thousand. Limit of detection, 10 ng/mL, once again very good precision and onboard stability with extended refrigerated claims since this is not a high volume assay.

As you can see, this is the anti-Xa application, specific for the rivaroxaban. The anti-Xa application that we use for low molecular weight heparin and unfractionated heparin is a three-point calibration curve. And you can see that, once again, we have a spliced curve here because the reaction at the high levels of the drug -- at the lower levels of the drug is different to the reaction at the high level of the drug. So we don't have to give up accuracy at the high or low point because we can use multiple curves. This is the comparison to an on-market assay and you can see down in the right-hand side, we've got the method comparison. And you can see that there's a little bias in terms of the line of unity there. And if you look at the one on the left-hand side, this is the comparison of our assay versus the HPLC tandem-mass spec. And you can see that that's an excellent correlation of 1 and the slope is very close to unity.

So moving on to, you know, the customer needing to know what's going to happen to their normal samples in the presence of these drugs, and there's literature available, which gives you sort of a qualitative feel for what happens. And this is what we've heard before, that thrombin time is very sensitive to dabigatran. PT/INR has some sensitivity to rivaroxaban. And apixaban is not particularly sensitive to anything, including the PT/INR. So as we decided that we would take sort of a more in-depth look at what was going on when we

added -- we spiked samples into normal plasma and then measured them using a series of our PT reagents on the top line there and PTT reagents on the bottom line. And you can see that top left panel, that rivaroxaban in the 200 ng/mL range has about a 1.7 times the normal clotting time.

What we've -- the first line you see there is 1.52 times the normal clotting time and this represents the normal clotting time plus about two standard deviations. You can see that apixaban spiked into normal plasma using a series of PT reagents really doesn't have very much -- very much of an effect all the way through the therapeutic range. And dabigatran, while PT is not the normal assay to be used with this, you can see that there is some effect. But it's at a very high level. Looking at the APTTs on the bottom row, rivaroxaban, there's some effect, not particularly dramatic. With the apixaban spiked into normal plasma, there's very little effect on an APTT. And for dabigatran, we have the -- you know, the characteristic curve that we've seen before that shows that for levels up to about 200, it is sort of a qualitative way to see the activity of that agent.

So Bob Gosselin mentioned the possibility of performing a global screen. And his approach was to use thrombin time and an anti-Xa assay. And we've actually looked utilizing a modified dilute RVV-based reagent. Since RVV -- Russell's viper venom -- will activate -- intrinsically activate the factor X in a sample, Xa will be present. And because it's a clotting-based assay, thrombin will be present and there's the opportunity for Xa inhibitors and IIa inhibitors to act throughout the assay. This is relatively early information. But you can see that there is quite a sensitive response to dabigatran, apixaban and rivaroxaban using this approach. And it also has the ability to look at indirect inhibitors such as unfractionated heparin, low molecular weight heparin and Arixtra. This is something that we're currently working on. And

we -- it's in a prototype phase.

So just to summarize, IL are committed to bringing system-specific products to the market. And the despite the fact that the test volumes are low, we hear from the customer that there's a strong clinical need to be able to measure these agents. And we've seen some of that in the ISTH subcommittee guidelines. We've implemented testing solutions in Europe and we expect the apixaban to follow through in Q1. And while sales have been quite modest, you know, we're looking at our regulatory plans for the United States. We've submitted a pre-IDE. We'll be talking to the agency later on in November.

And you know, we're interested to hear what the agency has to say after today. And we'd very much like to be able to provide our customers with an FDA-cleared product. And so would our U.S. customers. They want an FDA-cleared product. RUO, there are some issues with that. The regulations on them are relatively difficult to follow. But they shouldn't be used for directing patient therapy, patient management. And it's not our line of business. We don't do RUOs. And I think that, you know, one of the points that I was trying to make was that the applications for these assays are very important and they're very complicated. And I think that the manufacturers are quite often best left to do those in a, you know, system, ready-to-use solution for these products. So IL customers continuously inquire about the status of an FDA-cleared solution and we'll tell them that we came here to speak to the FDA about it. So thanks very much for the opportunity. Thank you.

[Applause.]

MS. GUITY: Thank you, Dr. Triscott. Our next speaker will be Marc Doubleday, from Haemonetics. Marc Doubleday has served as a director of product development for Haemonetics Corporation tech franchise for nearly six years. Prior to joining Haemonetics, Mr.

Doubleday held senior leadership positions in development operations and quality in the regulated biological products industry. He holds a bachelor of science degree in chemical engineering from the University of Michigan and a master's in management from Northwestern University's Kellogg Graduate School of Management. Marc Doubleday.

DOAC DETECTION ON THE TEG 6s

MR. DOUBLEDAY: Thank you, Niquiche, for that introduction. Thank you all for being here today and giving me 20 minutes of your time, and especially thank FDA for the opportunity for Haemonetics to come and present our vision for a DOAC assay on the TEG 6s. These next 10 slides that follow this, I'm going to talk about the Haemonetics vision and our development progress on the TEG 6s. I'll talk briefly about the TEG 6s, for those that aren't familiar with the analyzer or thromboelastography. I'm going to focus a good part of the time on seeing what we see as an unmet medical need and how we determine that a qualitative assay is something that's really needed today. We'll briefly touch on development hurdles, some regulatory uncertainty, as others have. And then, I'll wrap up with clinical applicability and how we think patients on DOACs should be dealt with.

The TEG 6s is thromboelastograph, and thromboelastography measures the strength of a blood clot. So it measures its resistance to deformation and how that resistance changes over time. So originally, you know, as liquid blood, there is no resistance. There is no clot stiffness. That will develop over a period of time and that's called the reaction time, or the R time. And that's how long it takes to get a measurable amount, a minimally measurable amount of clot stiffness. And that's important because that's the only parameter that we really use here in the DOAC assay. Classic elastography usually used what was a cup and a pen. You would introduce relative motion in those with blood in the annular space and measure the

stresses as they developed as the clot developed itself.

The TEG 6s is really a new generation. We measure the same phenomenon, clot stiffness, but we do it differently by shaking the cot and actually optically seeing how that shaking affects it directly related to that stiffness. What is important here is that we use a whole blood sample. So not only does it collect the cell basis of coagulation, incorporating micro particles and procoagulant membranes, there's no sample preparation involved. So the sample can be introduced into the device very quickly. And the feature here that's really critical is there's a reagent cartridge that's used in the device into which the sample's introduced. And the cartridge contains four reagent channels. And we can then optimize each of those reagent channels for a specific purpose. And in terms of the DOAC assay, we have two channels and they're specifically for DOACs. One is sensitive primarily to anti-Xa agents and one to direct thrombin inhibitors. So in the same cartridge, the same test, we can run and determine if either of those agents are on board.

I don't think this is really a surprise to, you know, anyone in this room since we're not practicing medicine on Mars. But what I would like to point out are a couple of points here. Recently, or actually last April, there was an anticoagulant-induced bleeding and reversal agent think tank the FDA cosponsored. And in there, it was indicated that, you know, currently in the United States, there's 4 million people with atrial fibrillation. Of those, 2 million are still left untreated and not on anticoagulants. And it's estimated with a 5 percent annual stroke rate, that 100,000 of those will experience a stroke that is preventable. And if those patients were on these oral anticoagulants, they think that it was estimated 60 percent of those strokes would be prevented.

And I think that's very important because, you know, not only is the revolution

here but it's ongoing. And I mean, we're going to see increased use of these agents as we go forward. You know, along with that really comes a widespread concern. I was pretty surprised. I don't know if there's any scuba divers in the room. But I opened my summer edition of *Alert Diver*, and *Alert Diver* is a publication of the Divers Alert Network, DAN as it's called, which is probably one of the premier dive safety organizations in the world. And here, lo and behold, you know, they had a nice article on anticoagulants in diving. And they see the concern in here of, you know, potential bleeding risks. So I mean, this is really -- it's getting very widespread, even in the lay press.

I'd like to talk a little bit about these clinical concerns. You know, there were some really nice groundwork laid all morning on this. So I'm not going to spend a lot of time on it. I mean, Dr. Korley did a great job with some of this. What I do want to focus a little bit more on is in stroke because what that ended up being is sort of an ah-hah moment for us. As we know, and probably most people have seen this, in 2013, the American Heart Association and the American Stroke Association came out and more or less contraindicated intravenous TPA if patients are on DOACs until they either get to low measurable levels or have not received a dose for 48 hours.

This was really kind of the start of our ah-hah moment, as I call it. Dr. Rybinnik, in 2013 -- so he published a study. He did a survey of stroke specialists and presented them with various scenarios of when they would treat or when they wouldn't treat. And what he found is if a patient was on dabigatran and presented with stroke, would they treat or not treat, given absence of any other information. And fully 60 percent would not treat or they were either unsure of that. And for us, you know, this was -- how do they know the patient's on dabigatran, right? I mean, they're not -- you know, they're not alert. Even if they are, they may

not be cognizant. Can you trust what they say? I mean, this person presents to the emergency department. How do you know they're on this drug? And you could have family members around that maybe would say my family member's taking a blood thinner or maybe even identify one.

But there was another study done just published in 2015 by McHorney that found that the noncompliance rate on these drugs is upwards of 30 percent. And so, even though you think that person may be on that drug, they may well not be and would be available for TPA therapy, which, you know, we talked about earlier, the very bad outcomes associated with the ischemic stroke. So we really saw this as an unmet medical need. That there needs to be a way to have a rapid and reliable determination if a patient is on either a DTA [direct thrombin activator] or an anti-Xa. And we can see this provides critical information for the patient. It provides it early. It gives them an opportunity to aggressively control or treat bleeding. It can be used to adequately look at reversal of drug activity after treatment's given. And importantly, as I pointed out, to really get really TPA therapy in ischemic stroke.

So at this point, it's still a qualitative assay and we really feel that this fulfills this medical need to get that information to the clinician as soon as possible. And why does this work well? It's very easy determination. Is the DTI [direct thrombin inhibitor] there or not? Is the anti-Xa there or not? And I think this is a little analogous to Dr. Korley this morning, where he said the first decision he makes is, is the patient sick or not. Right, it's fast and it's simple. As I mentioned, it's a whole-blood assay. There's no sample preparation involved. There's no calibrators involved to get the machine set up and the results are available in minutes. In fact, literally in five to seven minutes from a cold analyzer start, you can have results. And we've focused again on this because we feel accurate quantitation is not required to meet this

medical need.

So I'll talk a little bit about development hurdles. The current TEG assays that we have on the device lack sensitivity, especially at low levels, at low on-therapy levels to these DTIs and a little more so to the anti-Xas. So we had required development of novel reagents and we wanted to develop a reagent sensitive to each so that we could differentiate their presence. We wanted to do this at therapeutic, or what I know now as on-therapy drug levels. And lastly, in this part of the reagent development, we needed to really titrate these to get maximum sensitivity and specificity. And this was a little trickier with the anti-Xas as there's some variability in response between them. So we really had to sort of dial that in to, you know, make sure the board wasn't too hot or too cold. And then, of course, you know, formations needed to be stabilized with stabilizers and excipients for a long shelf-life to give us commercial viability.

And also, you know, we have had some discussions with FDA. And it's a little bit of an uncharted territory in front of us. We agree that there really is no lack of a clear predicate device that's been approved for measuring or monitoring these agents. So there is some uncertainty associated with that, as we follow the direct de novo approach. And primarily, right, in determination of safety and effectiveness, you know, we're discussing the required bench studies. Fortunately, as a qualitative assay, the requirements are a little lighter than they are for quantitative assays and things like linearity and accuracy really aren't required for qualitative. And the clinical trial design we think will be a little more straightforward than quantitative assays and that, again, we look for sensitivity and specificity.

I'm going to talk about two areas of clinical applicability. The first, as I mentioned, is in potential DOAC patients. I mean, we see this as a screen for everyone who

may be on the agent that presents to the emergency department with either hemorrhage or a stroke. If they're in need of emergent surgery, that would be another reason to test them to find out. I mean, knowledge is power for these physicians. And, you know, we see this as really one of the primary areas.

