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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

CURRENT TOPICS IN IMMUNOHEMATOLOGIC TESTING

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C O N T E N T S

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C O N T E N T S (Continued)

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P R O C E E D I N G S

Welcome

DR. ZOON: I want to welcome everyone to this Workshop on Current Topics in Immunohematologic Testing. I am Kathryn Zoon. I am the Director of the Center for Biologics.

When my staff asked me to introduce this particular topic, it is sort of our boutique topic, one which is a very small niche but one that is extremely important in terms of blood safety because if we don't do this right, then we really don't have the appropriate skills and opportunity to help people with blood transfusions overall.

So I want to just, one, tell everybody that the Center does view this as a very, very important aspect of blood safety. In saying this, we are excited to be able to support this workshop and have you all here, and I particularly want to take the time to thank my staff who planned this particular workshop, Joe Wilczek, Helen Morrow Worst, and Sheryl Kochman. They worked very hard putting this together and I hope it provides the opportunity, and I would like to encourage your input during the course of the workshop today to make sure that all these very important issues get discussed.

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In recent years, FDA has participated in a nationwide effort to enhance the safety of the blood supply in this country. I think there is no doubt that blood, whether it's this year, last year or will be next year, is always under a large spotlight.

We work in a fishbowl in the blood industry, you do, we do, and it's very important that the communication between the FDA and the people in the industry is very clear and that the expectations are clear, because if they are not, on either side, the fishbowl tends to gravitate downtown into the halls of Congress. So we will continue to have these workshops and help to support the efforts in improving blood safety.

We have made very great strides over the past several years in the area of blood safety, and I think overall the public can and should have confidence in the safety in our blood supply. But in the same context, the blood banking industry, as we know it, has evolved over time and has become far more complex than it has been just even 25 years ago, which for some of us seems like a long time, but in the real time world, actually isn't that long ago.

One of the issues that we would like to focus on is in terms of the immunohematologic testing, this is one of the safety layers in the blood supply. The development of

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new standards for these products, which will be discussed today, is really an essential element in one of those layers of safety.

It ensures that patients are typed and screened correctly to assure compatible cross match, and if you look at the history of the standards that have been set, you will see that the standards have come from three different sources: the Public Health Service Act, which CBER has worked under for the past almost hundred years; the Food, Drug, and Cosmetic Act; and FDA guidance documents, which are currently ones that we will continue to use to get the appropriate interpretation of regulations out to the industry as needed.

They do a great deal in our mind to define our role in what the standards are, and they also have a lot to do with the business that we have framed today. FDA's role often is to set standards for products that have been made by multiple manufacturers, so that when you read the labeling of the product, it describes the set of properties and that they are consistent and that the standards that we set determine those properties.

Today, we have the participation from the academic community, the manufacturers of blood grouping reagents, the anti-human globulin and reagent red blood cells, and most

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importantly, from people who use these products, so that we can see if there is a way to better define these standards.

We have designed the workshop with a significant amount of time to allow open public discussion and enabling exchange of information and ideas, so I very much would like to welcome you today and thank you for your participation in advance and look forward to the outcome and products of this workshop, so thank you very much.

I now have the great pleasure of introducing Mary Gustafson. Mary is head of the Division of Blood Applications in our Office of Blood Research and Review. It is always a delight to introduce Mary.

Mary, thank you very much.

Opening Remarks

MS. GUSTAFSON: Thank you, Dr. Zoon, and we are glad that you were here today to welcome the participants of this workshop. I would also like to reiterate the welcome. It's kind of a crummy day outside in Bethesda and it is also very, very close to the holidays, and we are very happy to have your participation today.

We are very pleased to sponsor this workshop for a small but important segment of the blood industry. With all the emphasis today on infectious disease testing, we

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sometimes forget that if the red cells aren't compatible, nothing else matters after that.

That is probably an overstatement, but we have become so accustomed to the quality of your reagents and the safeguards that are built into testing that we sometimes overlook the critical importance of the reagents in transfusion medicine.

In case there is not time later, I also want to reemphasize Dr. Zoon's thank you to the staff members who put together this workshop today, particularly Sheryl Kochman whose vision and persistence in working towards having follow-up workshops after the very, very successful 1990 reagent workshop has made today's workshop a possibility; and Helen Worst, who works with Sheryl, and has worked tirelessly to organize and plan today's program; Mr. Len Wilson, who is the branch chief of Sheryl and Helen, and whose branch manages the CBER regulated devices that vary everywhere from refrigerators to nucleic acid testing, and in that vast area of medical devices, he always seems to find time to give his attention to each product line; and last but not least, to Joe Wilczek, who is a member of the office's Policy and Publication staff, and he is the program coordinator for today's workshop. Sometimes when a workshop comes off without a hitch and seems to be effortless, we

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forget that it takes a lot of logistics and planning to really develop each part of the workshop.

Today's topics have been carefully constructed to bring public discussion to areas of interest and concerns. First of all, the morning will be spent on anti-D reagents. We could probably spend three or four days and not get through all of the topics involved in anti-D testing, but it is a reality that because of the success of the Rh immunization programs, we are really not dealing with a number of polyclonal anti-D reagents and we have to look towards the future.

Of course, there is the problems that are related to the weak-D and partial-D phenotypes in donor and patient populations when we talk about the monoclonal reagents. There is also the polyclonal-monoclonal blends and how they detect the partial-D phenotypes, the clinical impact basically of switching from polyclonal to monoclonal-D typing serum.

We know that there is not consensus in the area of D typing and we hope that we have a spirited discussion and come to some agreement by noontime today.

The afternoon, we have several topics that deal with performance, primarily starting off with the performance of antiglobulin control cells. We all pretty

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well know what this control should be, that a weak control is the best control for a test, but we also know that we have users that expect that 4-plus clump, and if they don't get that 4-plus clump, they think there is a problem with the reagent.

So with more emphasis today on pharmaceutical processing in the blood bank and process controls, perhaps we can talk about user education and see if we haven't reached a time when we can make the control really a good control for the users.

There is also the issue of saline - saline whether it's homemade or whether it is bought and whether it has claims for buffering or whatever, we all know that there is the icky-stickies that sometimes pervade all kinds of serological testing, and is this background noise that we just have to put up with, or are there strides and advances that we can make in the area of saline.

The labeling that goes with the products. It seems that there is a lot of maybe overreading of the package inserts in trying to make distinctions from very small wording differences, and I think we would like to have discussion today to see if there is not a way to level the playing field and to really have claims of superiority based

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on true superiority, and not just subtle reading of package insert differences.

And last but not least, we want to talk about the validation and the use of the blood grouping instrumentation, and I hope we are not all worn out by the time we get to this topic, because it is very important as we are becoming more and more automated in the laboratory to discuss what is the vendor's or the manufacturer's responsibility in developing and validating the equipment, and what is the responsibility of the user in making sure that that equipment is appropriate for the user environment.

We have a very, very full day planned and so without further ado I am going to turn the program over to Sheryl Kochman.

Introduction

MS. KOCHMAN: First, I have a few brief announcements.

As you probably all noticed, we are not allowed to bring any food or beverages into the hall, so if you need refreshments, you are going to have to step out.

Restrooms are to the right and left outside of these doors. There are telephones across the hall and upstairs. There are soda and snack machines out to the left. There is a food court upstairs where we expect most

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people will probably be getting their lunch. There is a cash machine in the lobby upstairs if you came not quite prepared to dish out the money. So that kind of handles the mechanics of the place.

Again we want this program to be very lively. We have come here with lots of questions, not necessarily many, if any, answers, and we are hoping that that is what today's session will do, is get us some of those answers.

