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PERSPECTIVES ON IN VITRO DIAGNOSTIC DEVICES,
REGULATED BY THE OFFICE OF BLOOD RESEARCH AND REVIEW

FOOD AND DRUG ADMINISTRATION
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TUESDAY, JULY 16, 2019
8:30 A.M.

APPEARANCES:
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WELCOME:
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INTRODUCTION:
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KIMBERLY BIGLER, MLS(ASCP)CMSBB, DBCD/OBRR

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CONFERENCE

TUESDAY, JULY 16, 2019

8:30 A.M.

DR. LATHROP: Okay, everybody. Thank you for coming to the second day of our workshop which is going to be focused on considerations for IVDs regulated by the Division of Blood Components and Devices in OBRR.

Again, the slides will be available on the website in about two weeks, so take a look for them there. And moderating today's session is Teresita Mercado from the Division.

MS. MERCADO: Good morning. Welcome to session three of the IVD workshop. Our first speaker will be Dr. Wendy Paul. She is the deputy director of DBCD who will give us an introduction to devices in DBCD.

DR. PAUL: Good morning. Good morning, everyone, and thank you for attending the second day of this workshop. My name is Wendy Paul and I am the deputy division director for the Division of Blood Components and Devices within the Office of Blood Research and Review.

Our division is led by Dr. Orieji Illoh and we consist of five branches, all engaged in the review of biological products and medical devices. The DBCD mission is to assure the safety, efficacy, and availability of blood and blood components and related biological products.

We accomplish our mission through the review of applications from device manufacturers and blood establishments that manufacture blood and blood components for

transfusion as well as the collection of plasma for further manufacture.

We also review devices used in the manufacture of blood and blood components. And that consists of blood collection sets, the blood storage bags, including their anticoagulants and storage solutions, apheresis instruments used in the automated separation of blood components, and the devices used for pathogen reduction of blood components.

We also review immunohematology reagents for pre-transfusion blood compatible testing between donor and recipient. In addition, we look at things like plasma volume expanders which include things like albumin and hetastarch solutions, as well as hemoglobin-based and fluorocarbon based oxygen solutions for therapeutic use.

In addition to reviewing applications for licensure and approval, we also are engaged in developing regulations and guidances which govern establishing blood donor eligibility. This is both for the safety of the blood supply as you heard about yesterday from Peyton, as well as for the safety of the donor.

We also are engaged in establishing standards for product manufacturing, including acceptance criteria for quality control testing. And you may remember form the organizational structure that I showed you, we also have two branches within our group that conduct mission relevant research which facilitates innovation, both through the evaluation of novel products and the development of tools and methods which help us to evaluate those products.

In today's session of the IVD workshop, we will focus on the following products which are reviewed in the device review branch led by Teresita Mercado. You will hear about blood grouping reagents, reagent red blood cells, anti-human globulin, the molecular erythrocyte typing test.

You'll hear about human leukocyte, human neutrophil, and human platelet antigen and antibody test kits, as well as the automated instruments which are used to run those tests.

This slide is a table summarizing the products that are reviewed and regulated within DBCD. On the left you can see a list of the products and then on the right you can see the applicable laws and regulations.

You will note at the top of the table, top left, blood grouping reagents, anti-human globulin, and reagent red cells are regulated as biologic products under the PHS Act and as medical devices under the Food, Drug, and Cosmetic Act.

You'll note that the rest of the tests from molecular erythrocyte typing down, including the automated instruments, are regulated only under the FD and C act. So the first three reagents and instruments are regulated, the regulations are found in Section 21 CFR Part 600 and Part 800. And those products require the submission of a biologic license application, or a BLA.

The remainder of the tests, tests and devices, our regulations are found in 21 CFR Section 800. And depending on the complexity of the method, they either require a premarket approval application, a PMA, a traditional 510K, or in some cases can be 510(k) exempt. And you will hear more about that today during some of the sessions.

This slide, I just wanted to give you some background on the regulatory pathways and sort of highlight some of the differences, some of the things that are unique about the, in this particular slide, unique about the molecular-based assays.

So for the molecular erythrocyte typing test, even though this is a test that's used to determine the red cell antigen phenotype, it does not meet the definition of a blood grouping

reagent as per 21 CFR 660.20(a). And therefore it is not regulated as a biologic. It's regulated as a Class III medical device and so it would require a premarket approval application and a preapproval facilities inspection.

And of note, the HLA, HNA, HPA, antigen and antibody tests are currently unclassified and are reviewed under a premarket notification requiring a 510(k) submission.

In terms of the automated instruments that are used to run the assays that we review, they're, both for the automated immunohematology and the automated molecular erythrocyte tests, the devices themselves are considered Class II medical devices but they each have specific regulations.

So for automated immunohematology instruments, the regulation is found at 21 CFR 864.9175, while the instruments for molecular erythrocyte typing are found at 21 CFR 862.2570. And these instruments are considered 510(k) exempt, the molecular erythrocyte instruments.

I've put together just, I've covered this in previous slides, but just so that you would have a point of reference, biological products. The regulations can be found in 21 CFR 600 to 680, with specific subparts for the various reagents. And medical devices are found in 21 CFR 800's through 898.

I also included some things that you may not hear about today, but may be helpful for you when you're considering what to include, what kind of quality systems information. And with regard to PMAs and 510(k)s, there are guidances which tell you when do you need to submit a supplement after you've made a change to an existing device.

So today you're going to hear from three DBCD reviewers who will provide really invaluable information on what you should consider when you're putting together your

submissions. Our first speaker will be Kimberly Bigler, who will present licensed immunohematology products, part one. Then we'll have Annette Ragosta. She will present licensed immunohematology products, part two. And the final speaker of today will be Jason Liu, who will present the molecular-based devices regulated by DBCD.

At this time I would like to present Kimberly Bigler. I've included my email address if you would like to contact me with any questions. Thank you for attending and thanks for your attention. Kim.

MS. BIGLER: Good morning everybody. Thank you for coming back to day two of our IVD workshop. I am Kimberly Bigler. I'm a scientific reviewer in the Division of Blood Component and Devices.

So today this is the outline of my presentation. I'm going to speak about the licensed immunohematology reagents. I'll talk about bundling, submission organization best practices. The bulk of my presentation will be chemistry manufacturing and controls and product testing. And Annette Ragosta, who will speak after me, will focus on performance studies. And finally I'll talk about CBER lot release. That's specific to our immunohematology reagents.

So for the first topic, Dr. Paul presented the overview of our division, so our licensed immunohematology reagents again include blood grouping reagents, reagent red blood cells, and anti-human globulin.

And she also talked about the applicable rules and regulations that govern these devices. So currently our licensed immunohematology reagents utilize the following test methods. Slide and test tube methods for traditional reagents, column agglutination or gel technology, microplate, and solid phase adherence.

All of these methods can be performed manually or using automated instrumentations. And Annette and I will discuss automation as applicable.

So now we're going to move on to bundling, which is my second topic. So FDA has a guidance for bundling. It's listed on this slide. In the guidance, bundling refers to the inclusion of multiple devices or multiple indications for use for a device in a single premarket submission from the same manufacturer.

But for our licensed immunohematology reagents, bundling is more appropriate for multiple individual premarket device submissions that include the same indications for use and clinical or analytical data. And devices that present similar scientific and regulatory issues that can be addressed during one review.

So when I'm talking about bundling here, I'm talking about bundling submissions together for the purpose of one user fee.

But we also use another terminology which we see frequently with our submissions, and this is companion submissions, which are submissions from different manufacturers that are on related products. So where each review, each product is reviewed separately and on its own timeline.

But one submission cannot be approved or cleared independently from the others. So I've included an example on the slide. So company A has a BLA with us for a new anti-D antigen typing test and company B has a 510(k) for the instrument. So the 510(k) review will be completed in ninety days, but it will be placed on AI hold until the BLA review is completed.

In this slide I've listed examples of submissions that are appropriately bundled. So the first example includes reagents and instruments that are used together as a test system from the same manufacturer. So an example of this would be a new anti-Kell antigen typing test

that's added to an instrument where the reagent BLA is bundled with the instrument 510(k).

And my second example, blood grouping reagents, for example, anti-D, anti-Kell, or anti-Jk^{a} could also be bundled together because they have similar manufacturing processes and clinical data. And in this example above, if one of the reagents in the submission was holding up the review of the other reagents, we would discuss possible mechanisms for de-linking the problem reagent.

So, for example, if the anti-D was holding up the review of the anti-Jk^{a} and the anti-Kell, we would talk about removing the D from the submission so that the other two could be approved. And then going back and finishing the review for the anti-D.