Second to that, the other area -- and you know, I'll say here pharma is actively developing DOAC reversal agents. I'll have to make some verbal edits because last week Boehringer Ingelheim had their reversal agent approved. It's called Praxbind. So I congratulate them and I thank them for having a nice short name that's easy to say. And what's key here is that direct thrombin inhibitors and anti-Xas today, as we see anyway, require differentiated reversal agents. So the question is, first, how does a physician know which one to give and the TEG 6s can differentiate those two, and then, the second question is should it be relevant, how does the physician know that the reversal is complete. And again, the TEG 6s can monitor that for both of the agents.

So in my final screen here, I'd just like to talk about the approach that we have really developed, how we envision dealing with patients on DOAC. And we really think the first critical piece of information the physician needs, regardless of the therapy they may employ, regardless of the reversal agent they may employ is that is this patient's hemostasis affected by either the presence of an anti-Xa or by a direct thrombin inhibitor. So thank you for your 20 minutes.

[Applause.]

MS. GUITY: Thank you, Marc. We will have our next presentation from Stago. It will be a double team. So we will have Francois Depasse and Daniel Kaczor. Francois Depasse is a clinical pathologist by training and is a board-certified European specialist in laboratory

medicine by the European Register of Specialists in Laboratory Medicine. He practiced at LCS, which became Biomnis, one of the most important reference laboratories in France. He has 20 years of experience in testing for new oral anticoagulants, having collaborated with pharma companies since the mid-1990s. He coauthored several publications and speaking engagements in this field for over 15 years. Dr. Depasse has been the clinical director for development at Stago headquarters since 2009.

Following Dr. Depasse, you will hear from Dr. Kaczor. So Dr. Kaczor is a board-certified by the College of American Pathologists. His entire professional career has been dedicated to the field of hemostasis and thrombosis. Dr. Kaczor is currently the director of scientific affairs at Diagnostica Stago, Incorporated. Prior to this, he held the position of director of technical service department as well as director of advanced support group. His 35-year career has been highlighted by numerous national, as well as international publications and speaking engagements related to hemostasis and thrombosis. So Dr. Depasse and Dr. Kaczor will be presenting on testing for DOAC of anticoagulants: an IVD perspective.

TESTING FOR DOACs: AN IVD PERSPECTIVE

DR. KACZOR: Thank you very much, Niquiche, for that nice introduction. On behalf of Stago, I would like to thank the FDA for putting together such a great program and inviting us to participate. As was mentioned, I'll be sharing the podium with my colleague, Francois Depasse. And the title of our presentation is "Testing for Direct Oral Anticoagulants: An IVD Perspective". The areas we would like to discuss are the Stago DOAC Initiative. We'll review CE regulation. We'll also discuss the Stago experience with DOAC assays in Europe. Then, we'll switch back to the United States and talk about potential DOAC testing market here with a little different spin than we've heard so far. And that is putting the challenge of how do

we deal with a test that appears to have high importance, but a low volume, into the clearance process. Is this a case for a least burdensome pathway? Perhaps it is. And then, we'll conclude and make some next step remarks.

So what is the Stago DOAC Initiative? Is it an initiative to provide IVD measurement tools to clinicians to assist them in decision-making? In this way, we want to contribute to patient positive outcomes. We want to offer a tool for management of high risk situations. And, as was discussed throughout the day, our focus is not in monitoring, as it appears at this point that it is not needed. The Stago DOAC assays have been developed, have been CE marked and are currently used in many countries throughout Europe. U.S. studies are ongoing at this time. If we take a specific look at the assays themselves, they are quantitative assays that are differentiated one from each other by a drug dedicated calibrators and controls. They are chromogenic in nature and, by being chromogenic, specificity is enhanced and interfering substances are reduced.

For the Xa assay, we utilize -- for the Xa DOACs, we utilize the same chromogenic assay as currently being used to test for heparin. For the anti-IIa DOACs, there's a specific ecarin chromogenic assay. And at this point, this assay is not cleared. The assays are fully automated and they have a very fast turnaround time of six minutes or less, which meets the requirement of most testing. They have a wide measuring range, from 200 to 500 ng/mL, depending on the assay. This allows covering very low to very high concentrations and certainty incorporates the concentrations of interest that were mentioned today. An the calibrators and controls are traceable to LC-MS.

So now, I'll ask Francois to come to the podium to give us his unique perspective on the European experience of DOACs.

DR. DEPASSE: Good afternoon, ladies and gentlemen. I would like first to thank the FDA for giving me the opportunity to present DOAC testing from a European perspective. I will first start with the CE process registration. There is a European directive which is quoted here that applies for CE marking. Indeed, the directive provides in an appendix a list of high-risk devices. And DOACs are not listed in this appendix. This means that self-declaration will apply. The directive also indicates agency requirements that have to be fulfilled for self-declaration.

But there is flexibility for the manufacturer to use its own expertise to define the protocol to implement to demonstrate analytical and clinical performances of the assay. Stago is using more and more the CLSI guidelines as a reference in CE marking procedures. However, one particular point of the CE directive is that there is no requirement regarding the use of native samples for verification. IVD companies are yearly subjected to inspection by an independent certification body to check the manufacturer compliance. And at the time we speak, there is an audit in Paris, at Stago in Paris. And it's very likely that the DOAC files will be inspected.

I will now report our experience with rivaroxaban receiving CE marking regarding the validation study. So the primary objective of the study was to demonstrate the performances of the assay for quantitative determination of rivaroxaban concentration in plasma samples. This indicates that the validation study was independent of patient clinical conditions. So the study design was based on a method comparison using the EP09a guidelines that was applicable at the time of the validation study in 2011. The results obtained with the Stago assay were compared with the results obtained with the reference method, liquid chromatography with mass spec detection.

We included 87 plasma samples for the method comparisons. This includes

native samples obtained from patients and assay volunteers as well as contrived samples for high concentrations in order to cover the assay's average range. So covering the range of high plasma concentration is difficult. On the one hand, patient samples containing such high concentration are very rare. On the other hand, a healthy volunteer study may not solve the issue. First, there are ethical aspects to consider before administering your high dose of anticoagulant in the assay volunteers. And second, due to the settling effect, the plasma concentration reached after a single dose administration may remain limited even in the case of a very high dose of drug. So use of contrived samples helps cover the high end of the human range.

In this slide, I would like to focus on the intended use of our DOAC CE-marked assays. The intended use denotes quantitative determination in plasma of the considered DOAC by measuring its direct anti-Xa or anti-IIa activity, depending on the nature of the DOAC, in a competitive assay using a [inaudible] chromogenic substrate on a Stago instrument. It should also be mentioned that this concentration is helpful for clinicians to assess the extent of anticoagulation in situations requiring further characterization of the clinical picture.

This statement is supported by many papers. However, no threshold for result interpretation is provided. Providing such information would not be feasible to date because of the absence of such recommendation in the DOAC labeling since they have not been defined in pivotal studies during drug development. In addition, determining such thresholds is beyond the sol capability of an IVD company. This would require a collaborative working party involving experts, pharma companies and even IVD manufacturers. There is therefore no possibility for Stago to document the guidelines for DOAC measurement interpretation with thresholds.

There are several European guidances that recommend DOAC measurement in specific situations. There is only one reference, to the best of our knowledge, issued by the GIHP that comments on thresholds with a proposed management flowchart. However, according to the authors themselves, this is only a proposal with threshold determined by extrapolation of existing data without validation. There are also proposed algorithms including DOAC measurements, but without thresholds such as the one issued by the European Heart Rhythm Association and the one issued by the European Society of Cardiology. Finally, the added benefit of DOAC assays in specific clinical situations is mentioned in the European DOAC labeling and supported by national associations.

I will now focus on our experience with marketing DOAC assays in Europe. So the first CE-marked Stago assay was launched in 2012 and was dedicated to rivaroxaban measurement. We then launched a dabigatran and apixaban assay this year, in 2015. So Stago DOAC assays are marketed in more than 40 countries worldwide, including Europe, Latin America, Middle East, Asia Pacific and Oceania. As an example, 50 percent of university hospitals in France use Stago assays and this is balanced by the fact that they are also other DOAC assays that are marketed by competitors in Europe, and especially in France.

The demand is growing each year and, looking at rivaroxaban sales volume, it increased by almost 200 percent in 2014 compared to 2013. So most frequent use of DOAC assays in Europe relates to investigation, either for evaluating the bleeding risk before surgery or invasive procedure or when searching for a bleeding cause while on anticoagulant treatment. We got very positive feedback from the field, as the assay is available 24/7, in routine settings. The assay uses a universal reagent for all anti-Xa agents. The assay is easy to use. It is fully automated. Reagents are barcoded. Results are obtained in a few minutes. So

test is considered as a useful tool by clinicians as an aide in clinical decision. Finally, there are scientific studies that support the accuracy performances of our assays and it is considered that DOAC assays can continue to generate more knowledge. I will now hand over to my colleague, Dan Kaczor, for a special focus on the U.S. situation.

DR. KACZOR: Thank you, Francois. And in particular, we're going to be focusing on an attempt to figure out what testing volumes could be for testing DOACs in the United States. So as we heard today, DOACs are now a very important player in the U.S. market. If we take a look at 2014, they accounted for approximately 25 percent of all anticoagulant prescriptions.

In addition, for new patients who are put on oral anticoagulants, the DOACs actually exceeded VKA [vitamin K antagonist]We know there are several specific situations in which DOAC measurements can be helpful. It was mentioned many times today. Perhaps the largest potential is in patients with atrial fibrillation, in an attempt to prevent stroke. It's estimated that in the neighborhood of 3 to 4 million patients with atrial fibrillation exist in the United States.

So if we take the information from the previous slide, in addition to the information from this slide, we've put together a table here where we're trying to make a best estimate as to the amount of tests per year for DOACs. I call your attention to the last line. So approximately 25 percent of patients getting anticoagulation prescriptions will get DOACs. If we apply that to the number of atrial fibrillation patients in the United States, that leaves us with approximately 750,000 patients. It's been published that 10 to 20 percent of the patients on anticoagulation require surgery at some point. If we use this figure then, the patients that may benefit from testing per year falls in the neighborhood of 75,000 to 150,000.

Using a modest estimate on the number of tests per patients per year at less than three, we can come up with an estimate of about a half a million tests per year in this patient population. Now, this is a significant number of patients that may benefit from DOAC measurement. But there is a big but here, especially for in vitro diagnostic companies. And that is the realization that DOAC testing of a potential market is limited compared to VKA. Again, I call to your attention a half a million as compared to right above it, 9 million, or 20 times less the amount of testing for these new anticoagulants. It's a very big consideration that will carry into our next slide.

So the Stago objective is to have DOAC assays marketed worldwide to contribute to the improvement of patient outcomes. Of course, the U.S. is targeted as a high priority. The challenge is developing the most efficient pathway for clearance, which balances the investment of test development and the burden of regulatory clearance while knowing all the time that the number of testing -- the testing volume will be very low. So in conclusion and next steps, it's made very clear that clinicians request DOAC assays to improve patient outcomes and to get an idea of what is going on in their patient population.

Stago has a strong expertise on anticoagulation measurement. It's clear that IVD tools have been and are commonly used worldwide for anticoagulant drugs. To that end, Stago DOAC assays are already cleared, CE-marked and used in many countries. They can also offer DOAC measurement tools to the United States. So Stago is looking forward to continuing to work with the FDA to find a least burdensome pathway to have these assays cleared in a timely manner to improve patient care and answer so many of the questions that were presented today. Thank you very much for your time.

[Applause.]

QUESTIONS FOR SPEAKERS

MS. GUITY: I will now open the floor for questions for our speakers.

DR. HOFFMAN: So in the morning session, there were a number of questions related to asking or saying things that will get you kicked out of the session. But perhaps I could ask what maybe the obvious question is and that is why is the U.S. so far beyond the rest of the world in having tests available for assaying or at least estimating the levels of these DOACs. And I don't know if that's a question for the industry representatives or for the FDA.

MS. GUITY: That's a very good question. And we could probably save that question for our panel discussion a little bit later on. Is there any other questions? From the webcast? Well, I had a question. So I think Haemonetics mentioned that your assay is qualitative. Do you have a threshold? Have you come up with some sort of level of a threshold for that assay?

MR. DOUBLEDAY: Yes, we do. We've done three different clinical studies, one in healthy volunteers, one in actual patients. And then, we actually did a trauma study and evaluated some patients there. In the patient study, we looked at 25 normal individuals to establish thresholds for the R time, which is reaction time. And it's right around two minutes roughly. And then, we looked at 25 patients on dabigatran and 40 patients on blends of rivaroxaban and apixaban. And so, we compared them against those thresholds and sensitivity and specificity in both cases were -- point estimates were greater than 90 percent.