We also want people's ideas about how we can solve some of the problems. You have the option of either coming to the microphone and giving your comments and concerns at the mike, or if you are a little more timid, there are some note cards in your pamphlet. You can put your question or comment on a note card and we will collect them at the aisles periodically.

With that, I would say we need to get moving because we do have quite a tight schedule.

Thank you.

Specificity/Sensitivity of Anti-D Blood Grouping Reagents

MS. KOCHMAN: I would like to first introduce Malcolm Beck, who is going to be our first speaker on the Anti-D.

Donor Center Issues

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MR. BECK: Good morning, everybody, and thank you, Sheryl.

The title of my talk is a bit deceptive I think. I wasn't quite sure what I was going to be talking about and Sheryl gave me a couple of different titles, but finally, I decided that she just wanted me to be provocative and develop some discussion, so it probably doesn't matter whether you agree with me or not provided we have got something to talk about at the end of the day.

I am going to be talking about anti-D from a blood donor center perspective. Just to start at the beginning, then, I suppose in the early days of D typing, 1940 and onwards, this was a relatively straightforward procedure, nothing too complicated, but this age of simplicity didn't last too long.

It was soon realized that D is terribly important secondary to ABO in terms of clinical significance, highly immunogenic and 80 percent of D-negative recipients of D-positive blood could be expected to product anti-D.

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Things got complicated in 1946 when Stratton, perhaps unnecessarily, clouded the waters by describing DU. I think it was Byrd who said that shortly after the

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description of DU, it was clouded with an unnecessary aura of mystique. I wish I had said that.

It was clear that the D antigen came in various strengths of activity and Stratton showed that some D's could be demonstrated by some anti-D's, but not others, and he was able to make a distinction between what he called high grade and low grade DU's, but it must be remembered that at that time, in Britain, the anti-D reagents most people were using came from single-donor sources, whereas, in the rest of the enlightened world, pooled entities were being used, so probably only the very lowest grades of DU's were being recognized in the USA at that time.

It was clear, too, that this was an inherited characteristic, two modes of inheritance most commonly resulting from a trans effect with big C and perhaps more frequent in blacks. As reagents got better and better, then, fewer examples of DU were detected until today only the very lowest grade DU is recognized at all, and the critical significance of this is still questionable.

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Things were even more complicated in 1951, Shapiro was the first to describe a D-positive person who apparently made anti-D in response to D-positive blood. There was some question as to the validity of Shapiro's results, but it

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wasn't long before other examples came along, and so the idea that D was a mosaic was soon accepted.

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So the point today then is that it's clear that D represents a continuum from the strongest D, which will be D--, that might have as many as 200,000 D sites per cell right down to the weakest partial D's, which may have only 100 D sites per cell and a continuum between the top and bottom of this curve.

In the early days of D typing, human polyclonal anti-D could make a distinction between what was clinically important D-positive, and what was clearly D-negative. Now that reagents are changing a bit, that distinction is not as black and white as it once was, and that perhaps is where our problem is.

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So what has changed then? Well, clearly, the reagents have changed.

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Why did the reagents change you might ask. Well, traditionally, anti-D was pooled human polyclonal material. We used it for many, many years, and, as far as I am concerned anyway, it made a completely unambiguous clinical distinction between D-positives and D-negatives.

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Unfortunately, polyclonal anti-D is becoming scarce for all the obvious reasons, because we make these distinctions between D-positives and D-negatives in our donor and patient populations. We rarely stimulate anti-D by transfusion because of the success of RhoGAM and other reagents of the like. It is rarely stimulated by pregnancy these days and we find that the deliberate stimulation of volunteer donors is unethical these days.

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So the sorts of reagents that are available right now it seems to me are there are still some solely human polyclonal material around. It seems that most people are using monoclonal-polyclonal blends and perhaps the future will be monoclonal-monoclonal blends.

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For donor testing -- and I must stress this is all I am talking about this morning -- for donor testing, today's polyclonal reagents and the polyclonal-monoclonal blends, particularly when combined with automated techniques, which is enzyme-treated cells, seems to me to detect all but the weakest weak and partial D cells, but it's this partial D that seems to be emerging as a special problem.

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Partial D then is a phenotype which lacks an epitope or more than one epitope of the normal D antigen, and the question is can we reliably detect these partial D phenotypes with monoclonal anti-D's, or perhaps a better question is why do we care.

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Well, I am told the reason why we should care is patients who lack a D epitope can be stimulated to make antibody to the missing epitopes if exposed to a complete D antigen. I shall say no more about this because this is the province of other people on the program this morning.

I am concerned with donors because I am told donors who lack the epitopes could be misclassified as D-negative with an inappropriate reagent, and if called D-negative, they might stimulate anti-D if transfused to D-negative patients. Someone in my position with a donor center can certainly not afford stimulating too much anti-D in the D-negative patient population.

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So the situation is -- and this is growing all the time -- these partial D's are being categorized first with the anti-D's that were made by D-positive people, but more recently with a series of monoclonal antibodies until at

at least nine different epitopes are recognized on the D antigen depending on whose model one follows, others can show you even a 30-D epitope model, but it is clear that there are an array of epitopes that may help to complete D antigen.

Of these partial D's, it appears to be D-VI that is stimulating so much discussion.

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We are concerned about partial D's because apparently human polyclonal anti-D will soon be unavailable. Monoclonal anti-D's are proven to be pretty epitope-specific. Category D-VI is the most common partial D, at least in white populations as far as we know right now, and D-VI seems to be amongst the least D sites, and most -- and this is the important characteristic -- most monoclonal anti-D fail to react with D-VI cells.

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So what, you say. Well, it is a question of frequency. Here is some studies that have been done to determine the frequency of D-VI, and they all seem to be pretty well agreeing that the percentage is about between .02 percent and .04 percent.

In our study -- I am sorry this is out of line, relying on computers to make slides these days -- but we

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find, and we regularly find, that over our DU population, about 5 percent or about 0.2 percent of the total donor population is of the D-VI category, and we tend to do this pretty regularly.

We like to keep about 20 examples of D-VI as a frozen cell panel because manufacturers often ask us to evaluate their monoclonals with D-VI, and this is something that we can soon build a respectable library of. So I am pretty confident that that is a population frequency in Kansas City anyway.

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Well, is D-VI really a problem? The factors that influence the antigenicity of D presumably include such things as the number of D sites, the number of the D epitopes represented on the particular phenotype, and the immunogenicity of the epitopes that are present.

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So if we look at each of these in turn, it has been calculated by -- and I have taken here the means of several studies -- the D-VI has relatively few D sites, in fact, very few compared to the R2R2, for instance, and may be as little as only 100 sites per cell. It is difficult to get too excited about an antigen expression where there is only 100 sites per cell perhaps.

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The array of epitopes on D-VI is exceedingly limited, looking at the 9-epitope model, only 3, 4, and 9 have been demonstrated. If you look at the 30-epitope model, there is rather more, but then there is more as a total.

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Sorry about that. What that says, if you stand on your heads, is that the D epitopes vary in their immunogenicity, and the important characteristic of D-VI cells is probably the fact that 6 and 7 are absent rather than what is present, because 6 and 7 are possibly the most highly immunogenetic.

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So looking at all this, it seems to me that two views are prevailing these days. There is the cavaliers who would look at all these factors and come to the conclusion that D-VI is of relatively little clinical significance, certainly in donor populations, and they would support their argument by saying that the immunogenicity of D-VI is probably low, although we don't really know.