And my last example includes a change that affects more than one previously cleared device from the same manufacturer. So an example of this would be adding a new antigen typing test to five different instruments. All of this could be bundled together.

This slide includes examples of submissions that are not appropriately bundled. The first example would be for further manufacturing use submissions where there is no associated user fee with a final product submission.

Because we cannot bundle a submission that doesn't have a user fee with a user fee submission. And this would be the same for any submissions that do not have user fees. But the submissions are companion to each other, so they will be tracked and reviewed together.

My next example includes company A, which is the manufacturer of a new reagent and has one BLA with company B that has two 510(k)s and they're the instrument manufacturers. So the BLA and the 510(k) cannot be bundled together because they're from different companies.

However, they will be companion submissions, so they'll be tracked and reviewed

together. But company B, the instrument manufacturer, since they have two 510(k)s, they may bundle those two submissions together.

And my final example of submissions that cannot be bundled include blood grouping reagents, ant-human globulin, and reagent red blood cell submissions because they have different manufacturing processes, specimen types, intended uses, and clinical data.

So some general information about bundling. Each submission must be complete and be its own record. So please perform all performance testing data for each reagent specificity in the bundle.

And then the submissions that will be bundled should be specified in the cover letter and FDA form should be included for each product in the application. We encourage you to submit bundled submissions on the same day. If they're submitted days or weeks apart from each other, it becomes harder for us to waive the user fee.

And the guidance states that the review time and user fee apply to the bundle is according to the submission type with the higher user fee and the longer review time.

So the next slide topic is submission organization best practices. So this slide includes recommendations for submission organization, the first two being a hyperlinked table of contents and searchable PDFs.

The submissions that we receive that have these two things are really easy for us to review because we can find the information that we need quickly and it leads to less questions from us.

Additionally, we encourage large file separation into subfiles, and I have two very specific examples of these in our submissions. The first being the product, the performance testing section should be separated into subsections.

So we could have a subsection for the clinical site report, a subsection for the protocol, a subsection for performance testing, et cetera. And additionally, each reagent in a bundled submission should have its own batch record subsection.

So for my example where the anti-D, anti-Jk^a , and anti-Kell are bundled together, each of those three should have their own batch record subsection. Please don't include one file with all three in a batch record.

We also ask that you submit your draft labeling as Word documents. Usually we ask for this later in the review, but it would just be helpful if you send this to us. And additionally the cover letter for companion submissions should cross reference each other.

So, for example, in my example where a BLA for a blood grouping reagent is bundled with a 510(k), or is a companion submission to a 510(k), the BLA cover letter should specify that company so-and-so is sending a 510(k) so we know that they go together. And then that way it makes it easier for us to track them and review them together.

I'm also going to talk about cooperative manufacturing arrangements as our IVD manufacturers are very familiar with these arrangements and they are engaged in frequently. But I just want to highlight two points, the first being 21 CFR 820.50, which is the purchasing controls.

Each manufacturer that's engaged in a manufacturing arrangement should make sure that they're following purchasing controls. And then as a final product manufacturer, you're ultimately responsible for all aspects of your product, so please make sure that they companies that you are in relationships with are following the regs and that you're auditing them.

Additionally, supply and quality agreements should be formalized between the arrangement parties. And in your submission, please describe the responsibilities of each

manufacturer. So we want to know who's responsible for shipping, who's responsible for complaint handling, et cetera.

So now I'm going to move into CMC. So this slide details a very basic overview of how our products are manufactured. We start with a source material component which in the case for blood grouping reagents and anti-human globulin could be monoclonal or polyclonal antibodies. And in the reagent red blood cells would be the donor cells.

This component, once it's manufactured, is formulated and then it undergoes filtration, filling, and labeling, and finally release testing. So the filtration, filling, and labeling that are grayed out on my slides, I will not be discussing at this time. I wanted to focus on other aspects of the process where we would like to see maybe clearer submissions.

So source material components for blood grouping reagents and anti-human globulin are the active component. And just some terminology. In the CFR 21 CFR 820.3(c) defines a component as any raw material, substance, piece, part, software, firmware, labeling, or assembly which is intended to be included as part of the finished, packaged, and labeled device.

It's also referred to as in vitro substance, for further manufacturing use, or antibody concentrate. So sometimes in our submissions we see all three of these terminologies, in vitro substance, FFMU, or antibody concentrate to describe the active component source material.

But sometimes they mean different things. So an in vitro substance might undergo a step and then it becomes an FFMU. So please in your submissions be clear on what your active component is. And for the rest of this presentation I'll refer to it as a source material component.

So for in the case of monoclonal antibody source material, a master cell bank, a

cell bank system is set up. That includes the master cell bank and the working cell bank and an adequate number of cells are established to provide enough over the product's lifespan.

So inventory records should include the location, identity, quantity, and give full traceability to the master cell and working cell bank. We recommend two different storage locations for your cell banks in the case of a device malfunction.

And when working cell banks are depleted, new ones may be created. But if a master cell bank needs to be reestablished, then a supplement to the BLAs should be submitted to CBER.

So for monoclonal antibody source material test methods, we recommend that you evaluate for potency and specificity. And please describe the test methods that you're using for potency and specificity testing. The red blood cell phenotypes you use in potency and specificity should support your intended use.

So, for example, weak heterozygous cells should be used. And if applicable, a certificate of analysis for cooperative manufacturing arrangements should be included.

So in the case of polyclonal antibody source material components, they're prepared from human or animal plasma that contains antibodies. This can be naturally occurring or produced following a sensitization.

So please remember that you're responsible for following the regs in 21 CFR 606, which is current good manufacturing practice for blood and blood components, and 21 CFR 610, which is the general biological products standard, and 21 CFR 640, which is the additional standard for human blood and blood components.

In your submission please list the name, address, and U.S. license number for all blood establishments. And then for any non-blood establishments that you obtain components

from, please provide the establishment's name and address.

Additionally for polyclonal antibodies you would perform the relevant transfusion-transmitted infection testing using one or more licensed, approved, or cleared screening tests. And you can see the applicable regulations in 610.40(a) and 610.40(b).

In your submission, please provide data that would support the dating period of our product based on the bleed date. And also describe your validated purification method. So if you're going to use an adsorption or adsorption-elution process as your purification method, please describe to us all the testing parameters.

So we would want to know the age of the reagent red cells that you're using, the antigen typing of those cells, if you're chemically modifying them, and then what your incubation and temperature ranges are.

Additionally, as similar to monoclonal antibodies, your red cell phenotypes used in potency and specificity testing should support your intended use.

And finally for the source material components for reagent red blood cells, they're prepared from human blood that meets donor eligibility requirements. Donor eligibility requirements are defined in 21 CFR 630.10 and then 21 CFR 630.15 for whole blood, red blood cells, and plasma collected by apheresis. And similar to polyclonal antibodies, you should perform the relevant transfusion-transmitted infection testing.

So formulation is the process of combining all the required components. So this would be your source material components, buffers, potentiators, colorants, anything you're adding to make your final product. Please include a narrative summary and a flow chart of the formulation process. This is really helpful for us when we're reviewing.

Additionally, mixing studies should be performed to show your product is

consistent and adequately mixed. And please include data that supports your mixing parameters for the lot volumes that you intend to manufacture.

Colorants, which are specific for blood grouping reagents and anti-human globulin, the property of the color should be determined based on the optimum wavelength. Please make sure the hue does not interfere with any interpretation of your test results.

Please follow 21 CFR 660.21(b) for blood grouping reagents, which states that anti-A should be colored blue and anti-B colored yellow. And 21 CFR 660.51(a) for anti-human globulin, both anti-IgG and polyspecific anti-human globulins may be colored green.

So in process hold times are the maximum time in process and bulk can be held. Please provide supporting data. So I've included an example on this slide where a source material component manufacturer defined a hold time between the harvest of the monoclonal antibody and clarification.

So they defined the maximum hold time as twenty-eight days in the submission and they provided potency testing results during that twenty-eight day hold time to show that their product met specification. And additionally the stability study they performed to support the shelf life was performed with the hold time, the maximum hold time of twenty-eight days.

So the date of manufacture or as we refer to DOM, refers to a time point in the process that's used to calculate the product's expiration date. So in your submission, please clearly define the date of manufacture.

There are some definitions in the CFR for blood grouping reagent. It's defined in 21 CFR 660.21(e) and for anti-human globulin it's defined in 21 CFR 660.51(c) as the date that you initiate the last group of potency testings.

So in your submission, please describe the maximum time between the filling and

potency testing. But you may choose another date of manufacturing. Sometimes we see filtration as a point that's picked as the date of manufacturing. That's fine as long as it doesn't extend your product's expiration date.