MS. GUITY: Thank you.

DR. ADCOCK-FUNK: I've got a question about the calibration of some of the assays and for those of you who have calibrators. So for instance, for dabigatran, do you know if your calibration is for total dabigatran or is it for free dabigatran, and the same thing for the

edoxaban. How do you account for the active metabolites?

DR. TRISCOTT: Dr. Adcock, I guess the dabigatran is the material supplied by Boehringer Ingelheim, which is the active form of dabigatran. And so, my expectation would be that it would be the correct calibrator to use to measure the active species.

DR. DEPASSE: I would like to mention that the calibrator at Stago is dabigatran, the active component. But the assay measures both activities of the parent compound of the kallikrein conjugate.

QUESTION: [Inaudible] from Germany. I have a question to Bryan Laulicht. Very interesting point-of-care methods. And you are using glass as a surface. And I am wondering about the normal coagulation time, which is four minutes, which is quite long. Can you explain that first?

DR. LAULICHT: Sure. So it is long compared to some of the traditional coagulation assays. And we attribute that, for example, to the large difference in surface area between a flat glass surface that we use as compared to, for example, particulate kaolin or celite, which is a very high surface area and very highly negatively charged surface. And so, we would expect longer clotting times.

But we also think that may be part of why we're seeing good sensitivity to, for example, low molecular weight heparin, which you typically do not. What we've found is as we have more and more activation, as we compress those times down, we also compress down the differences between normal and anticoagulated. And so, we've found that, for us at least, the sweet spot is to go a little bit longer. But what you trade for that time is increased sensitivity.

QUESTION: So it is a question of the surface of the glass?

DR. LAULICHT: Surface area to volume ratio, yes.

QUESTION: And the second point would be how can you differentiate between factor Xa and thrombin inhibitors and the effect of low molecular weight heparin or hirudin?

DR. LAULICHT: With this assay, you cannot. With this assay, it's broad spectrum. So the idea is whether you come in having taken Pradaxa, dabigatran or edoxaban, Savaysa, if you're elevated, you'll be elevated. And if you know beforehand, for example, that you are on one or the other, you can back out of concentrations if our linearity holds in the larger scale studies that we hope to do.

But in the emergency setting, we were really targeting this to be our hopeful assay for measuring the reversal activity of the ciraparantag. And the other interesting thing about ciraparantag is it also doesn't matter whether you're on a Xa or a IIa DOAC. You would use the same dose of ciraparantag to bring you back to baseline. So it's really meant to sort of look at things very broadly. And then, of course, there are all these other assays, which may take a little longer to do or, in some cases, are point-of-care and give you a sense of Xa versus IIa. But ours is really meant to give you a sense of where the patient is in terms of overall coagulability.

QUESTION: So there is another assay which is very simple, which is the activated clotting time. Can you explain the difference, except for the surface of the glass?

DR. LAULICHT: Sure. So my experience with activated clotting time, particularly glass bead-activated clotting time, comes from the hematocrit [phonetic] response unit. When we were testing that in our trials, we found it to be relatively insensitive to the DOACs. And in our hypothesis as to why that may be is that the unit is based on a rotating test tube which has a cylindrical magnet at the base of the test tube. And essentially, the test is triggered to have formed a clot. When that magnet moves a certain distance up the side of the test tube. And

that should only happen, in theory, once there is a solid clot.

But in our experience, what tended to happen was that you would get more of an adhesion of the magnet to the test tube via a surface clot rather than a complete clotting of the blood in the test tube. And in fact, in all of our DOAC tests that we ran on healthy volunteer blood, where they were taking the anticoagulant for this purpose, if you picked that test tube up at the end of the test and you dumped it, liquid blood would come out. And so, for us, this was part of why we thought there was some insensitivity to the DOACs and in particular to really measuring activated clotting time in that setting.

So we were looking more for a very reproducible setting where we could still use glass as the activator but where we could hopefully take advantage of microfluidics where you really have very precise testing conditions and very reproducible from one to the next. You know, these are the same sorts of technologies that are used in making computer chips. And we're all familiar with sort of the reproducibility of those features. So that was really what spurred us on to go the direction that we did.

MS. GUITY: Just to sort of follow up, is there a calibrator for the coagulometer?

DR. LAULICHT: So as of now, we're not targeting calibrators. I think we've seen they are fraught with some issues in doing so. What we're hoping to do is establish essentially reference ranges once we have a larger sample size to get a sense of what a clotting time of, say, five minutes means if you're on apixaban versus if you're on edoxaban or dabigatran, et cetera. But we think that really the major utility of this device is getting a relatively quick answer at the point of care. And we think that essentially calibrators may defeat that purpose. And you know, down the line, if we start to come to some good consensus on what calibration would look like in the lab, that might be something that you look at down the line. But I think

for the immediate use, specifically if you're particularly interested in reversal of a bleeding situation, which was really our reason for getting into this coagulation testing to begin with, we really just wanted to be able to get the answer of where is the patient in terms of coagulation status before you administer a reversal agent and then afterwards.

MS. GUITY: Okay. Thank you. If there are no more questions, we will take a 15-minute break and we will start our next session early, back at 2:30. So we'll start back at 2:30. Thank you.

[WHEREUPON, the foregoing went off the record at 2:13 p.m., and went back on the record at 2:29 p.m.]

MS. GUITY: Okay. We're going to go ahead and get started on our last session. And in this session, we'll be hearing about from the FDA, our perspective. And our first speaker will be Marina Kondratovich. Dr. Marina Kondratovich is an associate director for clinical studies and personalized medicine in the Office of In Vitro Diagnostics and Radiological Health at the FDA CDRH. She has been with the agency for 16 years.

Some of her interests are design of clinical studies for diagnostic devices, analytical evaluation of tests, personalized medicine tests and missing data. She has been an FDA spokesperson at multiple FDA advisory panel meetings. She actively participated in CLSI standards development and in the ISO. Dr. Kondratovich received her Ph.D. in mathematical statistics from the Department of Statistical Modeling at St. Petersburg State University in Russia. Dr. Kondratovich?

CDRH PERSPECTIVE

TEST OUTPUTS AND INTERPRETATION

DR. KONDRATOVICH: I will speak about some analytical studies related to

qualitative, quantitative and semi-quantitative tests. First, we need to understand what is the meaning of this term. And of course I will not discuss all aspects of analytical studies because you already heard about precision, carryover. So I will discuss only some I think maybe some confusing terms and aspects related to this test. Let us start with qualitative tests. This is very simple test. Usually this test has two outcomes, can call this positive or negative. Interpretation of this positive and negative result really depends on two basic points.

One is what you really measure, what we call a measurand. It can be, for example, drug concentration or maybe some coagulation activity. Most of my example here will be for qualitative test related, for example, to drug concentration. And second very important aspect is threshold for yes and no. If I consider drug concentration: then, what we call interesting is: not detected, what we can call like zero concentration or l absent, or detected, what we can call like some amount is present. Then of course cutoff for these two statuses, i.e. not detected and detected, really relate to limit of blank.

What is limit of blank? Limit of blank is a threshold for numerical values for these samples, what we call blank samples. And these blank samples are really samples with zero (analyte) concentration but from different patient. So we have zero concentration but metrics for this analyte of interest really can be different. So I have some set of patients and all these patients have zero (analyte) concentration. Then, we started to use our device under different testing conditions, different runs, different days, different calibrators, different lots. And then, of course, I obtained some even distribution of these results.

So we can consider this like on the scale, like you already provided some numerical value for your own test. And of course even if I know that true concentration is zero, your device in reality can provide some numerical output. I consider that (numerical output) as

a positive value then I can tell that all drug (analyte) is detected. Then of course I will be wrong because I know that this result is coming from the samples with zero concentration. So it really needs to have a threshold. That kind of a threshold that if the device gives results below the threshold, I can tell (that analyte is) not detected. And if above the threshold, I can tell detected. (For the example that we're discussing) I will be of course wrong because I know that the sample was zero. But I will be wrong for various small percent of the time. Usually it can be like 5 percent or even maybe 1 percent.

So this is exactly what we call limit of blank. And this can be like 95 percentile. It means that for subject with zero concentration, I can tell all drug is present and I'm wrong for 5 percent of the time. Or maybe it can be 99 percentile. Then I will be wrong only 1 percent of the time. Let us separate some kind of analytical evaluation of limit of blank and then we can discuss some even clinical study related to this. So blank samples is samples with zero (analyte) concentration. And according to CLSI, we have (the) last version (of the) CLSI (guideline) about limit of blank, limit of quantitation, limit of quantitation, EP17-82. And I recommend you to look for this study design. But the basic study design with point of view of analytical has said that we have at least four different patient samples with zero concentration. This is minimal requirement.

And then, I started to use this for patient sample and the different testing conditions. So each sample tested for three to five days with total number of 60 measurements. And then, we're thinking that because lot is really very important for the low concentration, you need to repeat this study with 60 measurement for lot one and then for lot, for example, two, or if you have, for a lot three. And then, your limit of blank will be the maximum value among lot one, lot two and lot three. Please pay attention that here I'm

talking patient sample, what we can call like between subject biological variability. But it's really very limited biological variability. Yes, it's more about random interferences among these patients. It's not biological variability, what was discussed today in the morning when you have different patients on the drug and there's some relationship. No, this is really very limited concept. Yes, all these patients probably will be healthy without drug and really what you're testing is more like what additional interferences you can see from these different patients, even if everybody has zero (analyte) concentration.

So when I have all these patients results, from four patients, 60 measurements, then we can establish this threshold, which is called limit of blank. And if the test gives results below limit of blank, we can call that analyte not detected. And if we have results above limit of blank, we can call the analyte detected and we are wrong 5 percent of the time if I establish 95th percentile. If you think you need to have 99th percentile, then what we call type one error, i.e. false positive rate for these people will be only 1 percent.

Another important characteristic is limit of detection for limit of blank. Again, this is more like about analytical performance. And here, right now samples when I know that they do have an analyte concentration. Yes, I know that these people are taking drug, for example. And I would like to find that kind of concentration that 95 percent of the time this concentration will be detected, detected, detected. So 95 percent of the time, I'm correct, yes? Because this sample definitely has (the analyte of certain) concentration. And this is distribution of all your results. And I can tell that only 5 percent of them are not detected. So this limit of blank and limit of detection are very important characteristics of the test.

And here, of course, again we're speaking only about the subject which has only four, like a minimum requirement, four patients in order to establish cut-off, a limit of blank.

We can consider even a clinical study when I would like to test variability of these patients without drug, yes? And maybe it will be not only health people. But it can be people without drug but from intended use population. It does not mean that they are healthy, yes? They have other (medical) conditions.

So they have other maybe some random interferences because they're from intended use population. And if I consider (that) Nzero (are) subjects from this intended use population, but without drug, zero concentration, yes? This will be like my set of patients, testing in some way limit of blank. But in a more precise way. Also, we can consider subjects from intended use population who are taking drug (N one). And in order to evaluate qualitative test, I really don't need to know maybe exact values, what kind of exact values of this concentration of drug. But if we know that they're taking drug, they have this concentration, then this is like I am checking limit of blank from other site, yes? If the people really have (certain analyte) concentration, what is the probability that I am telling not detected? Yes, this you can call sensitivity.

Cutoff here, it can be established in two ways. One way, (is that) you establish your cutoff, what you can call limit of blank in analytical study. For example, with these four patients. And then, with this type of clinical study, you really will evaluate it with my limit of blank. (This) is okay, yes? What is my false positive rate? What is the false negative rate? Another (one is a) little bit more sophisticated, it can be decided (, i.e.) you decided before the clinical studies that we would like to have, for example, false positive rate 99 percent. And then, when you have all the subjects who (are not on) drug, with zero (drug) concentration, (that) you tested under a different condition, then you can establish your cutoff based on your pre-specified level of false positive, for example 99 percent. This will be your cutoff, that only 1

percent of the subjects without drug will have results detected. So that kind of possible study for -- related for this presence or absence.