The reasons for suspecting that they might be low are the very low site density of antigens on the cells, which might be as few as 100, the few D epitopes present,

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the absence of the important epitopes 6 and 7, the relatively low frequency, only 0.2 percent in Kansas City anyway, or more significantly, there is no report of D-VI transfusion inducing anti-D in a D-negative recipient.

I could certainly be recruited to this school very easily, especially when one considers that we never take into regard other important antigens, such as K little c and things like that.

In fact, I did a quick calculation and we transfuse about 500 units a day in my area in Kansas City, so we might be talking about transfusion to about 250 patients If D-VI was not tested for, and masqueraded as D-negative, then, that would mean that a D-VI transfusion to a D-negative recipient would occur once every 30 days based on a two-unit transfusion to each patient.

It is difficult to get excited about an event that occurs so rarely when we completely ignore the fact that each day in Kansas City, we regularly transfuse 20 units of K-positive blood to K-negative recipients. Apparently, that's all right.

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Then, there is the more cautious view. Members of this school would point out the full D antigen is highly immunogenic, so therefore there is an emotional appeal to

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regard anything associated with D to be terribly clinically significant, and, in fact, they will say the immunogenicity of D-VI is totally unknown, and the reason for this is that experience with anti-D typing with purely monoclonal reagents -- and that is the environment one would need to estimate how frequently D-VI would be classified as D-negative, and therefore transfused to be negative -- that experience is exceeding limited, so how do we know how immunogenetic D-VI is.

They would also tell you that the site density of D-VI can certainly be more than the 500 that people like to quote. Type II and type III D-VI's have rather more D sites than this. There might be as many as 12,000 on some D-VI's. So I think their position would then be so why risk it, because the problem can be avoided by selection of monoclonal anti-D that does detect D-VI, and I could certainly be easily recruited to that school, too.

You would probably want to know, then, what I would recommend as an anti-D, and if I could go to the overheads now, please. I have got three overheads that I would like to show you because I struggled with this.

[Overhead.]

So what is the perfect anti-D for donor testing? Well, if you ask me what I want, I would love to have an IgM

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directed agglutinating monoclonal blend which detects all 30 epitopes currently defined. Then, I have got no problem.

[Overhead.]

Since it's Christmas and I have been good all year, why don't you give me an anti-D that includes all the other bits and pieces, too? Please note these included DHR, which only appeared in print yesterday, I think, so I am right up to date with this.

The final overhead, please.

[Overhead.]

But to be more practical, and join in the ranks of the cautious school, I would say that I don't have much choice, do I? I need an anti-D that detects D, weak D, and apparently D-VI, because while there is an opportunity to immunize someone, I can't risk it, and I am swayed, not so much by science, as I am by competition, because we have people in our area who would love hospitals to change their supplier, and they are going around pointing out the hepatitis tests vary in their sensitivity, and do you know that the Community Blood Center in Kansas City is using a 2.0 test when we have a 3.0 test. So what am I going to do, if they go around telling people that the anti-D used at the Community Blood Center in Kansas City doesn't detect D-VI, whereas, ours does, so I am not sure that I have a great

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deal of choice. If I have a choice and I can detect D-VI, then, I suppose I had better.

Thank you very much.

[Applause.]

MS. KOCHMAN: Thank you, Malcolm, for an enlightening talk.

Now, we would like to have Gail Coghlan come up.

Clinical Issues in HDN

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MS. COGHLAN: Since monoclonal anti-D typing reagents have become widely used, there is some concern that they may give different results than human polyclonal reagents when typing D-variant cells.

The concern is that we may be calling some people Rh-positive that are at risk of making anti-D. Of course, that problem also existed when we use polyclonal reagents. The question is whether the problem will increase depending on the specificity of the new reagents.

What I intend to do is review the record of Rh immunization in our prenatal population and discuss what impact the use of monoclonal typing reagents might have. When you do a lot of reference serologic work, as I do -- and I think probably some of you do, as well -- almost by

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definition we look at the unusual cases and not the bigger picture.

Since I am affiliated with the Winnepeg Rh Laboratory, we really have quite a big picture when it comes to Rh immunization.

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Our lab was founded in 1944 by Dr. Bruce Chown and Marion Lewis, and since its inception, has been responsible for all aspects of the management of hemolytic disease in the province of Manitoba.

That includes all serologic testing of all pregnant women in the province, which currently has a population of 1.1 million, the medical management of all immunized women, and the care of those babies at risk of erythroblastosis, and since 1969, the administration of a universal Rh immunization prevention program. So, in other words, we really have quite comprehensive statistics when it comes to HDN.

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This slide shows the total number of prenatal patients with a positive antibody screen that we have seen each year -- in the background color here -- and how many of those had anti-D -- in red.

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As you can see, since 1969, when our Rh immunization prevention program began, the number of women with anti-D has dropped from about 80 percent of the total down to about 15 percent in the 1990's. The top line here shows the total number of births in the province each year, and generally, that is between 16 1/2 and 17 1/2 thousand.

Now, obviously, we would like this number to get closer to zero, and it is not through lack of effort on our part that it does not.

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I will just through our prophylaxis program. I think it is safe to say that our Rh immunization prevention program is as rigorous as any. It includes a 28-week gestation injection of 300 micrograms of Rh-immune globulin for all non-immunized Rh-negative women.

If delivery has not occurred within 12 weeks of this first antenatal treatment, a second injection of Rh-immune globulin is given. If the woman has had two antenatal courses of prophylactic anti-D, no postpartum treatment is given unless a postdelivery maternal blood sample shows no passive anti-D or there is evidence of a significant transplacental hemorrhage.

Those women who have had a 28-week course of Rh-immune globulin then deliver before 40 weeks gestation,

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receive postpartum Rh-immune globulin, 120 micrograms if their babies are Rh-positive.

We also request a 36-week gestation maternal blood sample, which is screened for antibody, and all Rh-negative women who don't show any passive D in this sample are given another 120 mcg.

Of course all Rh-negative women who have an abortion, whether spontaneous or therapeutic, or are undergoing an invasive procedure, such as chorionic villus sampling or amniocentesis, are given protection.

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Given that rigorous Rh immunization prevention program, why do we still see approximately 20 to 25 cases of prenatal women with anti-D each year?

Well, first of all, you have to remember that the number of cases is cumulative. Once someone has made anti-D, they will show up in our statistics each time they are pregnant. In this table, I have just shown a breakdown of the probable reasons for immunization for all our cases between 1990 and 1995.

So if we look at this in some detail, then, in 1990, we had 11 cases in what I have called the external group. These are women who are immunized before they moved

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to Manitoba, so they are not counted in our overall treatment failure rate of approximately 0.25 percent.

Getting back to these ladies, of the 11, 4 of them were from other parts of Canada, 2 were from the U.S., and 5 of the ladies came from countries where they had no access to an immunization prevention program, and most of us I think are from North America, so we all probably see people that are fairly recent immigrants.

Four of the women were from South America, and one was from Africa.

The next group in the table are failures, and these are women who, as far as we can tell, were treated following our prevention protocol, but they still made anti-D. Six of those women were already making anti-D by their 28th week of pregnancy, and three of them had been given Rh-immune globulin and still made anti-D.

The next group here is women that have had large transplacental hemorrhages. There was five of those in 1990. Of that five, one, the TPH that occurred in that pregnancy, and four in past pregnancies, and, of course, as soon as we know someone has had a TPH, we give enough Rh-immune globulin to cover the estimated size of the bleed, but obviously, that isn't always successful in preventing immunization.

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The next group here is doctor error, and the doctors had a good year in 1990 because we couldn't blame any immunizations on them, but an example of things that would fall into this group, in 1991, we had two cases. One of those ladies had presumably made anti-D because she wasn't given Rh-immune globulin after a stillbirth, and another woman had had an Rh-positive transfusion in childhood.