Reagent red blood cell dated manufacturing is defined in 21 CFR 660.31(c) as the date that the blood is withdrawn from the donor. And in a pool of reagent red blood cells, it would be the date that the blood is withdrawn from the first donor.

So the CFR defines a lot as material that's fully processed and mixed in a single vessel and filtered. If you would like to use a different definition of a lot, please provide us your rationale in your submission. And please also describe sub-lotting processes.

So a lot of times this is missing from the submission and we have to ask, so we're looking for things like sub numbering, sublot number conventions, your testing requirements, specifications, and the lot release testing you'll perform to ensure that your sublots are identical.

So now I'm going to talk about conformance lots, which are manufactured to support the BLA. Your manufacturing process described in the BLA should be identical to the process that's used for the conformance lots.

So once you manufacture the conformance lots, this should lock down your process and you shouldn't make any more changes. So if additional lots are manufactured for the performance studies, then they should be manufactured the same way that the conformance lots were.

We recommend three lots are manufactured to validate your performance and manufacturing. And in your submission please provide us a summary of the three lots. This is usually missing. So we would like to know the lot numbers, the date of manufacture, and the expiration date for the conformance lots.

One lot should be manufactured from source material component that's nearing its expiration date. The conformance lots are not required to be in-date at the time of the submission. And in the case of a limited source material, we will usually allow one full scale and two pilot scale conformance lots.

For a monoclonal antibody source material, for blood grouping reagents and anti-human globulin, each conformance lot should be manufactured from a separate working cell bank, aliquot, or vial. So please don't take one working cell bank that you have and grow it out to make three lots. We suggest that you take three separate vials to make three separate lots.

So now I'm going to move on to product testing. So in your submission you should describe the test methods, specifications for potency, specificity, and any other biochemical properties you perform.

And then please remember that once your application's been approved, any changes to your specifications would require a supplement to the BLA. Additionally, please include each reagent for a bundled submission.

So we want to know the specs for, let's say an anti-D, anti-Jk^{a}, anti-Kell that are bundled together. Because each of those might have different specifications. Additionally, please provide a summary of all the testing data for the conformance lots. This is just helpful for us in our review.

So for blood grouping reagent, product testing includes but is not limited to the following on this slide. Potency testing which is described in 21 CFR 660.22 and 21 CFR 660.25. So the CFR lists minimum potency requirements for certain blood grouping reagents, but you may choose to exceed those requirements based on your own feasibility studies or the design of your product.

That's fine but we just want you to remember that once you're approved, those specifications that you have chosen super exceed those in the CFR, so you need to meet whatever you've decided that your specs are each time.

Specificity testing is defined in 21 CFR 660.26. Avidity testing, which is for our slide test method, is defined in 21 CFR 660.26, and we also recommend tests for spontaneous agglutination.

So potency testing for blood grouping reagents, some general information about a potency titer. So the potency titer value is the reciprocal of the highest reagent dilution for which a reaction is graded as one plus.

So in my example in the slide below, the anti-D investigational reagent would have a potency titer of one to eight, because that is the last one plus reaction seen. And the anti-D in house standard or reference would have a potency of one to four.

So for potency testing for blood grouping reagents, if there is a reference reagent available it's described in 21 CFR 660.22 that the reference reagent must have a potency titer value at least equal to your reagent.

We have some polyclonal reference reagents that are available. They have established potency requirements. They're listed on this slide. Anti-IgG, anti-D, anti-C3d, anti-C, anti-A, anti-E, anti-B, anti-c, and anti-e. These are only for lot release testing. They're not for stability studies.

So for blood grouping reagents where no reference is available, this is defined in 21 CFR 660.25. Qualified in-house standards are acceptable. Please describe in your submission how you qualify your standards that you'll use in your testing.

We recommend that for monoclonal blood grouping reagents the potency titer is

at least 1:8. And then we strongly recommend that you follow your test methods in your package insert.

So sometimes for potency testing, because it is so tech to tech variability, we see requests to do potency testing a different way. But just remember that you should be performing it the way that the end user would perform the test.

So we would, might want to see information from you that the potency titer wouldn't be affected by performing it a different way.

Additionally, we recommend that minimum parameters in your package insert are used for potency testing. So, for example, if your test method states you have a five- to ten-minute incubation, then we would recommend potency testing be performed at five minutes to make sure that weak examples are picked up.

Red cells that are tested should include cells with heterozygous or diminished expression of the corresponding antigen. So, for example, if you have an anti- Fy^a reagent, we would recommend that you would use cells that are Fy^a pos B pos.

The next test we recommend you perform for blood grouping reagents is negative specificity, which is to confirm the absence of contaminating antibodies. Standard laboratory practice would be to include a positive control to make sure that your test works appropriately.

Please follow the methods in the package insert again when you're performing specificity testing. And for specificity testing, we would recommend the maximum parameters in the package insert because we want to make sure that you're not picking up false positives.

So, for example, if you have a five- to ten-minute incubation, you would perform your test at ten minutes.

This slide just lists some general rule outs that we suggest you should rule out for

our blood grouping reagents, for monoclonal reagents and polyclonal reagents. So, for example, if you had an anti-D reagent that was monoclonal, we would recommend that you would rule out everything on the top of this chart except for the D, which is what the reagent is specific to.

So the next test we recommend for blood grouping reagents is positive specificity or sometimes called reactivity testing, which is testing performed to confirm the reactivity of each lot with antigen positive cells.

So we recommend at least four different heterozygous red cells. So in my example where you have an anti- Fy^a reagent, we would recommend four different donors whose phenotype is Fy^a pos B pos.

For anti-A, anti-B, or anti-A,B reagents, please test your reactivity with A1B, A2B, and also Ax cells if you have them available. Include a negative control because this is a positive test, to ensure that your test is performed accurately.

Again, follow the methods in the package insert. And since this is a positive test results, similarly to potency we recommend that you use your minimum parameters.

So avidity testing, which is specific for slide test methods, are a confirmation of reactivity. The red blood cells should be weak heterozygous cells. We recommend that you follow the package insert. And we recommend testing your undiluted reagent along with a reagent diluted 1:2.

And then you will observe and record the test results at two distinct intervals, usually halfway and at the end based on the time in your package insert.

The final test we recommend for blood grouping reagents is spontaneous agglutination. So red blood cells that are heavily coated with IgG molecules often spontaneously agglutinate with reagents that contain a potentiator.

So you should test for spontaneous agglutination and a control should be supplied or recommended in your package insert for the end users.

So moving on to reagent red blood cell product testing, the applicable regs are find in 21 CFR 660.33. Reagent red blood cells should be group O and each lot of product should be tested for at least the following common antigens. So D, C, E, c, e, Kell, Cellano, DuffyA, DuffyB, Jka, Jkb, Lea, Leb, P1, and then M, N, and S and s.

You'll confirm these using two or more blood grouping reagents, and if only one blood grouping reagent is available then we would recommend you would test the red cell twice, at least independently from each other.

We also recommend that reagent red blood cells are, have a negative DAT and a negative antibody screen. And this includes for both anti-A and anti-B. Any other release testing that you do for your reagent red blood cells, please describe in your submission and provide us with your specifications.

And then finally moving on to anti-human globulin product testing, it includes but is not limited to the following. Potency testing for determining your anti-IgG and anti-complement activity, which is described in 21 CFR 660.54. Specificity testing which is defined in 21 CFR 660.54.

We also recommend that your AHG product not have reactivity with normal red cells nor with enzyme treated cells.

So when determining the anti-IgG potency titer of an anti-human globulin product, we suggest that you use red cells that have been coated with dilutions of Fy A and anti-D. So, for example, 1:2, 1:4, 1:8.

Then you'll perform serial dilution titrations of your anti-IgG and the reference

reagent or in house standard with coated red cells. And then we recommend that your titer end point should be at least equal to the reference or in house standard.

So when determining the anti-complement activity, or anti-complement potency titer of an anti-human globulin product, we suggest that you use red cells that are coated with complement. So you may coat your red cells with complement how you choose, but in your submission just describe to us the method that you used.

Prepare serial dilutions for the anti-complement and the reference reagent or in house standard. And then some resul recommendations. So for anti-C3d it should yield two plus reactions and have a titer end point equal to the reference or the standard.

And then all other complement components, so anti-C3b, anti-C4b, and anti-C4d, the undiluted reagent should yield at least two plus, and the one to four should yield at least one plus.

So the final test we recommend for anti-human globulin is specificity testing. It should be performed using various sensitized red cells. I've listed a chart on this slide that includes all our anti-human globulin reagents and the cells that we recommend that you sensitize with.