We would like that you will discuss at our panel whether this type of study is useful or maybe question which I'm really asking, like presence or absence. Do I really need to ask that kind of question. Do you really have useful information for this type of study? And if you think that, yes, this type of study is really useful, then we would like that maybe you can discuss some details of this study because we can consider possible study design. For example, subject without drug, drug concentration is zero, yes? And then, subject with drug. Maybe it can be the same patient, at the beginning they were without drug. Then I have a subject with drug. Or we can imagine different study designs, that subject without drugs can be completely different set of patients. So should I measure patients with drug, for example, different time points or it may be already in this table, all those kinds of nuances of that kind of study design is very useful for us to understand what your opinion about these different study design.

Let us discuss quantitative testing. This is another extreme of the test. Qualitative, we have positive, negative, detected, not detected. Quantitative test provides numerical values and very important additional requirement, that these numerical values should have some good properties. It's not any numerical values and we can call quantitative. No, not in that way. There are two different aspects of these numerical values. One aspect, how well the relationship between device value reflects relationship between true values. And this aspect is really related to linearity and linearity is an analytical property.

So let me provide for you in plain language the meaning of linearity. Linearity formal definition is ability to provide measured quantitative value that is directly proportional to the value of the measurement in the sample. So device, and we consider that -- imagine that

this device does not have any random error. We already eliminated it. The device value without random error is proportional to the true value. Why is (it) very important? Because, for example, I have three samples and my device gives value 5, 10 and 15. And if the device has property of linearity, so it means that 15 is proportional to the true value, yes? And 5 is proportional to the true value. So I can tell that 15 is three times more than 5. You agree, yes? And the 3 meaning absolutely the same ratio of true values in the sample because really proportional K can be simplified.

So I can tell you yes, maybe 15 is not exactly true value. But relationship between 15 and 5 is the same like true value. Also I can tell that this difference is 5, this difference is 5 and this 5 meaning the same. So this is exactly of meaning of linearity, that when you have device with property linearity, you can do a lot of good stuff with these numbers. Doctor can take difference. Doctor can tell 15, 3 times more than 5. You can compare patient results today with patient results tomorrow or yesterday. You can understand what is meaning of difference. You can understand what is the meaning of relative change because in reality when you calculate percent of change, it will be exactly reflected in true change. So a lot of good properties if device is linear.

And of course, linearity is really related to calibration. Calibration is process that establishes relationship between assigned values of device calibrator and the raw instrument signal. So this is like hypothetical example with calibrators and value to assign calibrators. Maybe it will be not true value, yes? But of course, they are proportional to some true value. And we don't know K. But please pay attention. For linearity, I really don't need to know K. So linearity property is not about accuracy. We will discuss later. So we will create a calibration curve and we're using this calibration curve in our device. And if we use calibration curve which

are completely known without any errors, then of course this device will be perfectly linear, yes? But because in reality we never can have our true calibration curve. Always we have what we can call working calibration curve because there are some sources of uncertainty.

For example, how we assign value to calibrator? How many calibrators we have? How many number of maybe dilution I needed to do? How many replicates I did when I obtained this average of the signal? All these sources are very important. Of course, for example, mathematical model, I heard that some use a very complex mathematical model for calibration. Probably its device will be much better than if you use some one type of mathematical model. How you estimate all these parameters? So my point, finally your working calibration will be not exactly true calibration. So this in some way you will have deviation from perfect linearity. So linearity is really related (to) how well the device is calibrated. Frequently it's called calibration verification, or calibration validation. It's the same concept related to -- it's almost the same concept like linearity.

So linearity is also related to commutability because when you're doing your calibration curve and you use your own calibrators, usually these calibrators are not in the patient matrix. They are in some different matrix. So when you created your calibration curve, it's not obvious that it will work very well for the patient sample. So even if you check your linearity, for example, with contrived samples, it does not mean that it will work for the patient sample. So really, linearity study with the patient sample can show you that really your calibration curve is really very good also for patient sample.

Basic study design for linearity is like I can have some relationship between this level based on formulation. Very frequently like, for example, for drug concentrations, this formulation is dilution, that if I have one dilution, for example, two times compared to the

sample, what does it mean in linearity. It means that device needs to have results two times less compared to my original sample. So statistical analysis is very simple. You obtain in this sample with dilution. You try to find the best fitted line and then you see where is your deviation of this mean of replicant.

So for estimation of linearity, it's really not good to use coefficient of correlation because it can be very misleading. You can have very big range and of course you will have very good coefficient of correlation. No, it's not how you should evaluate linearity. For linearity, you need to construct this straight line and then consider where is the deviation. What is the maximum deviation of your device from this straight line? Of course, if you have no drug concentration but kind of like coagulation activity, or dilution, it will be not working.

So right now concept of linearity is more complicated and it will be like out of scope of my presentation right now. But if somebody decided to make devices which are not about drug concentration, then linearity is not so simple. We discussed quantitative test, like analytical, like the value which represented, value 100 and value 50. If the test has property linearity, 100 should mean two times more concentration than 50. Of course, interpretation of clinical numerical values, even with the property of linearity, it's very different how it's related to the clinical outcome because relationship between this numerical device value and clinical outcome is not like what we discussed through linearity. It's not what we discussed in the study absent or present, yes? This can be completely different study.

Of course, if you have linear relationship, between your device and to some kind of clinical outcomes is great because it's much easier to make interpretation. Even if you have some monotonic relationship, it's also may be not so bad because at least you can make interpretation, what maybe the relationship to some kind of coagulation activity using this

value. And of course, if you don't have any relationship with clinical outcomes, then this numerical value, it's only like you can make interpretation like analytical and that is all because what is the meaning of this from the point of view of the patient may be difficult to tell.

For quantitative test, measurement interval is very important. And limit of quantitation, this is when your precision is acceptable, your bias, what you compared to the reference method is acceptable. And linearity is acceptable. Sometimes maybe even you would like to evaluate limit of blank, then, of course limit of detection, then in reality your numerical value can have certain interpretation that if you observe results below limit of blank, not detected. If you observe results between limit of blank and limit of quantitation, you can make interpretation drug is here, detected. But we cannot quantify it because precision and bias is so huge it's no point for me to give you numerical value. And if we observe results above limit of quantitation, of course precision and bias is acceptable. You can provide numerical value.

Please pay attention, that linearity is not about checking what is the bias, yes? When in order to check accuracy, we need to compare to the reference method. And of course, the beauty of linearity that, look, it's really more about how well I calibrated my device with calibrators. And if this calibrator is really more like correct value, you almost start like this calibrator what we call traceable. Then you almost have good devices, not only linear but also is unbiased.

Last is semi-quantitative test. So we discussed qualitative, two value, positive/negative, detected/not detected, quantitative, there are a lot of numerical values. These numerical values have (this) very good property that you can compare this value. You can subtract. Also we can see the same in semi-quantitative tests, tests with ordinal categories.

Ordinal, it means that I only can tell that, for example, low is definitely less than on-therapy range, yes. And on-therapy range, definitely lower than high. But how much high, we cannot tell.

For example, we can have four categories: not detected, low, on-therapy range and high. What kind of advantages of this test? Of course assay can be less precise than quantitative assay. There are no really requirement for linearity. But still I need to have some calibration in order to get in this categories. Maybe it can be easy for interpretation. Maybe not. Maybe in some situation it's really more difficult to make interpretation. And of course, disadvantages that if patient has results which are really close to the cutoff but without these numerical values it's difficult to understand. Whether, for example, I call it an on-therapy range, it's very close to the low or very close to the high, still it will be on-therapy range. So semi-quantitative test has this disadvantage that we're losing this quantitative information about how close the patient results to cutoff.

Finally, we would like that in your panel discussion you will discuss this point related to question. What is better, qualitative, quantitative, semi-quantitative test? In what cases quantitative reporting more clinically useful than qualitative output? Is quantitative output more difficult to interpret and potentially prone to misinterpretation? Are quantitative claims always more informative? And whether ordinal categories may be in some situations helpful. Like, for example, not detected, low, on-therapy range and high. Thank you very much. So I hope that I clarified some aspect of all these three different type of test, qualitative, quantitative and semi-quantitative. And right now, Abe Tzou (will) discuss clinical aspects.

[Applause.]

MS. GUITY: So we will continue our FDA perspective with Dr. Abraham Tzou. So

Abraham Tzou received his medical degree from Northwestern (University) and clinical pathology at Yale University. Dr. Tzou is a medical officer in the Division of Molecular Genetics and Pathology. He will be speaking to us about some regulatory considerations. Just some technical difficulties. We'll have his slides up and running in a couple of minutes. Oh, at this time, our panel members, if you can please take your seats while we're working out our technical difficulties, we'd greatly appreciate that.

DR. KONDRATOVICH: [Off mic] -- if maybe somebody has questions for me, then I can answer --

MS. GUITY: We are a little bit ahead of schedule. So if our panel members, while we're working out our technical difficulties, if you can please take your seats here. Panel members, please? Dr. Cuker, can you please join the panel? Dr. Triscott? Marc Doubleday? Dr. Tzou?

REGULATORY CONSIDERATIONS

DR. TZOU: All right. Thanks for your patience. So unfortunately, I'm just going to tell you up front, unfortunately my slides aren't that exciting. So it's -- you should just be -- you should already be prepared to be let down for that wait anyway. So in any case, I am not going to have all the answers here. We've discussed a lot of interesting topics throughout the workshop today. So I'm just going to try and give you some perspective of, you know, we've heard considerations from the laboratory side, from the clinician side, from the manufacturer side. And I'll just try to give you some perspective of the regulatory decision-making of how some of the things that we've heard, how they factor in perspectives as far as making decisions regarding in vitro diagnostic devices.

So the first part, I will just discuss -- it's almost like a strawman-type scenario. So

routine therapeutic monitoring. I think we've had multiple speakers say that perhaps right now the evidence base does not support routine therapeutic monitoring. So even though that's not the case perhaps for right now currently or the immediate near-term future for direct oral anticoagulant testing, I do just want to bring up that scenario, you know, in the prototype case of, for example, INR of how in that context a particular regulatory oversight model might be applied and how, why in the absence of that context, different regulatory considerations may be altered.

And so, after that discussion of the routine therapeutic monitoring, how some of the things that have been brought up and raised as possible scenarios for other use settings that we've discussed and how that would factor into device development consideration and some of the general considerations that would apply across proposals for direct oral anticoagulant testing.

So for this routine therapeutic modeling scenario, so monitoring, people sometimes use monitoring colloquially throughout in a very casual sense. And so, sometimes a very loose sense that sometimes people say for monitoring is that, well, anytime a patient that has been diagnosed, after diagnosis, any sort of testing would be monitoring that patient. So sometimes people use it almost in the same as follow-up, right? So anything after diagnosis or anything after an evaluation, that's all monitoring. So in some people's eyes, perhaps in common usage, that might apply. For in vitro diagnostic devices, for what has classically been considered a monitoring in vitro diagnostic device has a more limited and focused in that it would involve individual patients being repeatedly tested over time.

Okay, so sometimes there are proposals that you take diagnosed patients. You just take a whole bunch of them, as many as you can get. And it may be that you just have a

single time point on each individual patient. That would not be within an individual patient repeated testing and results and how they change over time. And the rationale and reason for having routine therapeutic monitoring would be the idea that there is, for example, a therapeutic range. So the most prototypic classic example would be INR, that you would follow this result in an individual patient over time. And the rationale is because you understand how that results translate into meaningful clinical outcomes.

For example, some of the bleeding and stroke outcome curves that have been shown earlier today. And that understanding that adding individual patient level is sufficient to prompt the requisite clinical action -- so for example, dose adjustment. So although we recognize that the evidence for DOAC may not be at this level, although there may be interest in how things may pan out in the future, it's too early to say -- that some of the considerations may be that in the setting of tremendous variability at the inter- and intra-patient level, that understanding what a single measurement at an individual patient may not always be sufficient to inform clinical decision-making.

And so, just as a follow-up, in the setting of routine therapeutic monitoring, how that would affect the regulatory oversight from the total product life cycle, both in the pre-market, that is, before a product is authorized with marketing claims -- and in the post-market setting of how that would also factor in and the kinds of decisions that perhaps some people who -- in the audience who are not in the FDA world do not always -- will not always consider. So for the premarket setting, clearly if you were to support a therapeutic drug monitoring, you would want to take that intended use population, the patients who are being treated and undergoing routine follow-up and get samples from those patients.