Now, speaking of doctors, there are some cases that you would almost say were Acts of God, and I didn't think it was appropriate to put a God row on the table, so it is really quite amazing what happens.

One example would be a woman who was evacuated from her community because of a forest fire, and there was a lapse in her prenatal care and she didn't receive any antenatal prophylaxis. Again, natural disasters occur just about everywhere, so that is always going to be a little bit of a problem.

Another case was a woman who was visiting a nature preserve in Nepal and was gored by a rhinoceros. With only basic medical services in the area, someone decided she needed an immediate transfusion, and they used her traveling companion as the donor. Of course, he was Rh-positive.

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In 1995, this lady had a baby that required eight in-utero intravascular transfusions and three transfusions after birth, but thankfully, in the end, the baby did just fine.

Getting back to the table, we have some cases of immunization that can be attributed to the patient's own actions. The case in 1990, that was from a woman sharing syringes and needle to inject drugs.

Other examples in that category might be women who don't seek any prenatal care. We don't have too many of those. Occasionally, we have a woman who refuses Rh-immune globulin because it's a blood product.

The last group, unknown. It is just that those are cases where we didn't get information or we couldn't determine any likely immunizing event.

Until now, what I have been talking about are Rh-negative women who make anti-D. Presumably, whether we are using polyclonal or monoclonal reagents, the normal Rh-positive and Rh-negative blood will be classified as such with no problem, but, of course, there are always a minority of cases which fall into a gray area, and these are the variant D phenotypes, so the question is what will happen to this category now that we are using monoclonal reagents.

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I thought I would first talk about the group that we have always called D user, more appropriate now we are calling a weak D.

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I know that lots of policy state that women with a weak D phenotype should be considered Rh-positive, but it has always been our policy to give DU women 120 mcg of Rh-immune globulin if they have an Rh-positive baby, and we do this because there is no easy way to tell if someone has a partial D phenotype like Category VI or just a low number of normal D antigens on their red cells.

So, in 1979, we gave postpartum Rh-immune globulin to 29 weak D mothers who had Rh-positive babies, and then in 1980, we had 20, and, of course, in those years, we were using the polyclonal reagents.

In 1996, now using a monoclonal typing reagent, we had 18 women who were typed as weak D, and received Rh-immune globulin, so it seems that there is a slight decrease in our lab and using our methods in the number of people we are calling weak D since we have been using the monoclonals, but with the present monoclonal reagents and the way we do our testing, we should still be calling a Category VI a weak D.

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Of course, Category VI is not the only Rh variants that can make anti-D, so what about the other ones? Well, we have always typed Category II, III, IV, V, and VII cells as Rh-positive, and I expect that most people have.

This depends somewhat on what the specificity of your anti-D was like, but generally, that would have been the case. With the current monoclonal reagents, Category II, III, IV, V, and VII cells should still be called Rh-positive, so there is no change in the prophylaxis from past practice.

Now, as I already mentioned, with the current monoclonals, we should type a Category VI as a weak D and give postpartum Rh-immune globulin if the woman has an Rh-positive baby. Again, that is no change from past practice.

A case where we know monoclonal reagents can type someone differently is with the very rare RoHar phenotype. In the past, with our reagents and methods, we should have called these people DU's and treated accordingly, but the IgM component of the present monoclonal reagents will pick up the few epitopes of D on an RoHar cell and consequently, we would now call these people Rh-positive and they wouldn't get any Rh prophylaxis.

There are two reports of people with this phenotype making anti-D, however, we have never actually

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found a prenatal patient who has the RoHar phenotype. In our lab, we have always had a very strong research program, so unusual typing results that turned up in the routine testing were thoroughly investigated, so obviously, I can't say for sure that we never missed an RoHar, but I really don't think so.

I also asked Marion Lewis if they ever found someone of that phenotype in any of the hundreds of family studies done for various gene mapping and genetic studies in our research lab, and again the answer was no.

So, at least in our population, I would say the typing of an RoHar woman as Rh-positive is a non-issue. In fact, we have only ever found one pregnant woman with a partial D of any sort who made anti-D, and that was in 1966, before the Rh immunization prevention program.

The mom was a Category VI-D. The antibody was found in her fourth pregnancy, and although her baby became mildly jaundiced and somewhat anemic, no treatment was given and the baby was fine.

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So, we have a pretty paltry experience of D-positive people who make anti-D in our own prenatal population, but we have also had some referred cases. We don't have too many because we never really encouraged

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people to send us that particular serologic problem, but presumably the cases that we did receive weren't selected in any way, so I am hoping that this table should somewhat reflect the relative frequency of anti-D found for each of the partial D categories or phenotypes.

So, as you can see, we had one case of Category II with anti-D, four Category III's, nine Category IV, and all but one of those was a GOa-positive, so therefore they were a Category IV-A. There was five Category V's, and no Category VII.

So I put that group on this side of the table because I want to emphasize the point that these people have enough epitopes of D that their cells will be agglutinated by the majority of D's and anti-D's made by Rh-negative people. Therefore, we have always called these people Rh-positive.

We have also made the assumption that there was no point in giving them Rh-immune globulin if they were pregnant because the passive anti-D would bind to their own cells and offer no protection from immunization by fetal cells with the normal D.

We have also had 10 referred cases of Category VI that made anti-D, but, of course, these referred cases will be drawn from goodness knows how many people that were

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actually tested. So again in our own population, we have only ever had one Category VI that made anti-D. Also, these cases here are not necessarily all women. Some of them are transfused men.

We have never had an RoHar, a DFR, a DBT referred case where someone has made anti-D or these are quite newly described, at least we don't know of any of those types. Well, RoHar, that was described in '71, I think, but you can see, though, that we do have a fair number that we weren't able to classify, so it is possible some of the unclassifieds may now fit into the DFR phenotype, but I don't think any of them would be DBT's, since we routinely test any cells with an unusual Rh phenotype with anti-Rh32, and we would have followed up if any of the unclassifieds had been Rh32-positive, since Marion and Hiroko Kaita from our lab were the ones who described Rh32 and its association with the R--n phenotype.

So I really can't comment on how we would type these two phenotypes in our routine lab because we haven't seen examples, but whether or not we would call them Rh-positive, and not give prophylaxis, I think is really splitting hairs. If we haven't found one of them who has made anti-D in 50 years, I don't think we are going to have a sudden epidemic.

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Now, the other thing I put on this slide is the low-incidence antigens that are associated with several of the phenotypes. Despite the monoclonal panels of anti-D that Malcolm spoke about, that you can use to determine the epitopes present on a partial D cell, it is much easier to identify which of the categories the cell fits into if you can test for the low-incidence antigens that are characteristic of them.

For example, if I really wanted to make sure I never called a prenatal patient Rh-positive if they had the RoHar phenotype, I would test them for Rh33 if I had enough Rh33.

The same goes for Category VI. The best way to find one of them is to look for BARC. According to Patricia Tippett, almost all C-positive Category VI cells, which is the most common type of Category VI, have the BARC antigen.

So I have no idea if it is possible, but what I would do in a perfect world is make a blend of monoclonal anti-GOa, DW BARC, Rh32, FPTT, whatever I could get I guess, and test all the transfusion recipients and prenatal patients with it.

Now, since it would be a waste of time in our population to add another test solely to pick up the odd partial D, and since even in a perfect world cost

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containment would be a factor, I would try to make the diluent of the monoclonal blend I had created as close to that of my routine anti-D as I could get it, and then I would run the monoclonal blend instead of a totally inert control serum, something we have to do anyway.