Appropriate positive and negative reaction to be seen, so for example if you have an anti-IgG reagent, you should sensitize your cells with these components. And we would recommend to see positive results with the cells that are sensitized with IgG and negative results with the cells that are sensitized with complement.

All controls should yield appropriate reactions and no hemolysis should be observed. Additionally, we request that your anti-human globulin product not react with normal red cell samples or agglutinate enzyme treated cells.

And my last topic is CBER lot release. So yesterday you heard a presentation about lot release that was specific for donor screening assays. So in contrast to the donor screening assays, the licensed immunohematology reagents don't need samples sent.

So we just review the lot release protocols, templates, for each lot that you manufacture. So for new submissions, please submit CBER lot release protocol templates. The templates should perform test, the templates should include the testing you perform and your final specifications.

The testing on your CBER lot release protocol should be the same as your final release testing. So, for example, in your submission if you state that you run ten cells for positive specificity testing, please include all those ten cells in your lot release testing template.

CBER lot release is not applicable for reagent red blood cell final products because of their shortened expiration date. Additionally, yesterday Kori talked about surveillance, which is also applicable for licensed immunohematology products. So you may request to go on surveillance with us as a supplement to the BLA.

If you have a good history of compliance and no new major changes to your products, just formulation, you can request to go on surveillance. We actually only have a couple of companies that are on surveillance with us, but it's something that you might want to think about.

So in summary, please make use of the pre-sub program. So the recommendations in my presentation were fit on the majority of the products that we see in submissions, but they might not fit your product or there might be extenuating circumstances.

So that's why pre-subs are a really great way for us to talk about what's going on and then we can all agree before the submissions come in.

Additionally, I want to stress again that the quality of submissions lead to a much more efficient FDA review, so easy to read submissions lead to a lot less questions from reviewers. Please clearly describe all steps in your manufacturing and testing process and please keep your terminology consistent throughout your submission.

And then I want to strongly encourage you to follow the test methods that are in your package insert when you perform product testing. So this is a slide that lists the references that I used to prepare the presentation. Thank you for your attention and my email is on here if you ever have any questions.

MS. RAGOSTA: Good morning. My name is Annette Ragosta. I am also a scientific reviewer in the Device Review Branch in the Division of Blood Components and Devices.

So today I'm going to cover general requirements for labeling and then I'm going to also go over the studies that we recommend that you perform to demonstrate that your products are safe and effective. And these include accuracy, real time, stability, transport stability, post-approval stability, interference studies including sample type study, precision studies, and comparison studies.

Let's start with labeling. So because these products are both regulated under the FD and C Act and the PHS Act, they are required to follow the general label requirements for the medical devices that you'll find these in 801.

They're also IVDs, so they must follow the requirements that are outlined in 809.10. And here you'll see information on what needs to be put on the immediate container, the outer package, and a lot of detail on what needs to be put in the package insert.

And then you also should be following the unique device identification regulation

found in 830.20. And for the PHS part of it, they need to be following the general labeling requirements for biologic products, which are in 610.60 through 610.68.

And then product specific labeling requirements for the blood grouping reagents in 21 CFR 660.28 for reagent red blood cells and 660.35, and for anti-human globulin in 660.55.

So when we do our reviews, the first thing we're going to do is look at your labeling and we're going to identify the labeling claims you make. Then we're going to look to see that you validated all these labeling claims.

And these are just examples of what we're going to be looking at. So if you make a claim that you can detect rare phenotypes, so let's say you say for the anti-D that you are able to detect some of the rare types such as D6, we're going to look to see that you have done testing for that and there's sufficient sample size for that.

We're going to look at the intended use populations. Most of ours are donors and patients, but sometimes we have reagents that come in just for donors. And so what's the difference? It would be a difference in, say, if you did anti-D it would be low risk. If you had a false positive anti-D, if it was just donor population.

But as soon as you include patient for that, then you're going to have a high risk, especially for the prenatal population.

We're going to look at your testing procedures and we're going to ensure that you have validated the extremes of the time and incubation temperature ranges. And as Kim pointed out, positive samples should be run at the low end and negative samples at the high end. And then we're going to be looking at validation for sample types and sample storage that's listed in your package insert.

Then for automated methods, we're going to be looking at the instrument user

manual. Please be sure that you identify the reagent manufacturer and the applicable reagents. So for many of our companies they have very large portfolios of reagents.

And sometimes the instruments are only used on a small percentage of those reagents, so it's very important you list out the reagents in the manual that can be used. And sometimes you have to get down to the cell line, because if there's multiple cell lines for a specificity and you've only tested one cell line it needs to be indicated in the labeling.

And then for the companion reagent package insert, we should also see that you're listing the instruments that can be used with those reagents. And then it's very important that you don't put shortcuts in the package insert, how to run the user manual in one paragraph. You should be referring to the three hundred page user manual for the operator instructions.

And one of the things we see missing a lot is performance data. So it's important that you include the result tables and explanations of all your discordant results. And in later slides we're going to discuss what that should look like.

You should put a description of your expected results, and that should address all the test methods and include the expected reaction grades. And so an example, if you have hemagglutination and you also have solid phase adherence, a positive for hemagglutination looks like a negative for solid phase.

So it's very important that the end user can see the difference. And you should preferably put photos of what these reactions look like. This is especially important if there's a requirement for visual inspection.

The user manual. Please let us know how the end user is going to access that. Is it going to be a hard copy, an e-copy, or both ways. And state to us whether the user manual we're looking at is the exact same user manual that the end user is looking at.

Some of our companies have procedures that they provide to the end user in addition to the user manual and package insert. That's considered labeling and we would have to review that.

Okay, so let's start with performance studies. We're going to start with general considerations. So one of the issues we see for many years and almost all our companies is insufficient sample sizes. And this can occur with rare phenotypes, positive antibody screens, antibody identification tests, positive DAT samples, and incompatible cross matches.

It affects not only your original BLA, but it's going to affect all your future submissions for that product, such as supplements, lot release testing, and stability testing. And it could negatively affect your statistical analysis results. And we'll get into that in future slides.

So what do we suggest you do? We suggest that you identify the problem early in the design and development phase. And it's important that you do your due diligence and you try and find the samples that you need. And then you should plan to stockpile them, the well-characterized samples, and figure out how you're going to do the contrived samples.

So what do we mean by well-characterized samples? These are samples that have been extensively tested using a variety of immunohematology test methods. So it's important that you don't just look to see that you're using two different manufacturers to test, because many times a manufacturer uses the same in vitro substance.

So make sure that it's at least two cell lines when you're testing red cells. And then for the contrived samples, those are samples that are prepared or designed to express predetermined attributes. An example for that would be if you want to have a positive DAT sample, you would coat the cells with different dilutions of let's say Anti-Duffy A.

So the second issue that we see happen a lot is that people are using the

comparison study as a last ditch effort to do all your validations. What you should be doing is you should be performing in house prospective validation studies. And we're going to discuss some of those studies that you should be doing.

These identify the product characteristics prior to performing your external comparison studies. And you need that information to be able to design your comparison study. So if you've never done a sample type study and you're just guessing, well, the last reagent worked with these sample types, you're not going to know what should be used in your comparison study unless you did a prospective validation.

So let's start with accuracy. So this study will determine the measurement of agreement between the expected value and the investigational device value. This should be done early in the design and development phase as a feasibility study using well-characterized and contrived samples.

You also, then once you lock down your manufacturing process you would perform your prospective internal accuracy study, your formal study, using well-characterized samples. So for least burdensome approach, for an anti-A, an anti-B, we're not going to ask that you do a large number of samples for that.

Because in the comparison study you're not going to have any problem finding those samples. However, for something like a little e or a Cw positive, you need to have as many samples as possible that are tested throughout the submission so that we have enough to go by.

So it's very important in the accuracy study that you try and gather these samples. And we expect a hundred percent agreement to the expected results. And you should provide an explanation for any discordant results.

So let's look at stability studies. Here you demonstrate that the product can

maintain its performance characteristics over a defined time interval and within defined storage conditions.

This is applicable to both FFMU products and final IVD products. So there are three main types, real time, transport, and post approval stability studies. And whichever one it is, they need to have predefined acceptance criteria, and any out of specification result needs to be investigated and explained.

So let's start with real time. We need to see three conformance lots. You should use container closure systems that are included in the submission. And specifically for BGR and AHG, at the time of submission we need to see twenty-five percent of that data. If you're proposing a two-year expiry, that means we would need to see six months.

However, for red cells, because they have such a short shelf life, we expect to see a hundred percent of the stability data at the time of submission.