In the setting of having a clear therapeutic range, you would want to be that as

the basis to inform what are the key levels of interest. So if you're looking at INR 2, INR 3, a low 2, higher than 3, those would be a key rationale of saying these are the performance and bases to understand whether my PT/INR device is performing at the accuracy and consistency that would be expected. So clearly if you had a device where an INR below 2, sometimes at some frequency you were getting above 3, that that is probably not the type of device performance that would be supporting routine use. Having a comparison to a well-established comparator method, that also is linked to the translated clinical outcomes. Having applicable calibrators and controls, and if you have therapeutic ranges, having those targeted to key areas of the therapeutic range would be a sensible approach. And that this overall package of data therefore would support a marketing authorization for use in routine monitoring.

So probably most people may be more focused on the premarket setting and some of the discussions or questions may be more oriented to the premarket setting. But another consideration from FDA device regulation is also how this translates into post-market setting. So one of the compliance activities relates to whether the test is being promoted appropriately in a way that corresponds to what has been authorized for marketing. So a general sort of example I might say as a comparison is sometimes people would like to propose and request that a very vague, open-ended marketing authorization be granted.

So they might say, my test measures this and this is of interest for patients receiving whatever anti-hemostasis agents. So that would be broad. It would be open-ended. It would not explicitly say that it is established for monitoring. So some people might think that would be a reasonable approach. Well, one of the implications of that approach is sometimes then people would say, well, I have a test to measure hemostasis activity. And then, their marketing representatives would go out to clinicians who may be prescribing those agents and

say, oh, well there's a wide variability in how patients respond to anti-hemostasis therapy. So wouldn't it be nice if you take a lot of your patients and see how well they're corresponding based on this test? And then, some people would argue, well, that is within the scope of what FDA granted the authorization for because the FDA granting authorization that this relates to anti-hemostasis activity.

So however we market it, as far as it is patient related to anti-hemostasis activity, it is within bounds. So sometimes there's some push as far as what people might push towards the envelope of what is within bounds. So another thing that comes up as far as post-market activity is, well, over time there's different batches. There's different lots, whether it be calibrators, reagents. And sometimes those don't perform the same as what was evaluated in the premarket setting. So for INR, PT/INRs, there are different devices out there. Over time, there may be issues as far as how well their performance corresponds to other methods or more widely established or accepted methods. And there are some post-market considerations in that setting of what is the level of concern of that drift in performance.

What is the appropriate level of action that should be taken to respond when those things arise? And it could be that there are interferences. There are certain medications or patient scenarios where the test for PT/INR, there are some situations where patients with elevated INRs on some devices are not picked up consistently. So when that happens at some frequency, at some level, when is the appropriate level of response as far as notifying healthcare providers, as far as potentially correcting the device, as far as potentially taking products off the market?

Okay, so I'll just give you a sort of corresponding hypothetical situation. So sometimes people come in and they say, well, there's some situation, there's some drift in the

performance. And they will say, well, because there's so much variability in patients, it's not a big deal. So this morning some of you may recall there was sort of, you know, the analogy of if there was so much variability in height, it'd be very hard to pick, you know, basketball players or volleyball players if they shrink up and down in height all of the time. So -- well, so if someone comes in and they have a direct oral anticoagulant product and they say, well, trough levels of direct anticoagulants can be -- vary fourteen-fold. That may be true.

And so, therefore, if my performance in my lot drifts by fivefold, that's well within the variation that you see in people. So FDA should not be concerned about fivefold conformance, right, because that's a minority of the variation you see across people and individuals. So it's not a problem. Okay. So I don't know if, I'm assuming, much of the audience has a regulatory, necessarily, experience. But sometimes these are the types of things that come up. So if there are not clear expectations for what is acceptable performance, both in the pre and post-market setting, that can raise complexities as to whether the product is performing consistently and at the level that is expected by clinicians who may be using it to inform key decisions.

So just as that sort of -- although that was sort of a strawman argument, it may not be what manufacturers are expecting or proposing to claim for DOAC testing currently. A general question I would raise is that if the general consensus is that the evidence base currently for a so-called therapeutic range or routine therapeutic monitoring of DOACs may not be as yet supported, that perhaps it might be appropriate to state that in whatever marketing authorizations that might be considered, that the current evidence or the current evaluation does not establish this particular test for routine therapeutic monitoring and that that would therefore translate into some sort of boundaries or scopes of what might be appropriate

promotional activity for those products. And then, also what would be alternative appropriate premarket and post-market considerations in that context.

So I'll review some of the other use settings that have been raised. First, I'd just like to say that I'm not trying to say anything about any particular potential test. There's diversity and heterogeneity of potential tests that might be of interest. Generally, one could have tests or outputs that are more geared toward levels of drugs, particular drugs or they might be even more sort of broad as far as classes of drugs. They might relate more towards coagulation activity and not really be directly linked to levels of drugs or classes of drugs.

Or they may be in some cases looking at coagulation activity and translating them into some estimated or corresponding levels of drug. So those are all different scenario's. They may have somewhat different interpretations. One comment is that as you've heard there are different specimen types that are being considered. And in the field of hemostasis testing, there have been even considerations for non-blood, so testing that is not even blood-based.So those different specimen types all raise potentially different considerations as far as what the appropriate strategies and details of performance studies that would be appropriate. As you've heard from Marina in the previous talk, different outputs and interpretations may be relevant and even in some of the proposals from the companies, that whether they think a qualitative test would be appropriate, whether a quantitative would be appropriate. And then, the differing use settings as far as whether it's outpatient setting, inpatient setting, patient with a clinical presentation of bleeding or clotting, patient who is not otherwise bleeding or clotting but being considered for intervention. So those all may factor into the appropriate evidence.

So I would just break it down into a few sort of general topics. One is that it's not for all patients. But there may be particular patients where you are especially interested.

And so, some of these -- this, you know, altered metabolism, the body habitus, altered renal function, whatever the case may be. It may not be that every single patient needs to be routinely tested. But there are some populations of particular interest. So that would be a general, if that's at an operational level that makes sense. I guess the operational level of what is the appropriate device studies would be, well, does that mean different drugs depending on how much there is, you know, renal excretion. You might need different patient subsets or not. So how would you appropriately target the patient? If it's not all patients, then what are the right patients? And which of those right patients is it the same for every drug? So that's just an operational discussion as far as what would be the appropriate device studies to perform.

Another general category I would group them under is that it's not for all -- perhaps not for all routine clinical presentations but only under certain acute or urgent or emergent settings. So that could be in the emergency setting, as we heard from Dr. Korley. So is there an appropriate way to represent those underlying clinical conditions? So we heard that, well, the volume is low. It's difficult to get all these specimens. But it is not necessarily saying that a patient with presentation of concern for stroke has the same consideration or underlying coagulation profile as patients who may just be being consider whether they have invasive placement done with more or less concern. So their underlying hemostasis profile may be different. How much that influences the performance of the test may be an open question.

So this last part, I'll just try to run through some of the common themes that often come up for device development and some of the, you know, logistical issues that may come up. These three points, the first one is when you can't get clinical specimens. So people say, well, I can't get -- it's difficult to get the relevant patient specimen. So then I need to come up with alternative materials. And the last two bullet points are related to qualitative or ordinal

and numerical performance. This is sort of a follow-up to some of the points raised by Marina in the previous presentation.

So obviously if available, having the clinical specimens that reflect the intended use patients and whatever clinical presentation is relevant would be preferable. The concern of using alternative sources is whether those specimens really represent the performance of the device. So some of the issues that have been raised are, well, if you have free or if you have bound forms of the drug, does that reflect what your comparator method or your patient specimens really look like? In cases of clinical presentation of underlying conditions, presenting with bleeding or clotting, do those underlying hemostasis conditions, are those being reflected? So if you have a whole-blood specimen, the patient is bleeding. The hematocrit is lower or higher or their platelet function is not affected, how does that affect your overall coagulation assessment or hemostasis assessment as opposed if you look at healthy, normal people and you do or do not use spikes or use whatever calibrators or whatever you put in or manipulate the sample.

So one of the proposals you've heard from one of the manufacturers is that the qualitative detection might be an appropriate use. And so, the questions there -- so unlike Marina, I've somewhat combined qualitative and semi-quantitative as far as having a single qualitative threshold or multiple ordinal thresholds. So that could be presence or absence. It could be below on-therapy range, on-therapy range, above on-therapy range, depending on the design. And that some of these may have different clinical expectations as far as performance. So I'll just give you an example. So sometimes people might come up with, say, well, I'll just detect the presence or absence of DOACs. Great. So that's a fine concept. Then the question next is, okay, how well do you need it to perform? So people might say, well, I'll get it 80/80.

So 80 percent of the time, if something's there, I'll pick it up. And so it's non-random. It's informative. Okay.

So is 80 percent, is 80 percent picking up of when a direct oral anticoagulant present, is that really good enough? Now, if one of the settings we just discussed was, well, a patient has presented with a possible stroke and you're concerned about whether you give thrombolytics, and you say, well, 80 percent of the time, if something's there I'll pick it up. Not sure how good people feel about that. Okay. Now, whatever. You can always argue about the number. Then of course besides the number, there's point estimate, lower confidence bound. So is there -- are you talking point estimate, are you talking lower confidence bound as far as how often you're picking up presences of the drug?

Okay, and then of course how are you actually sampling these patients? So you're taking perhaps patients who are not having any presentation of hemostasis disturbance. And are you sampling them randomly? So if you're sampling them randomly, you're not getting all trough levels. You're getting -- you're throwing some higher levels in there and you're getting -- and that's contributing to your 80 percent-plus sensitivity. And then, if you're randomly sampling patients, well, there are all these factors that we mentioned that may contribute to variability as far as obesity, as far as renal status.

So do you want to take people with normal renal function? So do you want to have whatever percentile detection of presence of DOACs in normal renal function and normal body weight? You want to sample people with altered renal function? You want to sample people with altered body weight? You want to sample more frail, elderly patients? So all of these, as far as sampling strategies, do factor in as to what is the appropriate study you need to perform.

And then, as far as a numerical level, so I think at a high level there's some presentation of some of the, you know, Reilly or other work by our CDER colleagues as far as there is some exposure response relationship that can be derived. However, whether that in the context of the extent of variability that we've discussed at the intra-patient level, is that support [for] adequacy of expected values and reference ranges for clinical consideration? So again, similar to the qualitative discussion, expected values, let's say I know that 5 to 95 percentile falls somewhere, 5 to 95 percentile of what sort of patients? Of again, higher BMI patients, normal BMI patients, normal renal function patients?

Is that -- what is the expected value of right population to sample to say that this expected value and reference range supports the clinical use settings? So is 95 percentile enough? Ninety-five percentile, that does mean if you have 5 percentile, 95 percentile, there is 5 percent of patients that would be defined as outside that range. So is that reasonable expectation? Is that 5 percentile a point estimate, lower confidence bound? Obviously lower confidence bound means different things as far as sample size and power and all that implications too.

And then, you know, acceptable bias and variability in analytical performance. So I alluded to this more in the example I gave of, well, if someone comes in and argues, well, there's more than tenfold variation across patients. So my CV of a hundred percent is okay. Right? Because, well, patients vary all over the place. So therefore, I don't need to have really tight performance. And if in the post-market setting my lots vary by one-fold, twofold, that's okay too. So those are the type of -- I'm not saying anyone here has specifically said those types of things, that sometimes come up in regulatory decision-making. And then, something that has been brought up as far as even in the setting of the best available method may be

mass spec-based, that having the right mass spec, whether it picks up free or bound versions of the drug and whether those translate to clinical activity and so forth still requires some nuanced thought also.

So hopefully I just gave you some sense from the regulatory perspective for people who don't always sit as far as making decisions on pre- or post-market decisions, why the routine therapeutic monitoring may not be the case, but how that would inform pre- and post-market oversight, that other use settings, there's still heterogeneity and variability that may still need to be factored in thoughtfully and what are some of the other regulatory considerations that come up. All right. Thank you.

MS. GUITY: We will now take questions for Dr. Kondratovich and Dr. Tzou.

QUESTIONS FOR SPEAKERS

QUESTION: Dr. Kondratovich, please. I liked very much your presentation. I was just a little bit confused about the qualitative test and the Gaussian curve you have shown. So for me, the yes and no reply does not result in a Gaussian curve.