In that way, I would pick up the odd partial D without increasing the number of tests I have to do, and I think that is a better way of doing it than hoping for a single anti-D typing reagent that is going to solve the problem of the gray area in red cell serologies.

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But that was as bit of a digression. Really Rh-positive patients with anti-D are not a problem in our prenatal population and they are not a problem in the transfused population in Winnepeg either.

Our Red Cross has a centralized cross-match service for all of the hospitals in the city and according to their reference lab manager, they almost never see a case either.

But you have to remember that our experience with people that make anti-D, even though they have some D on their red cells, is a reflection of our population. So, as you can see from the ethnic breakdown here of -- this is

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from our provincial census data from 1991 -- most of our population is of European ancestry, 85 percent.

Now, these numbers total up to more than the total population of the province because, of course, once people have been in North America for a few generations, they end up usually with having a bit of a mixture of ethnic background, so people are allowed to put down more than one on the census.

Anyway, the main point is that 85 percent of our population is probably caucasian. We have a substantial aboriginal population, but most of those people are Rh-positive. Originally, they probably all were, but now with some admixture, there is a few Rh-negatives, but most of them are still Rh-positive, and as far as I know, a partial D phenotype has never been described in an aboriginal population, although there are a few partial D phenotypes in Japan, but in the Asian group here, most of those people would be Rh-positive, as well, although that group does include some people from India.

But really the important thing is that we have a very small black population, and you have to remember that Category III, IV, and V phenotypes are much more common in people of African ancestry, so you can't necessarily take

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our experience and apply it to every population or every city in North America.

So, in conclusion, then, I would like to say that in our prenatal service, virtually all of the women who have anti-D are Rh-negative. Despite the prevention programs, this is still the biggest problem, there is always people that slip through the cracks and for various reasons will make anti-D. In our population, production of anti-D by women with exotic D phenotypes is really not a concern.

Thank you.

[Applause.]

MS. KOCHMAN: Thank you, Gail, for those very interesting statistics. It is nice to be able to have some numbers we can put our hands on for a change, instead of having a lot of imaginary numbers out there.

I would like to introduce a man who needs no introduction, and that would be John Case.

Manufacturing Issues

MR. CASE: Thank you, Sheryl.

The cross I have to bear I suppose is that since I come after Malcolm and Gail, you are going to find some of my remarks to be repetitive. However, I perceive my role here as being to put things into perspective, so to speak,

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by reviewing the whole subject of anti-D blood grouping reagents.

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I am supposed to speak from the manufacturing point of view.

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I thought I would start by simply going over the varieties of polyclonal reagents as they have existed over the years with which I am sure we are all familiar.

First of all, there are the IgG reagents that are formulated in a high protein diluent, usually with a macromolecular additive to enable them to react reliably with cells suspended in saline.

Secondly there are the IgM low protein reagents that are made from human plasma from people who have made anti-D in response to immunization.

Finally, there are the chemically modified IgG reagents that because they are chemically modified, don't require a high protein diluent nor a macromolecular additive and therefore can be formulated in a low protein diluent.

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Now, if we consider, first of all, the most abundantly available kind of polyclonal anti-D, which is the high protein IgG with potentiators, they have certain

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obvious advantages, the first being that they give good immediate spin reactivity in a tube test with saline suspended red cells.

Secondly, they give good avidity on a warm slide using whole blood or a 35 to 45 percent suspension of red cells.

Thirdly, they give reliable detection of weak D or what we used to call DU cells at the antiglobulin phase, and are therefore abundantly available at reasonable cost.

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The disadvantages are associated with their tendency to cause spontaneous agglutination of IgG-coated cells, and for that reason they are unsuitable for D typing of red cells having a positive direct antiglobulin test, and secondly, you have to run a parallel control test on every blood sample tested using a potentiated high protein control reagent, and preferably that should be one that is distributed by the manufacturer that distributed the anti-D, so that you have exactly the same potentiator, the same level of protein, and, as a result of that, the same likelihood of potentiating spontaneous agglutination due to the presence of IgG on the cells.

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Now, when we come to low protein IgM reagents, they have certain advantages, too, in that the low protein formulation avoids spontaneous agglutination of IgG-coated red cells.

Needless to say, if your sample has cold agglutinins or something that causes rouleaux, you are still going to get a tendency to produce aggregation that doesn't have to do with the D antigen, but if you are sensible in those cases, you would be used washed cells.

But in any event, these reagents are suitable for testing IgG-coated cells, and they don't give a false result due to spontaneous agglutination, and so there is no need for a parallel control test.

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The disadvantages of such reagents are that there is a limited potency attainable with the human source material that is available, so you have to have a test that involves incubation, you can't have an immediate spin test. You have to incubate for 15 minutes or longer, and you cannot do the test on a slide.

Secondly, these kinds of reagents do not detect the weaker forms of D, so you can't use them for donors.

Thirdly, there is a scarcity of suitable raw material which influences the availability of it, and, in

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turn, inflates the cost. This kind of polyclonal anti-D has always been extremely expensive. In practical terms, this reagent is bought exclusively for the testing of cells that happen to possess a positive direct antiglobulin test.

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Now, when we come to chemically modified anti-D, the very first example of which appeared on the market towards the end of 1978, these kinds of reagents have certain obvious advantages.

Firstly, they have the same good potency as the high protein reagents because they are made from IgG and they show good, strong reactivity at immediate spin and good avidity on a slide.

Secondly, because the effect of chemical modification of the IgG molecule is to increase the flexibility of the molecule, saline-suspended D-positive red cells can be tested without the use of a high protein diluent or the presence of macromolecular additives, so you don't get spontaneous agglutination of IgG-coated cells.

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Like IgM reagents, there is no need for a parallel control test because your ABO test, providing it is truly done in parallel with your anti-D test, provides an adequate control unless the blood being tested happens to be Group

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AB. Unlike IgM products, these are suitable for the detection of weak D by the antiglobulin test.

Finally, since there is such an abundance of IgG raw material and the manufacturing procedure is relatively simple, there is an abundance of available product and at a modest cost. Incidently, I mention all of this knowing that, in fact, chemically-modified anti-D has gone completely out of fashion nowadays, largely because it is harder to get polyclonal source material in the first place, and secondly, because with the advent of monoclonals, it is no longer necessary to chemically modify IgG.

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The disadvantages of chemically-modified anti-D are that the modified molecule is vulnerable to enzyme action at the hinge region, so if you have any protease activity in your product, the potency tends to fall off steeply.

Secondly, if you have potency at too high a level, you can have a prozone. In fact, we put out in the very, very early days our initial chemically-modified reagent came out in 1979, about the middle of the year, and one of the early lots was so potent, and we were so proud of it, until somebody in New Zealand incubated the test for 30 minutes and found that it went negative with Rh-positive cells, and

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this had to do I think with the fact that when you chemically modify the IgG molecule, you leave some of those molecules unmodified, so the result is that as you incubate, more and more of the unmodified molecules go on to the cells which results in blocking of the sites and therefore the reaction tends to go negative over time.

Third, the stability of the chemically-modified IgG molecule is inferior to that of native IgG for reasons unknown.

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Now, when we come to monoclonal anti-D, which came into the picture a little over 10 years ago now, there is a relative abundance of raw material because once you have got a stable, producing hybridoma producing anti-D, there is no end to the amount that you can produce.

People often think that this ought to make it cheaper, but if, in fact, you are a manufacturer that does not grow your own hybridomas, and therefore you have to share your profit with somebody that does, in fact, the cost is not any less significant from the cost of buying human plasma.