Use the test methods in the labeling. You should describe your in house reference material, describe your testing intervals and study duration, and the tests should extend beyond the proposed shelf life.

You should challenge the actual routine use of the IVD in the user environment. This is in use stability. And an example would be the stability of your product after opening the vial and remaining at room temperature for a few shifts in the lab.

We expect to see at a minimum micro testing at time zero and end of expiry. And then for automated methods you should be performing on-board stability, and this would show us the maximum length of time that the IVDs can be loaded onto an instrument and still perform according to specifications.

You should submit additional stability data during our review as it becomes

available. So don't let us ask for it all at the last minute, especially if there's going to be issues.

So for BGRs and AHG, the stability indicating assays are potency and specificity testing. Include the phenotypes of the red cells used in the study. And as Kim stated, you should be using heterozygous cells.

The test results should meet the potency requirements that are outlined in 21 CFR 660.25 for blood grouping reagents and 660.54 for anti-human globulin.

So one of the big issues we see in stability is unanticipated potency titer variability between testing time points. So what do I mean by that? If you have a time zero potency of five thousand and then it drops to five hundred at month three and then it goes back up to five thousand. And as you can see, what I'm saying about variability, we cannot do anything, we cannot review that and make any sense out of that.

So what should you do for that? Make sure you control the variations in pipetting techniques. Try and use the same donor red blood cell throughout the study. And then be very careful that you're following your incubation times.

So this is especially important for immediate spin incubations. If an operator is pipetting out sixty tubes, those first ten tubes, first twenty tubes are not going to be immediate spin. And so it'll be this false elevated titer for that time point.

And then when the next time point comes and that operator decides to do it the right way and do it in sets and run ten and then spins ten, then it's going to be a false elevate, or decrease. It'll look like something happened to your product. So it's very important that you pay attention to variability and try to control it.

So for reagent red blood cells for stability, you should be checking for hemolysis, performing your direct antiglobulin tests, and you should ensure that the limit of detection is not

reduced over time and you can do this with, by diluting the antibodies you're using.

Let's look at now transport stability studies. It's important that you test the transport conditions that are going to be experienced between the time of manufacture and the delivery to the end user.

So to determine the effect of the conditions on the shelf life, you would use a stability study test. You can do it two different ways, and I'm not going to get into detail on how to do a transport study. There's a lot of stuff on the Internet that you can find.

You can do actual transport studies, but those are difficult to maintain and control the environmental conditions. Or you can do the preferred method which is a simulated transport study.

And here you would challenge the extreme conditions that could occur during shipping and handling of the product, such as high and low conditions for temperature and humidity. You would also look at what happens to the product when it's dropped, when it's vibrated.

For the transport stability study, you would use the same testing time points, same stability indicating tests, and acceptance criteria as you did for the real time study. If you have a bundle submission such as you have ten BGRs together, it may be possible that you could do reduced testing and use a family and matrix approach.

You don't have to include every single one of those specificities in the study. So you can make your proposal to us and provide a justification for that design.

And make sure you include all your packaging configurations such as single pack, ten pack, for the in vitro products and then also the type of packaging that you'd use for in vitro substance, which is very different than the final product.

And the third type of stability study is post-approval. FDA has a compliance policy guide called number 280.100 and it's entitled stability requirements for licensed IVD products. And in this document it states that you do not have to do post approval stability studies for these products unless they're required as a condition of approval of the license, it's due to changes in manufacturing or formulation, or as part of a corrective and preventive action plan.

If you do perform post approval stability studies, make sure that you report to FDA any stability study time point failures.

Interference studies. Here you would consider the substances that are likely to be present in patient and donor samples that may have the potential to interfere with the test. And I provided you with a CLSI document, EPO7.

We don't expect you to follow this exactly because it's not pertinent to immunohematology products, but it can give you an idea of how to set up the design of your study. But we do expect to see in this study common sample abnormalities such as hemolysis, icterus, lipemia, and Wharton's jelly in cord blood.

You should be addressing interference of anticoagulants, additive solutions, and preservatives. And any substance that could contact the specimen such as serum separated devices, specimen collection containers, and their stoppers.

Now if you do find in the study that something does interfere, then you need to include that in the limitations and warning section of the package insert.

And then later on, you remember this study's going to have small numbers. Later on you're going to do the comparison study which you have a very large number of samples. And that could provide some additional information to you on whether there are additional interferents. And then those would also have to be included in the package insert.

So as a subset of the interference study, we have the sample type study. And here you would demonstrate that the reagent is not affected by the recommended anticoagulants and sample age that are listed in your product labeling.

It's important that you include all sample types, specimen collection limitations, and sample storage conditions. That you list there. A lot of times we see only certain, some companies are only doing a certain percentage of what, they have a huge list of sample types, especially for the additives.

And we checked with our blood and plasma group and asked about whether in the segments are the additives there and they said yes they are in the segments. Maybe not for all the different blood establishments, but for some. So therefore you should be using the additive solutions in your study.

And here you would expect a hundred percent concordance with the expected results, because you're using well-characterized and contrived samples. Now if you don't have concordance and you find out a sample type cannot be used, make sure you let us know and that you amend your package insert.

So precision study. Here they would demonstrate that the test reagent generates repeatable and reproducible results using a panel of well-characterized and contrived samples. And the study should capture all possible sources of variation including within-run, run-to-run, day-to-day, operator-to-operator, instrument-to-instrument, site-to-site, and lot-to-lot variation.

Be sure to use the test methods that are listed in the package insert during this study. And your precision panel samples should cover each test listed in the submissions. So if you have a bundle, it doesn't mean, as Kim said it's bundling for user fee only, it's not bundling and you don't have to do the testing for each of the different reagents.

So if you have blood group reagents, there's ten of them, you need to have ten in that panel. And the lot-to-lot study could be performed in house. If you do it in house, make sure that the panel is the same as you used for the external sites.

This is just an example of a precision study design that many of our companies use. It's tested at three sites. Two of those are external. By two operators at each site with each operator performing two runs per day on five nonconsecutive days over a twenty-day period. And then each sample is run in duplicate for repeatability and then the lot-to-lot was performed in house.

So for the data analysis, the acceptance criteria, it should be a hundred percent between the different sources of variation. Make sure you provide the agreement results to us summarized separately for each panel member. And any discordant results, you'll have to investigate and provide a justification to us.

Oh, and I just wanted to make a point on the lot-to-lot study. If you, as you know we have the instruments come in as 510(k)s and then the reagents come in under BLA. So if you already had your reagents approved and you have a new instrument coming in, you will be sending us labeling supplements.

You don't need to repeat those lot-to-lot if you had already been approved previously for those reagents, say under a manual tube method. You do not then have to prove to us that you can still make the reagents consistently. So you don't need to do lot-to-lot if it's coming in as a labeling supplement.

So for comparison study, this evaluates the performance of the investigational reagent compared to a U.S. licensed reagent. And just as a note, reminder that BGRs, AHG, and reagent red blood cells are exempt from the IND requirements and you can find that exemption

stated in 21 CFR Part 312.2. And you may use de-identified leftover samples for this study.

And as we discussed about sample size, you can supplement the de-identified leftover clinical specimens with well-characterized and contrived samples for the following: rare phenotypes which would be for the BGRs. And the next four bullets would be contrived samples.

These are positive direct antiglobulin, positive antibody screen, antibody identification, and incompatible crossmatch testing. And make sure you include weak samples. We don't want to see all four plus reactions.

And in your report, please describe the methods that you're using to determine the samples are well-characterized and how you contrived the samples.

They should be performed at three external sites and the intended use population will determine the site selections. If it's donor testing only, you only have to go to a donor place. But if it's donor and patients you need to include transfusion centers.

For BGRs only, the sites should cover different geographic regions and include a representation of the major ethnic groups found in the U.S. And please provide a summary table to us for that.

You should be comparing two distinct lots of the investigation reagent to the FDA licensed products. Now if you're coming up with some new specificity that we have not yet approved and there's no FDA licensed reagent available, you'll need to discuss with us acceptable alternatives.

And as we stated previously, you should be doing validated prospective studies for things like sample type so you'll know which sample types to use for the study. Therefore, you don't have to include them all in the study. This is not going to be a validation.

But you do have to provide a summary to us of the sample types you used. And you should test the samples by all test methods and test conditions in the labeling. And if you're doing it at transfusion centers, you need to provide a summary to us of the patients and the various conditions and diseases, whether they're neonates and older patients.