DR. KONDRATOVICH: Yes. Yes, you're right. It's only like example.

QUESTION: Thank you.

DR. KONDRATOVICH: So usually, it can be very unusual shape, especially for samples without drug. You're right. It was only like example to show basic concept. It can be -- this is reason we need to consider 95th percentile, not how we call parametric assessment. So where is your 5 percent or 1 percent above this threshold? This will be your limit of blank or cutoff for your test.

MR. GOSSELIN: I'm going to be in trouble again. I'm a little disappointed in the last two presentations because they seem to want to relate a numeric value with a clinical

measure or a clinical outcome, which means we're not going to get something for a long time approved in the U.S. And I think that what we need is to be able to measure right now which is separate from a clinical outcome because right now we can't even get out of the box. It seems like we have a chicken-and-egg thing going where we have some people say, well, we need to do some therapeutic monitoring, versus having to be able to assess somebody who comes in where they need to make an intervention based as, in part, just a tool in the box saying, well, we have this level, we have this result and this result and this result. We're going to put them all together and look at this patient and make a clinical decision.

Like Dr. Moll said, he had results that went outside the range. But you know what? He gets paid a lot of money and he's a smart guy to make clinical decisions based on numbers and the clinical presentation. And it's concerning that we don't have tools right now for drugs that have been out for years to assess these levels and not make a correlation doing levels and clinical outcomes. I think that is a real struggle that we're going through right now. And it looks like it's not going to change any time soon because the last two presentations want to have numeric values equal outcomes. And that's difficult for the clinical industry to do. That's a huge, huge study for such a small yield. We don't do that many. But we need it. We've got to have it. And we can't send it to LabCorp to get it back two days later. We need it within minutes.

DR. TZOU: Right. So I'm not sure that is necessarily the interpretation or the intent. So I think what I tried to suggest is that depending on whether or not there is a correlation between numerical outputs and clinical outcome that would inform the appropriate regulatory pathway. So that if there is a relationship, then the basis of the relationship would inform the appropriate evaluation. I think the question I raised is if that relationship is not as

well understood, what then would be the basis to establish acceptable performance in the pre- and post-market setting. So it is not that only in the presence of a relationship. I don't think that's what I tried to suggest. What I suggested is in the absence of a relationship, what -- is there enough understanding and expectation of what appropriate performance would be. That's all.

DR. MOLL: I will add onto those two comments. I think the relationship between levels and outcomes has been clearly established for dabigatran, Reilly data and for edoxaban, the edoxaban study data that I showed. I highlighted that for rivaroxaban and for apixaban, those data are not available at this point. I fully agree with Bob Gosselin that it's not a role, in my opinion, of the testing companies to create clinical laboratory correlations where the huge companies have failed, the rivaroxaban, apixaban folks. This cannot be put onto the smaller companies. That's number one.

Number two, I think the anti-Xa assay has been well-established as a reliable, sensitive, well-correlating test with mass spec assays. The mass spec has been used for the correlation studies in dabigatran and with edoxaban, that I mentioned. I think the anti-Xa assay in my practice is ready to be used and I send the test. I don't need further correlation studies. There are plenty of clinical questions in every field of medicine about DVTP management, thrombolytics, monitoring, the meaningfulness of certain tests. But I don't think that can and should hold back the availability of the anti-Xa tests.

Now, the ecarin clotting time, very similarly, the chromogenic or the clotting time has been well-established as a well-correlating test with mass spec levels. I again refer to the dabigatran data of clinical outcomes. So in my practice, the ecarin clotting time or chromogenic ecarin time is ready for clinical use. And I think they should be available. The

additional test that may be needed for proof for the FDA probably has to do rather with the replication, the lot number issues and the variations that I'm not familiar with. But Dot and Bob, you can talk about that. But those seem relatively minor things. I don't think we need to go into special patient populations with the laboratory test development companies where that's really responsibility of investigator-initiated studies, drug companies, the NIH and other sources.

Now, I've taken up five minutes or 10 minutes. I have two questions to Dot and to Bob. Dot, do you think the anti-Xa assay is ready for clinical use at this point? Yes, no?

DR. ADCOCK-FUNK: Yes.

DR. MOLL: Bob, do you think the anti-Xa assay is ready for clinical use? Yes, no?

MR. GOSSELIN: Yes.

DR. MOLL: Thank you.

MR. GOSSELIN: Calibrated to the appropriate drug, not just random. Screening, yes. But quantifying it, it'd have to be calibrated to the drug. But yes.

DR. ADCOCK-FUNK: So to add to both of these comments, I feel that many of these assays have demonstrated that they can accurately measure drug concentration over a given range and they can do it with a linear response. And so, I think the companies have really shown that these assays are available. And certainly the companies can manufacture reagents that show lot-to-lot consistency. I think, you know, that's something that can certainly be accomplished. And it's really, I believe, up to the clinician to determine what to do with that number. It's important that we make that number available to the clinicians. And we can do that with a reliable, accurate assay that is available today.

MS. GUITY: If there are no further questions, we will start our panel discussion.

And Dr. Dollins will lead us out in that.

PANEL DISCUSSION

DR. DOLLINS: And so, they all leave me. Well, so, I guess I hear the sentiment in terms of disappointment. Dr. Tzou, I think he really outlined the difficulty with, you know, if there's a broadly defined intended use and a premarket authorization, what that does to us in terms of the post-market. So what we would like to know, , I think we had a lot of discussions on the intended use populations. I think we're kind of clear on like the kinds of patients that we would want to assess.

But you say you have a really good understanding of the types of -- you know, the performance criteria for these types of tests. What we want to know from you, we want your input on what those performance criteria would be. Like what is the acceptable amount of variability? Like where on the post-market side would we have to step in and say, well, no, at this point, even if you're reporting out a number, where would you draw the line? What is not acceptable anymore? So that's kind of what we're looking for. Anybody?

DR. MOLL: Can I respond to that?

DR. DOLLINS: Yes, Dr. Moll.

DR. MOLL: So Marina, I don't think there is a need. I think a quantitative assay is always better than a qualitative assay. A yes/no or the low/medium/high, what have you. The variability in individual patients is so high and the therapeutic range, on-therapy range is so large, to me it does not matter whether it's 200 or 300. I want to know is it 200 or is it 600 ng/dal. I want to know is it really within the therapeutic expected range or is it way outside. That's the one thing. I don't really care about values of 20 or 15 ng/dal, which is a really low level where I wouldn't expect any bleeding issues. I would not delay any surgery. I would not

not give TPA for that reason. So at the lower end, anything -- I'm grabbing this number now, less than 40 is fine with me. You can just report it's less than 40. That is almost sufficient.

DR. DOLLINS: So would you suggest like tiered acceptance for lower range would be not quite as important versus --

DR. MOLL: I don't need to say that. I just say give me the quantitative number and I will say, okay, it's 35. That's really low and go ahead with the LP. So if the quantitative number is sufficient, there does not need to be an interpretation. And I think they cannot and should not be because sometimes we don't know how to interpret these.

DR. KONDRATOVICH: [Off mic.]

DR. MOLL: For me as a clinician, less than a certain number is sufficient because I wouldn't quite understand what the laboratory folks are talking about anyway. What is -- if you say it's 20, how many percent of those could be at low level or are really negative. I just know, okay, this is either completely negative or not -- or is at such a low level, it's clinically not relevant to me.

DR. TRISCOTT: But wouldn't you --

DR. KONDRATOVICH: [Off mic.]

DR. TRISCOTT: No. What I was going to say was wouldn't you, you know, look at the analysis that you were discussing and looking at a limit of quantitation, a limit of detection and have the -- you know, have the assay labeled appropriately? It may go down to 20 ng/mL and therefore you could say it's still less than 40 but it's between 20 and 40, so --

DR. KONDRATOVICH: [Off mic.]

MALE: Nobody can hear you.

DR. KONDRATOVICH: Okay. So I'm like repeating what's suggestions, at least to

do maybe some analytical evaluation of limit of blank, yes, with some limited number of patients, like maybe like according to CLSI, yes, that at least you understand what is the threshold for presence and absence. Thank you.

DR. DOLLINS: Does anybody else have any opinions on the matter? Of course.

MR. GOSSELIN: Yes, I do. Again, I think it depends on the question being answered. When Dr. Moll is seeing patients, is it an outpatient service? He's monitoring patients. If somebody's coming into the emergency department with a stroke or somebody wants to do an intervention, it may be: is it gone? So wanting to know if they're going to put in some neural amnesia[anesthesia?]. Is it gone? That's a qualitative issue to say, yeah, it's pretty much gone. So I don't necessarily think that one test needs to encompass the zero to 1,000. It would be nice. But we have other things in our toolbox we can use. I mean, if somebody said can you tell me if you have something less than 50, that's the lower limit? Can you give me something else? Yeah, we can give you something else. So the lab can be pretty creative. So if it's not a zero to 1,000, we can figure it out. It depends on the question being asked.

DR. DOLLINS: Okay. As we --

DR. REILLY: Hi. Paul Reilly, Boehringer Ingelheim. I feel like I should add a couple of paragraphs to limitations of the study for the study that's been discussed today. Everybody showed that figure, that nice figure that we created. And I knew it was going to be very impactful. And looked at that as that's the population we're dealing with. But in fact, that figure represents the risk-benefit for a 72-year-old male with previous stroke. If you look at figure one in the paper, you will see that the variability in the benefit-risk curves, for instance, over decades of age, varied tremendously so that that risk-benefit proposition that is brought out in the paper is very variable depending on who the patient is. And that cannot be

overstressed because if you're trying to make a decision about dosing, that comes into play.

The second thing is, and what really I wanted to emphasize in the paper is that the demographic factors are as important or more important than the blood levels. And it may well be that based on age or renal function or history of previous stroke alone, you can make decisions about whether a patient is adequately dosed or not. And in fact, you see that in the European prescribing information, that we have those conditions as reasons to go to the lower dose of dabigatran. Of course, we can't here because we don't have that dose. So those issues are really important in trying to decide whether or not you're going to use this paper for, let's say, dosing decisions. And if you wanted to dose adjust, I will point out we did a study called the REALIGN trial in prosthetic heart valves. We tried to dose adjust and we failed abysmally. We also have a pediatric program where we tried to dose adjust, also failed.

So just because there's a relationship there on top of all the demographic factors, it's still a long way from being able to actually adjust a dose based on some measured parameter. And just one response to the CDER group, they showed the variability between within patient variation and across the population variation. What's really important for dose adjustment is within patient variation. And you compare the within patient variation on dabigatran of 30 to 40 percent with the tenfold variation for warfarin and you see very rapidly why you need to dose adjust for warfarin. But you may not do that for dabigatran. Thank you.

DR. HOFFMAN: I have a comment about clinical laboratory testing. So, relevant to the earlier comments, you can say that a lab test has a reportable range and therefore tell Stephan, oh, well it's less than our limit of detection, without actually saying there's none there. And that would be how we usually would report lab results: is it less than our reportable range or greater than our reportable range. And in the clinical lab, I think that what we try to

do -- our experts can comment too -- is that we try to make sure that we give results that help the clinicians make decisions at particular decision points within what we're reporting. So if you're reporting a fibrinogen value, the clinicians don't care if it's, you know, 500 or 800.

But they care if it's maybe less than 50 or greater than a hundred. So there are decision points where it's important to have some idea of what the accuracy of your result is. And then, there are other parts in the range where it's really not terribly important. And so, that depends on the purpose for which you're using the test. So I think saying, well, it's linear from zero to a thousand is nice. But that may not be what the clinicians are concerned about. And when you're asking questions about what should the precision be or what should the variability be, well, it maybe matters at what point along that reportable range that you're talking about.

DR. RAVIV: Gabriel Raviv, from Coramed. I just wanted to touch base on your statement, Stephan, that the numerical is always better than qualitative. And I just want to remind everybody what we're trying to test here is the coagulation. The numerical value of how much drug you have in the plasma doesn't always translate to what happens with coagulation and with hemostasis. You can have a little of a drug. But for you, that's too much. And I think the qualitative has a lot of benefit in telling you what potentially could be your outcome if you have this drug on board versus knowing how much of the drug you have on board. And that's proven many, many times over with products like the thromboelastograph and other products like that.