Secondly, the exceptional potency of monoclonal reagents is such in a low protein medium that you can get immediate spin results even though these are IgM and you get

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good slide test avidity with these IgM reagents in a low protein diluent.

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The disadvantages or the potential disadvantages of monoclonal anti-D are these. First of all, predictably, a single monoclonal antibody is not going to recognize all the partial forms of D, because they are directed as a single epitope.

Secondly, IgM is always potentially less stable than IgG.

Thirdly, your tissue cultures supernate that contains the harvested antibody doesn't contain other proteins, so it is naked, so to speak, and is not protected, so this may result in an unpredictable loss of stability on filtration because there is a tendency of IgG and IgM to stick to the membrane infiltration.

Fourthly, the availability of raw material could be subject to uncertainty in the case your hybridoma mutates, and that could be a big difficulty because the thing is that an FDA license is clone-specific, and if, in fact, suddenly your clone dies out, you are going to have to wait a long time. I hesitate in these premises to say how long, but you could wait forever to get the FDA to approve a new clone.

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Now, when monoclonals first became available, I am sure everybody is aware that the first licensed monoclonal reagent or the first licensed anti-D reagent that contained a monoclonal component was that introduced by Ortho some years ago now, and it took the world by storm, so to speak, in that it was, first of all, a mixture of monoclonal and polyclonal anti-D, the monoclonal component providing good, strong immediate spin reactivity, as well as slide test reactivity with most D-positive red cells.

The purpose of blending polyclonal anti-D in with that was in order to be able to detect the weaker forms of D, but not only the weaker forms of D, also the partial forms of D that the monoclonal component didn't find.

Secondly, the potency of the IgM component provided better reactivity with some of the quantitatively weaker forms of D. In other words, some of your "DU" cells came up by direct agglutination, and that is either an advantage or a disadvantage according to your point of view. If you think it's terribly, terribly important to give Rh-negative blood to all "DU" recipients, then, you might be alarmed by that.

On the other hand, if you are blase about that, then you think, well, it's a good thing.

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Thirdly, the polyclonal IgG component is there to provide antiglobulin reactivity with those forms of partial D that are not recognized by the IgM component.

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The disadvantages of these blends are that some partial D's are directly agglutinated by potentiated high protein reagents, but may not be by the monoclonal IgM. For example, Tippett's category V was not well detected by the MAD-2 monoclonal IgM that was present in the first Ortho monoclonal anti-D, not that that is a disadvantage I hasten to say, but it did result in the detection of some cells that used to be thought of as D-positive, classifying them as DU with this new reagent.

Those anti-D's that are based on the NELP-3 monoclonal, some category V's react and some don't react with it, so it is a mixture.

Secondly, most monoclonal IgM anti-D's give good strong agglutination of RoHar red cells, causing bloods formerly classified as D-negative to type as D-positive, which creates confusion.

Now, you already heard Gail say that in her Manitoban population of 1.1 million, she has never encountered an RoHar, but as manufacturers of an anti-D reagent that is capable of giving direct agglutination with

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RoHar cells, I have to say that we know of quite a number as they have been reported to us as discrepant results in D typing.

Thirdly, mixing IgM and IgG antibodies directed at the same antigen can diminish the reactivity of the IgM component because of competition for sites, so this blend has to be made very, very carefully. If you put in too much IgG, you can, in fact, impair the reactivity of your IgM component.

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Now, more recently on the scene has come monoclonal blend, which is wholly monoclonal, in other words, both the IgM and the IgG component are the same, monoclonal. Now, the advantages and disadvantages are exactly the same as those that apply to monoclonal-polyclonal blends.

You still have this potential for competition for sites because although presumably your monoclonal IgM and your monoclonal IgG are directed at different epitopes, nevertheless, there is a tendency, if you make the IgG component stronger, too strong, then, you can get, in fact, some diminution of the reactivity of your IgM component.

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I just saw a recent paper actually of somebody that had proved that to be the case in Japan, who is present in audience I noticed.

And additional disadvantage of these blends is that partial forms of D could exist that do not possess the epitopes against which the individual monoclonal antibodies are directed. The use of multiple individual monoclonal antibodies could diminish this likelihood, but do enhance the effect of competition for sites.

I should also mention that from a manufacturer's viewpoint, it is all very well to talk about putting in umpteen monoclonal antibodies. Each one that you put in adds to the cost. I mean everybody wants to buy cheap, but, in fact, you know, even though these are monoclonal antibodies, there is some cost involved in manufacturing each of the monoclonal source materials.

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Finally, you will hear more from me during our discussion later. I guess we have to be satisfied with what we have got, and Phillips Brooks wrote some years ago, "Dreadful will be the days when the world becomes contented when one great universal satisfaction spreads itself over the world; sad will be the day for every man when he becomes absolutely contented with the life that he is living, with

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the thoughts that he is thinking, with the deeds that he is doing."

With that, I shall finish. Thank you.

[Applause.]

MS. KOCHMAN: Thank you, John.

Summary

MS. KOCHMAN: You will find in your packet a publication that was recently sent to me by Dr. Marian Scott. I wanted to summarize some of the concepts that are described in that paper, because I think it could add a lot of fuel for our discussion today.

[Overhead.]

So, I am going to kind of add a few additional comments and bring up a few additional points to liven up the discussions.

[Overhead.]

First, to put some perspective on what testing has been done with the use of the various anti-D reagents, I just wanted to put up some figures on the new monoclonal reagents.

As you can see from this slide, we have had five different manufacturers of monoclonal-polyclonal blend anti-D reagents. The first was licensed in 1988 with a minor change to enhance consistency made in 1994. The second was

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approved in late 1990. Another didn't come along until 1992, and then we have a new one in 1996 and a new one in 1997.

I also listed the number of lots that have been manufactured and distributed under FDA license for each of those manufacturers. Now, some of them I didn't have records back to the initial approval date, so I know that there were in excess, for example, of 24 lots of the first produced prior to the change, and on the second reagent that was approved, there were in excess of 49 lots distributed.

It gives a rough idea of the kinds of usage we were seeing. This doesn't tell you how many vials of reagent were being used, but it gives a general perspective of how much reagent was being made and putting out there.

[Overhead.]

There has been mention of monoclonal blend anti-D's. We currently have two firms approved to manufacture those. The first was approved in September of 1994 with a recent approval in January of 1997, and as you can see, there has actually been very few of these lots that have gone out and been used in the market.

[Overhead.]

Lastly, we have one manufacturer who has been approved to manufacture monoclonal anti-D that is a single

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clone of IgM origin. That reagent does have special labeling with it. That one was approved in January of 1997, and again only six lots of this product have been submitted to FDA for release at this time.

[Overhead.]

So, bearing those dates and those numbers in mind, it might help to put some perspective on what we know about category VI, RoHar, and all the other issues that we are talking about here today.

In the paper that I have included in the packet, there is a description of one approach. It is predominantly a European approach to performing Rh grouping, and the main basis for this approach is that you would treat blood donors in one manner, and then treat transfusion recipients and pregnant women in a different manner.

[Overhead.]

Under that approach, the goal of donor testing would be to classify as Rh-positive any weak D or D variant that is capable of evoking an antibody response, and they specifically excited categories IV, V, and VI, and RoHar, including all the weak D's and the other categories, but then when it would come to doing the grouping on transfusion recipients or pregnant women, you would classify as Rh-negative any variant not detected by current IgM monoclonal

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anti-D's, and most specifically, this would be category D-VI.

[Overhead.]

Using this approach, the use of the reagents would also vary. In the donor population, monoclonal-polyclonal blended reagents would tested. I put room temperature. Some of them are immediate spin, some of them have a brief incubation, so I just lumped everything as room temperature. So those would be tested at room temperature and at the indirect antiglobulin phase to ensure that you are detecting all the weak D's and the variants.