So let's look at recommended acceptance criteria. And there's various criteria is going to be dependent on the products. So for antigen phenotyping, this is for BGRs, the low bound of the one sided ninety-five percent confidence intervals for the positive percent agreement and the negative percent agreement with the comparator reagent should exceed ninety-nine percent.

For antibody screening, non-ABO, antibody identification, and direct antiglobulin test, we reduce that to ninety-five percent. You're using random samples here and we all realize that when you're doing antibody screening and IDs, a lot of times if it's coming from a transfusion center they're going to be complex antibodies in those serums.

So we know you're not going to be able to detect that or, you know, identify an antibody on first try. So because it's random samples, that's the rationale for this coming down to ninety-five percent here.

So for ABO antibodies, and this would be the A and B reagent red blood cells, we go back up to ninety-nine percent. And just, and the rationale for that is for anything with ABO we're looking at it and making sure there's not going to be mistakes.

The same with the crossmatch, immediate spin, and indirect antiglobulin testing. This also has to exceed ninety-nine percent. This is the last time someone's getting tested prior to getting transfused. We want to make sure the test is correct.

So now if the study does not include a sufficient number of positive or negative

leftover samples, to meet the acceptance criteria as we stated before, you can use contrived or well-characterized samples to increase the sample size.

So instead of comparing to a U.S. licensed reagent result, you're now comparing to an expected result. And because it's different, you have to analyze separately from your random sample results. And here we expect a hundred percent agreement.

So we recommend that you look at this guidance, statistical guidance on reporting results from studies evaluating diagnostic tests. It goes into detail about repeat testing, resolution testing. And we also ask that you include two by two result tables for each reagent comparing the investigational test with the comparator or with the expected result.

You should include measures of positive and negative percent agreement and the corresponding competence intervals. Make sure you don't include overall agreement. We don't look at that.

So this is just an example, you don't have to do it exactly this way. And here this table shows for anti-K how many samples going across. It shows you the investigational positive and negative results. And going in the columns down, the comparator results.

And on the right-hand side you see the positive percent agreement and negative percent agreement point estimates and lower confidence intervals.

In your report, in addition to those tables you should be providing us with exclusion criteria. So is it insufficient samples, sample conditions, no type determined which is for automated test methods? You should be performing repeat testing only if it's allowed in the labeling.

So you shouldn't be seeing that in manual testing, but you would see that in automated testing. And your statistical calculations should be performed on the original results

if repeat testing is not allowed in the labeling. And then you perform it on repeat testing if it's allowed in the labeling. Again, that would be for automated.

So if you have discordant results, you're going to perform resolution testing. Here you're going to investigate a discordant result using a referee reagent, so that's your third reagent you're going to be using. And it's going to break the tie for you.

So a big mistake we see a lot of the companies making is we, they're saying that the comparator has false pos and false neg, and that's not true. The comparator results are always assumed to be correct, because we're comparing it to your investigational, which is unknown and unknown at this time.

You might remind yourself that resolution testing is not necessary. You see a lot of times you are going to be doing resolution testing for well-characterized and contrived samples. It's not necessary. You're going to only compare it to your expected result.

And then unlicensed reference reagents, because you can't find a third reagent at this time, then you would provide package insert to us in the submission. Now with resolution testing you can't redo your statistical analysis.

However, the results of that analysis could provide some additional information to us when we're making an assessment of whether the product should be approved or not. And we'll discuss that in a few slides.

So once you do resolution testing you can either do it in house or you can go to a referee lab. If you do go to a referee lab, make sure they're using an investigation, a method in the investigation that's equivalent to your method and that they don't use the same reagents as those used in the study.

So for an example where one went wrong, the company contrived an

incompatible crossmatch test. And so it should have tested positive or incompatible.

However, the investigational solid phase device result was negative. So they got a discordant result to the expected result. So they sent it out to a referee lab who also got it negative, also incorrect, using the tube method/LISS crossmatch.

So here were two problems in this. This was a contrived sample so the original investigational device result should have been compared only to the expected. There was no reason to do resolution testing.

But since they did, why didn't it work out? Why did the referee lab agree with their incorrect result? It was because upon further investigation they realized that the referee lab method was less sensitive than their own investigational method. So be really careful when you're choosing a referee lab.

And then another point I wanted to make for antibody detection and I.D. tests, your results need to be reported at sample level, not test level. So these two examples here in these tables were done correctly. This first one is a three-cell screen result.

They had 1,789 samples and they showed us the positives and negatives. However, if it was done incorrectly and we've had quite a few of these where they would have sent us 1,789 times three, which is over five thousand tests, and broken it out by positive and negative.

The same with the antibody I.D. We want to know were you able to identify the antibody. We don't want to know what every vial's positive and negative came out to. So if it was done incorrectly and this was a ten cell panel, we would have seen 2,830 tests. But again, these were done correctly.

And in reference to indeterminate and equivocal results for automated methods,

do not discard or ignore these. Follow your labeling instructions for repeat testing. And you really need to establish equivocal limits and it's helpful if they're in a table. So here in this table they compare their investigational to their comparator and gave us a P value.

So let's look at some examples of data analysis. The first one is that it did not meet acceptance criterion due to sample size. This is anti-e and there were only a hundred negatives and the NPA was ninety-seven percent. So this is the way you should write your assessments, these are examples of what we want to see in the labeling and in the reports.

The PPA met the acceptance criterion, the NPA did not meet the acceptance criterion due to the low frequency of e negative samples in the population. And the point estimate was at a hundred percent. So this was acceptable to us.

Now if those were only twenty samples, we would say it's not acceptable. But you should go out and do your due diligence and get more samples.

Example two, it did not meet the acceptance criteria due to sample size and discordant results. So it's a little more complicated and this is for anti-K. And so it didn't meet the PPA due to three false positive results with the investigational device. And also there was an insufficient sample size because it's the low frequency of K antigen in the population.

So on resolution testing, we found out that the referee method agreed with the investigational device result. And so they did do a calculation that's not going to be in the labeling. However, to show us that, hey, we met a hundred percent after we did resolution testing. So we looked at that and said that would be acceptable.

The third example, they just didn't meet the acceptance criterion due to incorrect result. And this is for B cell and here it was at 97.6 percent. And as I stated, ninety-nine percent is the criteria and for ABO typing we really want to see things high up at ninety-nine percent.

Most of our companies have no problem meeting that.

So after resolution testing, you still had one false neg and one false pos, which is why it stayed low. And what they did as justification is they said although it didn't meet it, the software would indicate no type determined due to a mismatch of forward and reverse typing results. So we realize, yes, that's true.

This needs to be put into the package insert so the end user knows about the performance of this product. If that number was really low, like at say ninety-five, ninety-two, it would not be acceptable only because we have plenty of reagents out there for A and B that don't have this problem.

And then this is an example of an antibody ID test. And in the assessment they let us know which reagents they used, I mean which antibodies they used. And it didn't meet the acceptance criteria. It was just under it, only because of sample size.

So in summary, make sure you identify and prospectively validate all your labeling claims. It's really important that the validation reports for the performance studies are well-organized, easy to navigate, and contain accurate information.

You should anticipate the number of samples needed for testing and identify solutions for insufficient sample sizes during product development. And please provide an assessment of the test results. This would be for precision and comparison, both in your report and in the labeling.

Okay, thank you.

MS. MERCADO: We are about fifteen minutes ahead of schedule, so let's take a twenty-minute break and be back at ten after ten.

(WHEREUPON, a brief break was taken from 9:51 a.m. to 10:10 a.m.)

MS. MERCADO: Our next speaker is going to be Dr. Jason Liu, who's going to be speaking on molecular based devices. HEA, HLA, HNA, and HPA.

DR. LIU: Thank you, everyone. Thanks for staying for the last session of the workshop. My name is Jason Liu. I'm a scientific reviewer from the Division of Blood Components and Devices.

I will discuss our regulatory review of molecular-based tests for determining human erythrocyte antigen, HEA, human leukocyte antigen, HLA, human neutrophil antigen, HNA, and the human platelet antigen, HPA. I will provide our recommendations that we think will be helpful for you to prepare your test, your submission of your test.

Here is an overview of the presentation. We will first discuss the molecular erythrocyte antigen typing devices which are subject to PMA requirements. We also call these types of assays as blood group antigen genotyping assay, or red blood genotyping assay.

I may use these names interchangeably in this presentation. I will first talk about the two previously approved PMA applications for the red blood cell genotyping assay and provide our recommendations if you also want to choose a modular PMA pathway for your future new assay.

I will then discuss the major content required for PMA submission including CMC information, nonclinical studies, software, clinical studies, and the labeling. And the provide our recommendations.