DR. DOLLINS: So I guess if I can follow up on that, I was kind of curious if there's any kind of clinical scenarios in which qualitative outputs would be more useful than quantitative outputs or is it always the case -- is it the feeling for you -- do you guys have the

feeling that quantitative outputs are always more advantageous?

DR. MOLL: Can I retrace that? I may have misunderstood, Marina, what you meant as qualitative. I thought you were referring to positive/negative, not qualitative in the pharmacodynamic sense or in the thromboelastogram, thrombin generation. And I think that's what you are referring to. That's a completely different story. And I would agree with you. The drug levels are in general inferior to a real qualitative assessment, what really happens physiologically. So I'm on your side and I think we've just had a different use of the terms.

DR. KONDRATOVICH: [Off mic] -- with regard to measurements, that what you measure, it's really representing all proportional to the true value. And if you well calibrate it, then it will be like your true value of drug level, for example. And if I measure very well and calibrate it very well, then this will be like quantitative from analytical point of view, that what I measure, it has good properties with regard to my measurement to the drug level, how it's related to the, for example, clinical outcome, we don't know. How it's related to coagulation activity, we don't know. This is not related to analytical, yes? So what I call qualitative is really more like with threshold, positive/negative or maybe very simple present/absent. Yes, and of course in most situations, we don't have relationship to clinical outcome. What is your opinion? That there are no points to give quantitative values in the situation, yes? This is your point because there are no relationships, why you giving all these numerical values, especially if you're telling that, for example, precision should be 10 percent or 20. So can you clarify a little bit your way of thinking, why you think it's not important?

DR. RAVIV: So specifically thromboelastograph measures the coagulation, the strength of the clot. And that's the final product, irrespective of what caused it, et cetera. And if you can associate that with safety or non-safety of a drug on board and you know you reverse

the drug -- let's say if you have a reversal agent, that is more powerful than figuring out how much drug you have on board in the plasma. And as you see, the numbers are all over the place. But what Marc has described, the product he described specifically showed you that you are hypercoagulable or not or hypocoagulable and where you are. And that's the strength of the test like that.

DR. MOLL: That's the potential strength, as you say, if there are data on the clinical correlation with it. And unfortunately, you know, often with tech and thrombin generation, we don't have that or it's --

DR. RAVIV: Not thrombin generation. Specifically, for these two drugs, where you are compared to normal, for example, or compared to patients before they took the drug and after they took the drug.

DR. CUKER: I just wanted to make a comment. So there's something that -- and I may not be able to articulate this well. But something that makes me think that when we're talking about a qualitative assay, where we're turning one or more continuous variables into a dichotomous result, that then there is a greater burden to somehow connect with clinical outcomes. I buy the idea -- I drink the Kool-Aid that if we have quantitative assays that correlate very well and very reliably with tandem-mass spec, I'm comfortable with that. But when I get a dichotomous result, we're synthesizing all sorts of complexities into a yes/no answer or, if it's semi-quantitative, a few categories. And how do we know what that means without clinical outcomes? So I mean, how do we set this threshold? That makes me uneasy as a clinician.

DR. MOLL: You lose information. We have that with anticardiolipin antibodies, with HIT testing that some people just say the anticardiolipins are negative or positive or

intermediate. Give me the value because then you really have the range and you know it's impressively positive or negative. And with HIT, we've had that too. Some people report the HIT test as it appeared before as negative or positive. And we've learned that the quantitation matters in the prediction of HIT.

MR. DOUBLEDAY: Right, and if I can comment on that. I mean, the purpose isn't to predict outcome. It's to guide treatment and therapy, right? I mean, right now in terms of knowing if a Xa or a DTI is on board on that patient, we have no assay. We have a sensitivity and specificity of zero, right? And here, so this empowers the physicians, you know, for thrombolytic therapy to really guide them and help them. You know, we talked to one neurosurgeon and we said, you know, what do you do if you think this person might be on a NOAC. And he said, I pray. And I said, you know what, we have to do better than that, right? I mean, we really do.

DR. CUKER: And I'll just add, so by no means do I question the motivation for this and the clinical use. I mean, it's really important. But boy, where you set that threshold is so important, right? If you get it wrong in either direction, you're putting patients at risk. You're either -- if you're too conservative with your threshold, then you're depriving patients who should be getting thrombolytic therapy and in whom the benefits outweigh the risks of potentially life-saving therapy. If you are too liberal with your threshold, you put patients at serious risk of bleeding. So where you set that threshold is so important. And it's hard for me to imagine how you do that with no clinical outcome – no knowledge about clinical outcomes.

DR. ADCOCK-FUNK: But the threshold --

MR. DOUBLEDAY: Can I -- just one more? So I mean, I presented some information from the paper where if a patient has been on dabigatran 60 percent of the time,

they won't get TPA. Right? I mean, we don't have to make that a hundred percent. If we can make that decision-making so 80 percent of the time they get TPA or 90 percent of the time they get TPA, that's a marked improvement.

DR. MOLL: There's been one study. Dr. Reilly, do you recall that it's a thrombolysis study in stroke where people on one of the anticoagulants, and I thought it's dabigatran, received thrombolytics and there were some outcome reporting. And it seems like from that, one could -- I thought they also calculated back how long prior to that they took the dabigatran so that one could come to some conclusion what this lower level of safeness is where you could give thrombolytics. Do you recall that?

DR. REILLY: I do not. I can only tell you we treated one patient in the antidote trial who had thrombolytic onboard. But other than that, I don't recall what data you're talking about. Sorry.

QUESTION: I would like to make a scenario which is you have a patient in terms of care [in an] ambulance and you want to know the result immediately. He has on board new anticoagulant or not and you have a point-of-care test which is very sensitive and very specific and accurate, maybe 99 percent or 98 percent. And then, it comes out that this test, after 10 minutes, is negative. And so, he has no drug onboard and clinical decision can be made and patient can be treated according to what is necessary. I think this would be a good example for a qualitative test. And if the test is then positive, then you always can go on with the quantitative testing.

DR. MOLL: So I would agree with you. That would be nice to have. And if that's easy to do and you can carry that on the ambulance, great. I think the general statement that a quantitative assay is preferable over a yes/no assay would apply to that situation as well. If this

test that you have on the ambulance could be quantitative assays, even more power to you because then even with low levels, I would feel comfortable giving TPA. But in the absence of having a quantitative assay, yes, I think there is a role for these yes/no tests.

DR. CUKER: And I would just add, so I think there's a role for an assay like that too. But I would imagine that you would decide to set that threshold between negative and positive as very, very conservative. So that relatively small concentrations of drugs would register as positive. So if somebody gets a positive result, that doesn't mean that they're unsafe to undergo emergency surgery when the ambulance brings them to the hospital. So you still in some cases need more than just yes or no, depending on where you're setting that threshold.

QUESTION: I like all the discussion. I would bring in another point, which is repetition of the blood sampling or of the analysis. And if you have -- if you have another scenario, you can get sampling which is not invasive, like from urine, you know. Then you can have repetitive measurement without an invasive procedure, which is also of harm for the patient always sampling or every two hours or so. And so, this is another situation where qualitative tests, in this case from urine, might be helpful.

DR. DOLLINS: If I could steer this in a slightly different direction, so there's a lot of concern about the potential need for clinical studies that are associated with outcome. We were wondering, like, what [is] the difficulty -- obviously there's a difficulty with performing these largescale trials as were done as part of the drug approval. But are there any alternatives that you can think of like, you know, bank samples and things like that and what is -- you know, what is the difficulty of obtaining samples. And I think we touched on some of the issues, like the frequency of testing. But if you could kind of go into that as well a little bit?

DR. MOLL: Well, I can make a statement because it came up in the lunch break, that one of the test developing companies approached me and said would you be interested in a site to test our assay in the patients on the DOACs that you see. And I said, that sounds awfully boring. It just means having to draw plasma samples from the patient without any scientific question really behind it, just to get your test FDA-approved. Give me a study that looks -- or let me create a study that looks at a special population, at the morbidly obese patient, treat them with DOACs and then get a peak/trough level in a structured manner. That would be interesting to me. But just these general tests or studies to test samples for FDA approval for me as an academician, clinician is not interesting. And it's a lot of work to do, a lot of work indeed.

MR. GOSSELIN: And why are we doing this?

DR. DOLLINS: What do you mean?

MR. GOSSELIN: I mean, why are we doing this --

DR. DOLLINS: Well, we talked about --

MR. GOSSELIN: I mean, there was a concern about -- again, you're talking about a clinical outcome study.

DR. DOLLINS: Right.

MR. GOSSELIN: You're doing a clinical study versus doing a traditional comparing method x to method y, less robust but does the methodology work. So those are two different questions being asked here. Are we talking about measuring and monitoring patients over time and doing dose adjustments or are we talking about a lab test that can assess the value? What question are we asking?

DR. DOLLINS: Well, so the concern is obviously we would like to see the kind of

validity -- we brought this up in connection with the qualitative claim, like what is at the cutoff. So sometimes, you know, if you would have clinical outcome data, especially for samples right around the cutoff, it'd be really beneficial and giving some level of confidence that, yes, that cutoff was set appropriately, right? So I just wondered if there's -- you know, aside from doing -- like what's the evidence, aside from doing these largescale clinical trials? Like are there samples available that have these outcomes, you know, like maybe from the previous clinical trials? Or what is the literature evidence that's currently available to support any of that?

DR. ADCOCK-FUNK: Well, one of the issues is that it really depends on when the patient is drawn. I mean, would you have to have different levels for peak, trough, renal failure? You know, we don't always know the situation or when the patient's been dosed. So I think that's really a tall order.

DR. DOLLINS: Right.

DR. MOLL: So these samples that are banked, that I don't know where they are banked, but obviously the Boehringer Ingelheim folks had samples. The edoxaban people had samples. I don't know whether the rivaroxaban and apixaban phase III clinical trials did collect samples. But if they did and if those are somewhere or if the data are already available, they should be published. And Dr. Reilly, you had difficulties publishing it. But you succeeded. And if those -- people just need to be encouraged to contribute that to the scientific knowledge pool. And maybe that's a role of not your branch of the FDA, but of the drug approval folks that have approved rivaroxaban and apixaban, to talk to the companies and say, look, there's post-marketing, you need to provide additional data. I don't know how that works.

DR. DOLLINS: Right. I guess we could also say maybe for future trials, maybe it would be beneficial to also collect additional data. Do you guys agree?

MR. GOSSELIN: My little comment about the samples we've tested at UC Davis, maybe less than a handful have been -- we want to see what a peak level is. Most has just been random. They come in through the door. So getting peak and trough levels, you know, again, from a random standpoint, which is the majority of the testing we're getting, I'm not sure why we're waiting for clinical outcome studies because we need to know if what we're doing is accurate. I don't have a problem with what we're doing. But I mean, there's got to be more people in our neighborhood doing this kind of testing because we're the only ones in probably Northern California. And we're doing nobody else.

So it's again I think there's a reluctance to do something that's RUO, that's not FDA-approved. That is a certain need because a lot of people are going to these drugs. We need to do something now. We need to do something four years ago and we're still kind of waiting to do just a simple measurement, not an outcomes study but just to measure and give these clinicians something to work with, something as opposed to nothing. And I think that's where we need the emergency -- we need it right now. So if there's a fast track, by the end of the year we should have something approved from this company saying, yes, go ahead, and you can do it. And then you would see clinicians maybe saying, well, now we have other questions to be asked. But at least we've gotten out of the box a little bit.

DR. MOLL: So not to branch out too much to other branches of the FDA, but I was surprised that the dabigatran reversal agent was FDA-approved without really solid clinical outcomes. I'm [appalled] that the Andexanet folks seem to get FDA approval without any meaningful clinical outcomes. Yet here with the anti-Xa, these companies are being asked to provide clinical outcome data. That just seems over the top.

DR. DOLLINS: Right, I mean so I guess I want to kind of wrap this up, you know,

since we have been discussing for a while. I think from our side, we understand that there's a need for testing. And we certainly want to do, you know, everything we can in order to facilitate the clearance of these types of devices to make them available for our clinicians. We just want to make sure that they're also useful. So I guess do you guys -- maybe everybody -- do you guys want to have some sort of like one additional statement that we should maybe take into consideration, or maybe the main point, that if nothing else, maybe, you know, the one thing that we should take away from this day?