The monoclonal blends tested against donor cells would also be tested at room temperature and at the indirect antiglobulin phase. I am sorry, there is a mistake on the slide. The monoclonal IgM's would be tested only at room temperature in the donors if they were used at all.

In a recipient or a pregnant mother, they are not recommending the use of the monoclonal-polyclonal blends because of the need for the indirect antiglobulin tests to detect all of the examples and because of the inherent error in performing an indirect antiglobulin test.

They would also recommend that if you were using a monoclonal blend, that you would perform that test only at room temperature to avoid the error introduced by performing

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the indirect antiglobulin test, and mostly they are recommending use of monoclonal IgM, testing only at room temperature by whatever the manufacturer's directions for use are, and in general, you would pick two reagents in each of these categories.

[Overhead.]

So, as result of that type of testing, if you had category IV and that person was a donor, they would be grouped as Rh-positive. A recipient or a mother would also be grouped as Rh-positive. The same would go for D-V, and ironically, the same would go for RoHar.

The D-VI's are the only ones where the donor would be classified as Rh-positive, but then a recipient with that same phenotype or a pregnant mother would be classified as Rh-negative.

[Overhead.]

I think this introduces a real area of confusion or uncertainty. They are now going to have D-VI individuals who have two Rh groups depending on what they are. If they are a donor, they are going to be Rh-positive, and then when someday they become a patient and they are told they are Rh-negative, they are not going to understand how their type has changed.

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Other areas of uncertainty are that if we are treating RoHar patients as Rh-positive, we are not certain whether or not they will be able to develop anti-D from an Rh-positive transfusion or from pregnancy.

Lastly, it is also unknown whether Rh-negative patients might develop anti-D's if they receive blood from an RoHar transfusion.

[Overhead.]

If we are looking for solutions to problems, I think we can say that category VI may not be a medical problem. Yes, it is going to be confusing to the patient, but the approach described in the paper doesn't present any medical problem, just problems of confusing the patients, so there will be necessarily no action except to counsel the patient why they have two different Rh phenotypes.

The RoHar issues do have potential medical problems, though. My question is do we, as is recommended in the paper that I have handed out, wait and see what happens if we are going to call RoHar's Rh-positive, or should we be proactive and assume that RoHar is immunogenic and could cause problems, should we be working on something to eliminate these problems.

One of the suggestions in the paper was that some reagents could be optimized, so that they won't detect

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RoHar's. Apparently, some of them have thermal amplitudes that, with variation, they detect different variants to different levels.

[Overhead.]

One thing I noted in reviewing the approach that was published is it does not describe how the fetus should be managed. They apparently are using quite a bit of advanced technology in Europe, and they have pretty much skimmed over how you would test and group the fetus. Presumably, they would be treating the fetus in the same manner as you would treat a donor in terms of how they are classifying them.

[Overhead.]

Ironically, the paper brings up some timely additional concerns. Under the European approach described, there are higher minimum potency standards than the FDA's current minimum potency standard.

It is expected that the European Union is going to adopt that higher standard under the Medical Device directives that are forthcoming.

Coincidently, FDA's two anti-D standards are running out and we are faced with having to replace them, and I think now is the appropriate time for us to ask ourselves, since we have to replace the standards anyway,

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should we consider the European approach and raise the potency of these standards or should we go forward with what has already worked in the past.

That is all I have.

I think now we can open the floor for discussion.

Open Discussion and Proposals

MR. CASE: Who is going to speak?

[No response.]

MR. CASE: Well, perhaps I should.

MR. BYRNE: One question with regard to the standards. The current standards I believe are human based, if we are going towards a monoclonal base, reagent base, I assume that the new standards would be monoclonal?

MS. KOCHMAN: I think that is not an automatic assumption. I think that the FDA views the standards as minimum potency standards, and if we can achieve that minimum potency with a polyclonal source, we might well do that, but I also think that we are not tied into one versus the other. I think that we would go with what is readily available and what consensus could be reached at in terms of minimum potency.

Again, I would not view these standards as being appropriate for fine specificity, but merely for the potency.

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MR. CASE: Perhaps I could address that issue. My own feeling is FDA should adopt a higher standard now since we have monoclonal reagents available that, in fact, exceed the existing standard for IgM material by a very substantial margin, and my feeling is that the level we need is not consistently attainable with polyclonal material, so I think they are going to have to adopt a monoclonal standard.

I do believe that if we are measuring the potency of an IgM reagent, we should be measuring it against an IgM standard.

MS. RAY: I am Molly Ray. I am from the FDA, but I have a question as a blood banker. Once we determine the best protocol for accurately typing the donors, infants, et cetera, how are we going to ensure that the RhIG that we administer will cover the partial D and prevent stimulation of the anti-D to do that?

MS. COGHLAN: We have never treated anyone -- well, most of the partial phenotypes, like a category II, III, IV, V, VII, we have never given our Rh-immune globulin to those people, because we call them Rh-positive, and we have never seen anyone that has made an anti-D, and we only ever had one case of a category VI that made anti-D, and that was before we had any treatment program.

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MS. RAY: Right, but the question was the RhIG that you administer, will that contain anti-D-VI?

MS. COGHLAN: Oh, I see.

MS. RAY: To prevent stimulation by the mother.

MR. CASE: Well, for as long as it is made from polyclonal material, I am sure it will.

MS. RAY: That is declining.

MR. CASE: But when it comes to monoclonal, I don't know.

MS. RAY: Since that source is dwindling.

MS. COGHLAN: Certainly the polyclonal do, I mean because the people that we use for typing have done the serum that we have used for typing for quite a few years in our lab are actually the women that were plasmaphoresed to go, and their plasma was used in the Rh-immune globulin, so I know for sure that most of them, probably all of them would, their individual plasmas would detect a category VI.

MS. RAY: I guess this is more of a point to consider as the sources of the polyclonal or HIG are dwindling?

MS. COGHLAN: Right.

MR. CASE: If you want my opinion, I think category VI is a non-issue.

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MS. ROLIH: Susan Rolih with Immucor. I wanted to agree with John Case. As a manufacturer, I think with our anti-D standard as it exists is not an accurate representation of what we want to do with the monoclonal reagents, and certainly would support a more potent standard.

I think at the same time, since we are all dealing with a blend of IgM and IgG components now to prepare our reagents, to have a standard that only measures the potency of one of those two components, if we go to a standard that is just an IgM antibody or against which we only test at an immediate spin phase, it is not only a direct representation of what you expect to see as far as potency in an antiglobulin test with that same anti-D reagent, and, in fact, if you look at a lot of products, you don't always get consensus on a lot-to-lot basis or from the manufacturers of what activity is represented there at the antiglobulin phase, so that perhaps we need to look at a standard that is formulated a little bit differently or two separate standards for both activity areas of that anti-D reagent.

MR. CASE: I think two separate standards is appropriate. In fact, the approach we took when we submitted our application for monoclonal-polyclonal blend, the approach we took was that we would attain at least the

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level of the IgM standard in a saline test and that we would attain the level offered by the IgG standard at the Coombs phase, because that is the phase at which that became operative, and that was the approach we took.

Now, I think what is needed now is two separate standards. The IgG one could be polyclonal, I feel, but it needs to reflect the reactivity of your product. In other words, if you product is reacting only at the Coombs phase, then, it is at the Coombs phase you need to measure to compare the reactivity with the standard.

George is next, right?