After that I will briefly discuss quality control materials required for the blood group antigen genotyping assay. After that I will talk about the PMA supplement required for modifications to an approved PMA.

Lastly, I will discuss HLA, HPA, and HNA genotyping devices which are subject

to 510(k) requirements.

Let's first discuss molecular erythrocyte antigen typing devices. We have previously approved two PMA applications for molecular determination of blood group antigen phenotypes. Both are multiplex molecular assays designed to detect genetic polymorphisms and predict blood group antigen phenotypes.

The first one is called PreciseType HEA Molecular BeadChip Test. This test detects and reports thirty-six blood group antigen phenotypes plus a mutation in the beta-globin gene that is related to hemoglobin S.

The second one is called ID CORE XT. This test detects and reports twenty-nine polymorphisms, fifty-three alleles, and thirty-seven antigens from ten blood group systems. Both case were submitted to FDA as modular PMA.

As both of the two previously approved blood group antigen genotyping tests were submitted to us as modular PMA based on our review experiences we have some suggestions if you also want to submit a modular PMA for your new test.

For a modular PMA, we generally suggest you submit, you include not more than three to four modules. We find this is helpful for the review process if you can submit CMC information in module one, submit nonclinical studies in module two, software in module three, and clinical studies in the final module.

For each module, please make sure to submit complete information based on the PMA shell agreement. For the deficiencies identified by the FDA during the review, although you can choose to address them in the final module, we encourage you to resolve the deficiencies early in the review process.

We have a few additional recommendations specific for the first module. In

addition to the CMC information as discussed yesterday by DMPQ, we suggest that you also include the intended use, the instructions for use, and detailed device description in the first module.

The device description should be in detail including information such as the principal of creation, the genetic variance targeted by your assay, major assay steps, data calculations, software, and any other relevant information.

We found it is helpful for us if you include a detailed information of the new test, otherwise we may ask a lot of questions about the new test which may slow down the review process.

We also have a few suggestions for the final module. When you are within ninety days of submitting the final module, please notify FDA. FDA needs to know the upcoming final module to schedule the preapproval manufacture facility inspection.

Please remember to include to the final module your responses to all outstanding deficiencies related to previously submitted module. Please be aware the final module is a complete PMA submission. You need to provide any additional information required for complete PMA.

Please, we recommend that you compare using the PMA acceptance and filing checklist when you prepare and submit your final module.

You may choose to submit your new test as a traditional PMA. For a traditional PMA you will submit all PMA data at the same time, regardless of when the testing is completed.

PMA review timeline. If FDA needs to hold an advisory committee, the review timeline is 320 days. That was the case for the PreciseType. We went to an advisory committee

meeting for recommendations because that was our first molecular test of its type.

If advisory committee input is not required, the review timelines are 180 days. We didn't go to advisory committee meeting for our second PMA, ID CORE XT.

The major PMA content is the CMC information. For the CMC section, in addition to the quality system information as we discussed yesterday, we highly recommend that you include a detailed summary of device manufacturing.

Without that summary, it may be difficult for us to fully, to quickly and fully understand all of the information provided in each individual method, specification, or procedure document.

You also need to provide us a device master record, DMR, of the subject device such as production process specifications including the final manufacturing procedures. An additional flow diagram would be very helpful.

Other information include but may not limit to quality assurance procedures and specifications, packaging and labeling specifications, installation, maintenance, and servicing procedures and methods. You also need to include information about facilities and utilities.

We recommend that you manufacture at least three distinct validation lots using the final manufacturing procedure. One of the validation lots is manufactured using raw material near its expiration date.

I would like to emphasize it is important to lock down the manufacturing procedures before making the validation lots. If you later make change to the procedure, you may need to make new validation logs and repeat the performance studies with the new log.

If your test kit components contain preservatives, you will need to submit preservative effectiveness studies for applicable components. Such study are typically

performed at the end of the stability.

If your product is microbiologically controlled such as filtered for microbial reduction, you need to provide bioburden limits for applicable components and determine pre-filtration bioburden level.

You also need to submit microbial interference studies. In these studies the functionality of the product is assessed. Other information include procedures for in-process or release testing for bioburden and the procedures to assess the level of microbial contamination in the facility during manufacturing.

For nonclinical studies, we recommend that you submit them to module two if you choose the modular PMA pathway. Here is a list of nonclinical studies recommended for the blood group antigen genotyping assays.

A study validating the storage time of blood samples before DNA extraction. DNA sample preparation study in which several DNA extraction methods could be validated. Purified DNA sample stability study. Assay limit of detection study, LOD study. Assay guard band studies. Carryover/cross contamination study.

Interfering substance study. Shipping studies and reagent stability including open vial stability. Cross hybridization studies. Lot-to-lot reproducibility study. Accuracy study. This may not be a complete list. Other studies may be needed to fully validate a new assay's performance. Generally speaking, you need to submit a study to validate all labeling claims.

The blood group genotyping test tends to be multiplex assays. Based on our review experiences, we recommend that you submit information to support the prediction of each claimed phenotype from the corresponding genetic data, such as evidence from literature or book chapters.

Please carefully select samples that should be tested in the recommended nonclinical studies. The samples tested in the studies should challenge as many primers and probes as possible and also these samples should represent different types of genetic variants targeted by the assay.

For multiplex assay, you need to determine how many invalid calls within a test would declare the entire test, entire sample invalid. For example, you could recommend that one invalid call would declare the entire test, the entire sample invalid.

If you want to claim a different number, you need to provide adequate justification including testing data to support the proposed number.

Again, because of the multiplex nature, you need to determine the negative control run validity criteria. Need to consider the signals from each individual target amplicon to ensure the negative control would alert the user in case of contamination from individual PCR product.

A major nonclinical study is internal accuracy study. This study tests well-characterized samples to demonstrate that the test can accurately identify the phenotypes listed in the intended use.

In the PMA submission, you need to describe how the samples were characterized. The phenotype of these samples should be characterized using FDA licensed reagent approved molecular test if they are available. If they are not available, you, the predicted phenotypes from bidirectional sequencing can be used.

If your assay additionally reports genetic data such as polymorphisms, alleles as final results, you need to additionally compare the genetic results from the new assay to the genetic data from bidirectional sequencing or FDA-approved molecular test.

DNA sequencing may be used to characterize the samples or to investigate discrepancies. Please note, please remember you shall not use the parameters from the new assay for the sequencing assay.

The primer of the sequencing assay should be designed and validated independently. They may end up to be the same parameter, but you cannot use the primer from the new assay. You have to design them separately.

Again also for the conversion from genetic data to phenotype data, you shall not use new assay's software to do that work. The conversion from the sequencing data to phenotype should be done independently. These two recommendations also apply to other performance studies.

So acceptance criteria for the accuracy study are that for each antigen phenotype the lower bound of the one-sided ninety-five percent confidence interval should be more than ninety-nine percent.

You need your best effort to test adequate number of samples to meet this criteria. For rare antigen phenotypes which you may not able to locate and test 299 samples, we expect one hundred percent agreement by point estimate.

Although a multiplex blood group genotyping assay reports many antigen phenotypes in one test, you should analyze data and apply to acceptance criteria to each antigen phenotype separately. So calculations should not be based on each blood group system.

In this slide we have a couple of recommendations for DNA quantity and DNA quality required for the new assay. Technically your LOD study, your limit of detection study, would tell you a DNA concentration that maybe work for the new assay.

You may suggest a nominal DNA concentration for the new assay based on this

range. The benefit of this practice is that you can test this single DNA concentration in other performance validation studies such as the interference study, the guard band studies, and the crossover, and the contamination study.

Otherwise, you may have to test the entire DNA concentration in some other studies which could be very resource demanding.

For DNA quality required for the new assay, we may accept commonly recommended OD A260 or A280 ratios for well-established technologies with additional data such as 1.7 to 1.9. If you want to claim a much wider range, this range should be supported by adequate testing data.

Based on our review experiences, it could be very challenging to design a study to validate a much wider range.

So assay guard band studies are performed to validate all key assay parameters outlined in the instructions for use. You may choose to design and conduct this study together with assay QC material to demonstrate the QC material are sensitive to anticipated analytical variables. You may save a lot of time with this approach as compared to conducting a separate study for the QC material.

For the shipping, drop test, and the stability studies, we recommend that you use the actual packaging configurations in the study and challenge the worst case shipping conditions. It is, at each time interval until the end of the study, you should show the functionality of the kits, not just the visual inspection.

For the software, we recommend that you complete all the development and software testing before submitting the PMA software module. Modification to the software, we require additional testing.

Limitations of the software should be clearly stated in the user manual. For any test samples in an invalid run or for any individual invalid test sample, the software should not provide genetic or phenotype results.