DR. HOFFMAN: I'd just like to make a comment. So I've been in the clinical laboratory business. I'm the director of a clinical lab. I've been in this business for about 30 years. Here we have potentially an assay that measures a specific analyte. We have a gold standard method, namely the chromatography mass spec method. We have standards that are intended for at least the chromogenic assays. So I really don't understand why an assay that measures a specific analyte can't be approved for the measurement of that specific analyte when it correlates well to the mass spec gold standard method and has linearity in the relevant range.

DR. DOLLINS: Right. I think we're trying -- the point we're trying to make is that everyone said -- you know, I keep hearing the term it correlates well. We're trying to define clinically what is well. Is it 10 percent deviation, is it 50 percent okay? I guess that's what --

DR. HOFFMAN: But the correlation's better than 50 percent CVs for the mass spec to the anti-Xa activity assays.

DR. DOLLINS: Right. So where would you draw the line? What is clinically acceptable?

DR. HOFFMAN: Well, what's clinically acceptable for any other analytical

method?

DR. DOLLINS: Well, it depends on what you're using it for, right? I mean, it's not -- yeah, it's risk assessment-based.

DR. HOFFMAN: Well, to some extent it does. But you're setting the bar way higher for this assay than for other assays of specific analytes, as far as I can tell. I mean, if you're measuring cyclosporine levels or gentamycin levels or something like that, where would you set the bar?

DR. DOLLINS: Well, that's the thing. It depends on the intended use. So the problem is not every assay is the same. So sometimes the variability of, you know, 50 percent, even a hundred percent can make no difference in clinical outcome or in clinical treatment. But we're trying to figure out what is appropriate for this type of device. I mean, we have the same criteria as Abe outlined. You know, we have the same criteria for evaluation of devices. It's just we're trying to figure out what the appropriate measures are in this case, what the appropriate allowances would be. Does that make more sense?

DR. HOFFMAN: Not really. No, it doesn't. It doesn't make more sense. But --

DR. DOLLINS: So what don't you --

DR. HOFFMAN: But there are other tests where you set -- yes, where -- exactly, where you know that there's a toxic level and the test needs to be able to detect that level with a CV of 10 percent.

DR. DOLLINS: Right.

DR. HOFFMAN: Well, so if there is a level which is toxic here or which is a clinical decision point, then why can't you decide that at that level, you have to have performance with the CV of 10 percent?

DR. DOLLINS: Right. So where would you -- that's a time --

DR. HOFFMAN: But you keep talking about 50 percent or a hundred percent. That's -- in no test in my laboratory is that considered acceptable performance. That isn't what we're talking about.

DR. DOLLINS: Right. S o I think you're getting bogged down by the numbers. So what I'm trying to refer to is, you know, we are trying to establish exactly that, like what are the criteria for this. You're saying, okay, there is a toxic level. What is a toxic level for this type of test?

DR. HOFFMAN: Exactly.

DR. DOLLINS: Right.

DR. HOFFMAN: I don't think there is a specific toxic level. But the thing is that if you don't have a measurement method available to you out in the field, you're not going to figure these things out. You're not going to do the tests. You're not going to correlate with patient outcomes because you don't have the tool available. And we need the tool available, the tests. At least some of these tests will give you accurate values, reproducibly. And we can then at least have something to work with to figure out where our clinical decision points ought to be more precisely. That information is simply not available in great detail, as Dr. Reilly pointed out for dabigatran. So I'll stop ranting.

DR. MOLL: You asked for a final, maybe summarizing comment. And this is mine. I think your group should not be asking for -- and you phrased it -- you don't need data that these tests are clinically useful. You should not be asking for the usefulness. I think you should be asking is this test reliable in showing what it's supposed to show, which is, in this case, a laboratory correlation with mass spec and not with clinical outcomes. The clinical

relevance of the issue, that should be left open. There's a lot of open questions, as we discussed. And that should be left over to Maureane Hoffman or Adam Cuker or Bob Gosselin and the clinicians and the future clinical trials.

DR. REILLY: I echo that. I was intimately involved in development of the reversal agent for dabigatran. And the FDA approved last week a label with purposefully included APTT, thrombin time, dilute thrombin time and ecarin clotting time and the profiles, not necessarily knowing what their value is in an individual patient, but so that the clinicians have some information about what happens to these tests. Now, in our case, we have 100 percent reversal almost immediately in almost all patients. But if it was only 50 percent reversal based on one of these tests, we don't know what that means in terms of, you know, the risk of bleeding or the risk of clot. But the numbers are there for clinicians to use and interpret how they like. But at least we collected the data on it.

MR. GOSSELIN: And we only have two of those four tests you just mentioned. We can only look at two. And Claudia, I don't want to gang up on you here.

DR. DOLLINS: Right.

MR. GOSSELIN: Like it seems like you're doing a little pushback here. I'm just kind of curious what measures you folks are using when you say, well, we want to look and see about this and we want to look and see about that and then we're going to decide. So what yardstick are you using? Because there are a lot of published data, like Stephan was saying, that you see linearity. You see good correlation. You see, you know -- so what's holding up? Who's driving the bus here? We should be already saying, oh yeah, that stuff's good. Let's go. So kind of what are you folks using? Do you have committees that are making recommendations? Do you have advisory panels? Because if you do, I'd jump on one saying,

yeah, here's what I think we need to do. Here's what we don't need to do anymore. Here's what we've already done.

And some of this stuff's been done by published studies, reputable journals, not, you know, impact factors of 0.5 or something, but good journals that shows this stuff works for what we need. You know, again, some other stuff, we don't know about the future and the use of these tests. And you will never control the appropriateness and the usefulness of tests ever, ever, ever, ever. You know, that's -- you know, when it shouldn't be ordered and that kind of stuff. So I think that we just need to get something going, again, quickly.

Not to beat a dead horse. The data's out there. The data's published. We have it. Everybody here would be willing to share it, I think, with small studies. But again, we don't -- we're talking about method A compared to method Y and do they match. And if they don't, what are the bias and can you live with that as a clinician and as a laboratory? We would inform our clinicians here's the bias. If you see it here, do something else or we'll do something else. But we've just got to move forward. Five years since dabigatran, five.

DR. DOLLINS: Understood. So I mean, our process is obviously very similar. So you know, we try to solicit input from the sponsors. Obviously, you know, they're supposed to justify their acceptance criteria for linearity, what is considered, you know, precision, like what is acceptable precision, what is acceptable stability.

DR. TRISCOTT: Just in terms of acceptable precision, you know, when we do a submission to the FDA, you will ask us for a limit of detection, limit of quantitation. You will ask us to get controls that surround decision points and it's usually three or four controls. And they will be run, you know, in times over 20 days according to the CLSI guidelines. And that will come in at, you know, around 5 percent or something like that. And so, you know, you will get

to see the precision of these assays. And we will have specifications for the correlations, for the R values, et cetera. So they'll all be right there. I think what it gets down to is what kind of clinical, you know, scenarios will you accept.

If we were to put in something like an urgent or emergent situation where you would need to measure this, you know, that could be conceivably taking a patient and looking at a peak or a trough and seeing if it was on-therapy. But, you know, I think that the real issue is, you know, how the clinical trials get structured. I think that somebody may have asked you for samples because they're all in the one spot. Otherwise we would have to go to multiple hospitals, get IRBs, set up contracts, set up, you know, protocols, et cetera, for each one of those, get them approved, which is a months' long process to do it under good clinical practices.

DR. DOLLINS: Right. I mean, that's why we have this workshop, right? I mean, if you want to say, okay, you have to do this giant clinical study and you have to have these acceptance criteria then there's no need to have a workshop and discuss this. The point of having this workshop is to find out what your comfort level is. Obviously you feel very comfortable with the qualitative output. I mean, we hear that.

And we're trying to figure out like, you know, if you would need -- for certain types of claims, if you would need more data versus other types of claims. So we're trying to find -- we're trying to define like what, you know, is relevant information to gather from our side. So you mentioned literature. Bob mentioned literature references. Well, you know, it'd be fantastic if you could share those so that we have an added comfort level. So that's the kind of evidence we're looking for and that's what we're trying to get out of the discussion.

DR. TRISCOTT: And just one other thought that sort of crossed my mind while

Dr. Tzou was going over his presentation was how would low molecular weight heparin stand up to that or unfractionated heparin, you know, to those sort of criteria as you were going through measuring it? I think I wonder if these are being set to a higher standard than some of the other drugs that are already through in being measured.

MR. GOSSELIN: Or even some of the tests we have now would never pass muster, PTs and PTTs because there's just no correlation to clinical outcome. DR. ADCOCK-FUNK: And if I had one thing to say, I would hope that if the FDA does consider approving assays, that they look very closely at the calibrator and how it's been referenced and has it been referenced back to the pharmaceutical drug or what it's been referenced against.

DR. CUKER: And I would just add that I think that there's a real need here to balance rigor with pragmatism. And I think, you know, it may be important to have detailed discussions about the standards that we should set for assays for each specific drug with a group of experts. And this may not be presented with evidence and this may not be the appropriate setting. But we have the on-therapy ranges. And we know that overall clinical outcomes were very favorable within those ranges from the trials.

And so, we have those as an anchor. And we can -- you know, think about it a little bit and set some arbitrary standards that say that if we -- as long as the assay is accurate and reliable, within 10 percent variability or less, across the on-therapy range and then plus or minor some amount on either side of the therapy range, then I think pragmatically that is all that we need as clinicians at this point.

DR. DOLLINS: All right. Well, thank you guys for the lively discussion. I'm going to hand the mic over to my division direction, Lea Carrington.

MEETING WRAP-UP AND ADJOURNMENT

MS. CARRINGTON: Okay. Well, just to summarize today. First, I'd like to start with some very special thank-yous. I would like to again thank our presenters and panelists for attending today and for that dynamic discussion. It has definitely raised some issues that we can look at and discuss further internally, hopefully partner and get information that we can use to decide what's an appropriate regulatory path. So that is certainly our homework and takeaway from our discussion today. I'd also like to thank our DOAC workshop committee, especially Claudia as the lead and for the hard work that went into preparing this program. It has been a long process. But we think it has definitely developed into very fruitful discussion and very good information that we need to move forward. So I believe all of that is open.

I think that just in terms of summarizing what we've seen today, clearly we understand that there is a need to have some method to measure the DOACs. And so, one of our, again, homework assignments is to figure out what's going to be reasonable in terms of trying to find that appropriate pathway. I think one of the messages that came across from everyone was there is a high degree of intra- and inter-variability between patients and within the same patient. Some of that is something we'll have to reconcile here as we look for a value that will be necessary.

We also have to think also as globally as we can in terms of saying if someone gets a measured value, will everybody be able to interpret it. We have a panel of experts here today and we want to make sure that anybody else who's obtaining that value can also come to that same conclusion that our experts who are here today would also do. So we have to think of that in terms of public safety. So that's a very important factor that we look at. And we also have to look at it across technologies. We have some whole-blood assays. We also have plasma-based assays. Those are things that we need to consider as we consider our regulatory

approaches.

I would say, again, so those were the main highlights that I think we took away was definitely the variability, definitely that we need to act swiftly and quickly to try to work together to come up with a regulatory path that will be necessary and useful. I don't know if there's any other questions that we have here today. Otherwise, we will adjourn for the day. And I do want to point out one other thing. The people asked about the slides and whether or not they would be available. So I am told that the slides will be available in two weeks and they should go to the confirmation that you got for signing up for the workshop. So please look forward to the slides coming out.

And as I pointed out when we started this morning, interact with us early and often. And I think some of the discussion we had today, we'd certainly be willing and ready to discuss with our individual manufacturers to re-approach some of these things. So thank you all. Have a very good day. Safe travels if you're traveling. Thank you.

[Applause.]

[WHEREUPON, the foregoing adjourned at 4:24 p.m.]

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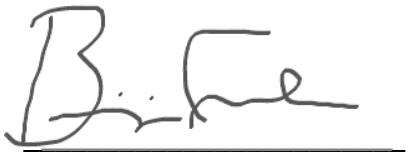
I, BENJAMIN GRAHAM, hereby certify that I am not the Court Reporter who reported the following proceeding and that I have typed the transcript of this proceeding using the Court Reporter's notes and recordings. The foregoing/attached transcript is a true, correct, and complete transcription of said proceeding.



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