Here are a few software related FDA guidance documents which may be helpful for you to prepare your software section.

Clinical comparison studies. These studies should be conducted using at least three sites representing U.S. population. Please test the random samples in the studies which could be leftover de-identified samples from each laboratory's routine clinical analysis that would be normally discarded.

You may need to test samples collected from both donors and patients. Depends on the intended use. If your test is not limit to be used on donors only, you should also include patient samples.

We recommend that you use at least two reagent lots from the new test in these studies. To determine concordance, please compare to phenotypes from antigens if FDA-licensed reagents or approved molecular tests are available. For other antigens, compare to phenotype results predicted from bidirectional sequencing.

If you also claim genotypes, genetic data as the final results, you need tocompare, additionally compare to the genetic results from the new assay to the genetic results from bidirectional sequencing or from FDA approved molecular test.

In the study you should follow a pre-defined algorithm to resolve discrepancies. Any discrepancies should be investigated and reported. Please make sure your clinical set will conduct the study in accordance to the study protocol.

For example, they should use FDA licensed reagent if they are all molecular, use

a molecular reagent, or serological reagent if they are available. They should follow the predefined algorithm for discrepancy resolution.

They should not contract the work out. Protocol deviations should also be investigated and reported. For final studies analysis, please calculate all agreement using initial test results prior to discrepancy resolution.

The study's acceptance criteria is similar to the accuracy study. Please remember you need to apply the acceptance criteria to each antigen phenotype separately.

For the precision study, the precision study is designed to assess reproducibility and the repeatability. So test sample in the precision study should cover different types of genetic variance targeted by the assay and most if not all phenotypes.

We recommend that you use at least three sites for the study. So studies should be designed to capture possible sources of variation including within run-to-run, lot-to-lot, day-to-day, operator-to-operator, instrument-to-instrument, and site-to-site variations.

You may use one lot of reagent in the precision study if you have a separate lot-to-lot study. The separate lot-to-lot study can be performed at an internal site. For the study acceptance criteria, we expect one hundred percent agreement. Please investigate and report any disagreement.

The genotyping assays are also subject to the labeling requirement outlined in 21 CFR 809 Section 10. Please follow these CFR section carefully to prepare the labeling. Please included the polymorphisms, alleles, and antigens that the device interrogates and reports as final results in the intended use.

Please pay attention to the limitations section of the package insert. Many genetic assays are designed to target specific, predefined genetic variants. In the limitation section, you

should discuss any other genetic variants that are not targeted by your assay but are known to affect phenotype prediction.

Your assay may be submitted as a system. In this situation, please remember to include the labeling of other components in the PMA such as the user manual for the software, the user manual for the instrument.

The blood group antigen genotyping assay are also subject to the requirement of the UDI rule, unique device identification. You can refer to 21 CFR 801 Section 20 and find more information on FDA's UDI website.

For the quality control material, this material may not be human genomic DNA. For example, it could be plasmid DNA. You need to demonstrate the QC materials are as sensitive as human genomic DNA to anticipate analytical variables.

The labeling on the QC materials should clearly state all limitations. If the QC materials are not intended to monitor the DNA extraction step, please say so clearly. Please also make it clear how often the QC materials should be used. For example, each test run and for each reagent lot used in one run.

You can refer to FDA guidance document, assayed and unassayed quality control material for more recommendations regarding the studies and the information requested for the QC material.

After your test is approved by the FDA, you may have different reasons to make modifications to the test. There are several different types of PMA supplement that you can use to submit modifications to an approved test, such as panel track supplement, 180 day supplement.

In addition to 21 CFR 814 Section 39, you can refer to FDA guidance,

modifications to devices subject to premarket approval the PMA's supplement decision making process to determine when to submit a new traditional 510(k) or submit a specific type of PMA supplement for the modifications.

PMA applicant also required to submit periodic annual reports. Generally speaking, reportable changes that do not affect test safety and effectiveness can be submitted in the annual report.

For the blood group genotyping assays, identification of new molecular variants after PMA approval may affect how the results should be interpreted and whether any modifications should be made to the test.

So it is important for you to monitor any new molecular variance that may affect your assay's performance. The information about the new variance may come from feedback from the customers, complaint investigation, or review of literatures.

Based on the impact of the new variance on your assay's performance, you may have to update the applicable package insert. For example, you may need to add a new limitation statement to clarify that incorrect test results may be obtained in the presence of a new clinical variance.

Whether this update may need supplement and FDA review and approval depends on the significance of the change to the package insert. New molecular variants and markers should be evaluated through the design and development process and potentially incorporated into the device following FDA review and approval.

For example, you may later need to make change to the existing primer and the probes or include new primers or probes based on the finding of a new clinical variant.

Let's move on to the HLA, HPA, and HNA genotyping devices. As Wendy

discussed before, this type of devices are currently unclassified. FDA has held device classification panel meetings and has proposed to classify them as Class II medical devices.

These tests requires 510(k) submissions. FDA has published guidance document regarding 510(k) submissions for HLA genotyping devices used to, used for donor and recipient matching. Some of the recommendations in this HLA guidance document may also apply to HPA and HNA genotyping devices.

Here are a few highlights from this HLA guidance document. In the 510(k) for HLA genotyping assay, we recommend that you submit internal accuracy study test that was nationally or internationally recognized well-characterized samples.

The benefit of using the well-characterized samples is that their HLA typing result that do not contain ambiguities. It is common that a new assay may report HLA results with ambiguities. In this situation, the concordance is determined if one pair of the alleles from the new assay is the same as the known result. For precision study, the list of ambiguities, if there's any, should also be compared.

Regarding changes to a previously cleared assay, if you introduce a test kit for different HLA locus, you should submit a traditional 510(k) for the new test.

Here's a summary of what we have just discussed. For new molecular erythrocyte antigen typing devices, you can choose to submit a modular PMA or a traditional PMA. The two previously approved cases were submitted as a modular PMA.

So major content of a PMA includes CMC information, nonclinical studies, software, clinical studies, and labeling. We have provided our recommendations for each major content.

For the QC materials, you need to demonstrate they are sensitive to anticipated

analytical variables. After your test is approved, you need to monitor new molecular variants and make changes to the kit as needed.

So HLA, HNA, and HPA typing devices are subject to 510(k) requirements. Thank you for your attention. You can, if you have questions for the presentation, you can ask in the next Q and A session or you can contact me directly using the email on this slide.

MS. MERCADO: Thank you, Jason. So now we have an hour, about an hour to answer your questions. So if you have any questions, please come to the microphone.

AUDIENCE QUESTION: Hello, yes. Hi, I was wondering, we have the two guidance documents for recommended methods for blood grouping reagent evaluation and AGG and I was wondering are there any plans to update those guidance documents, particularly with some of the new requirements and/or also issue one specific to reagent red blood cells?

MS. MERCADO: Yes, we have plans to update the two guidance documents, the draft guidance documents that we issued many years ago.

Questions from the audience? So we just want to talk to you briefly about the 508 compliance. Annette?

MS. RAGOSTA: Okay, so we're going to be asking that your labels meet the 508 compliance requirements. Now that's not part of our determination for, to make sure that the product is effective. It's just a requirement that you guys have. So because we post your package inserts.

So please when you send in the initial package insert, let us know if they have been looked at for 508 compliance. If you're having any trouble getting them to 508 compliance, let us know. Because I know you have a lot of pictures and, but we have companies that do it successfully. So just to let you know, we will be doing that.

AUDIENCE QUESTION - MS. WILLIAMSON: So for, back to the reagent red blood cells. So for the three conformance lots, do you expect to see all the kinds of stability done on those three conformance lots or will you take data from previous in house studies? Oh, sure, Elaine Williamson, American Red Cross.

MS. RAGOSTA: So it should be done on the conformance lots. I think that's what, we put that in the submission.

AUDIENCE QUESTION - MS. WILLIAMSON: So all of the kinds.

MS. RAGOSTA: Yes.

AUDIENCE QUESTION - MS. WILLIAMSON: Okay, thanks.

MS. RAGOSTA: And I would, just to let you know, for the guidance that Teresita talked about, it will address red cells, so.

MS. MERCADO: Okay, if there are no questions, thank you for coming.

DR. LATHROP: Well, I was just going to say thank you for coming. I think there's been a lot of interaction during the meeting, so a lot of questions have already been answered and don't need to be addressed here in the Q and A. So thank you. Again, if this has been useful we appreciate feedback. If you'd like to see it again, other topics that you would like us to address. The slides will be available, so check the website in a couple of weeks. And thank you and safe travels for everybody going home.