DR. GARRATTY: I have been a little slow getting up because I am still a little confused on what we are trying to decide on, but if I could just talk generically about the subject and about the weak D's, I just thought that there has been far too much emotion over the years spent on DU's and weak D's, as we are calling them now, and I still think that.

I really don't understand the paranoia that seems to exist in Europe about the weak D's and this driving force that Doug Voak has to convert everybody to this is a major problem.

I think it was put in perspective very nicely about Malcolm and by Gail, and I agree with everything they

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said, and just wanted to add some stuff that I am sure everybody is aware of here in terms of the donor, and Malcolm said that he didn't seem too concerned, but I also agree if you wanted perfection, you could do what he said, but nobody quoted the old work of Paul Schmidt, and I am sure everybody is aware of it.

Paul Schmidt, years ago, in this institution, transfused over 60 people, D-negative people with DU-positive blood, many of which were perhaps category VI, and not one of them made anti-D, and they received whole units of blood.

They did make, however, antibodies, so they were immunologically responsive, most of them, although it has been argued some of them were undergoing treatment that might have made them immunologically unresponsive, but they made other blood group antibodies, but not one of them made anti-D.

So, I think there is no doubt some individual may make anti-D, but I think it is going to be exceptionally rare, so I think, as Malcolm said, we haven't worried about C's and D's and JKA's over these years, and I really can't see why we are too concerned about that.

Having said that, I agree with everything they said about perfection, and this is why I say I don't quite

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know what we are trying to decide here. I just hope the FDA won't mandate that we do some of these things.

On the other hand, I would like to see the companies producing different monoclonal antibodies that are capable of doing some of these different tricks, and we can make our own decisions in the hospitals and in the blood centers.

I think that is where I have always envied Europe, that they do have access to many, many monoclonals and can play these games and do what they want in their own institutions if they make a decision, make their own risk-benefit exercise just like we do with compatibility testing in different hospitals, whether we want to go through a risk-benefit exercise and do certain testing.

I hope we can apply some of the principles that Malcolm spoke about if the reagents are available. I just hope we won't be driven into having to do some of the things that they are suggesting in Europe.

MR. CASE: One of the things, you know, it sounds easy to get manufacturers to do things. One of the difficulties I think, when you come to read this paper by Douglas Voak and Marion Scott, et al., that is in your package, you will see that it is written in rather emotional terms. It is very important, they say, that D-VI people

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should receive Rh-negative blood and that D-VI donors should be classified as D-positive.

As I said, I can't see why that is so important when it is okay to give K-positive blood to K-negative recipients, and c-positive blood to c-negative recipients, and, in fact, most of us go through our entire lifetimes without seeing a category VI person that has made anti-D.

However, the good side is that all of the monoclonal IgM reagents currently available in the United States do not detect category VI. Now, they do detect your quantitatively weaker D's, most of them, what used to be called DU's, they will usually detect them, not necessarily at immediate spin, but they give a weak direct agglutination reaction, and maybe those people are going to be called D-positive and given D-positive blood and no harm done.

They don't detect category VI's, so category VI's are always going to be classified as D-negative among recipients. Now, when it comes to donors, one of the things that Voak and Scott say in their paper is that to do an anti-D test by the antiglobulin test is, quote, unquote, "dangerous." They actually use the word "dangerous," and they quote as their justification for that, that in the surveys that take place in Great Britain from time to time,

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there is a high percentage of people that classify coated Rh-negative cells as D-positive.

Now, my view is that if they do that, then, we are dealing with incompetence, because they should not be classifying as D-positive anybody that has a positive direct antiglobulin test. Isn't that so?

So, my view is that among donors, it is very hard to find IgM's, monoclonal IgM's that react by direct agglutination with category VI. I think in the recent workshop, there was only 1 out of some 26 or 27 anti-D's presented that actually gave direct agglutination of category VI.

So, if you think that manufacturers are going to be able to produce a monoclonal anti-D that directly agglutinates category VI, so that you don't have to use an antiglobulin test, forget it.

MS. ROLIH: Susan Rolih again. I just wanted to mention that there are a number of monoclonals currently under evaluation by the FDA that are going to broaden your ability to detect certain of the partial D or weak D phenotypes, so that at some time in the U.S. marketplace, as well, you might have a selection as to the performance criteria you want in your reagent.

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Nevertheless, I think that as we are talking perfect world, and we are looking at trying to eliminate rare occurrences from happening, that we have to remember that as long as human beings are involved in testing, we will never approach zero.

I think from all manufacturers we have reports in our files indicating the number of patients that have been incorrectly classified as an inappropriate D type based on a technical error as opposed to performance of the reagent, so even if we come up with that perfect cocktail that picks up all the lows, et cetera, you will never, by formulating a reagent, come up with a perfect reagent.

The only way you can come up with zero problems is to make sure Rh-negative females only marry or have children with Rh-negative men. That is the only way you can make it 100 percent safe.

MS. MALLORY: It didn't work for me. I have I guess the honor of speaking for both the Red Cross and the AABB at this point, and I would just like to say that I think that in the past, with polyclonal reagents, we typed weak D's as D-positive.

We typed a lot of the categories as D-positive in both patients and donors, and I don't think I have ever seen a great deal of evidence to show that this has been a

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monumental problem, and I agree with John, I think that it tends to be a little more emotional, I guess, or they get too focused in Europe.

I personally think that we might end up with a whole lot more problems if we try to have two different kinds of anti-D, used in two different institutions, that might get mixed up or confused, and then we would have real errors if we mistype someone instead of the very rare problem of a person who makes anti-D.

The times that somebody would make an anti-D would be primarily a male, a female who is not of transfusion age or who is not going to have children, or a patient who is going to die, to me it just seems we are blowing this whole thing out of perspective, and if we could get the appropriate reagents, use them appropriately in all circumstances, I think we would be much better off.

I believe in typing donors using automated equipment, we don't pick up the very weak D's as it is, and I don't think that we are having any problems at all in those situations. I have not ever seen anything reported, and I am sure this goes back to the work of Paul Schmidt, has shown that people just aren't going to make anti-D when they are stimulated with very weak D antigen.

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So, I think I would rather see one anti-D, or if we are going to have to have two, then, we are going to have to have some very strict standards about how they are going to get used, and I would just as soon not go to those extremes and to continue as we have been.

MR. BECK: Well, you are right. One consequence of the European approach would be that there would be two reagents formulated, one for donor use and one for patient use, and I thought that that was a potential cause of concern or at least would be for institutions testing both donors and patients.

We recently had a meeting of all the blood bank supervisors in the Kansas City area. We do this on a regular basis. So, I posed the question to them, and I was expecting them to resist that, but they thought that would be just fine, they thought they could handle two reagents. In fact, I thought they were a bit offended when I suggested that perhaps they couldn't.

MS. MALLORY: Until they make an error and then get caught.

MR. CASE: You will notice that the European thing, what it says in Scott and Voak, et al.'s, paper, is that you should be using two anti-D's, and I think in Germany, there is a rule that you have to use two anti-D's,

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one of which is capable of detecting category VI, which is rather weird actually, because that is for patients, and what they really mean to say is at least one of which is incapable of detecting category VI.

MS. COGHLAN: I just wanted to say to Delores, talking about the category VI, as I said in my presentation, we treat category VI women postpartum, we give them Rh-immune globulin, but we only ever had one case, so really, you know, looking at our data, I am not sure why we still keep treating those women.

I think although you can't say, well, we only ever had one before, so, you know, we would only have one in the next 30 years, it doesn't really work like that. We would probably be entirely justified at calling those people Rh-positive, which would mean we would be calling all the category cells, prenatal patients, Rh-positive.

As you say, we have always called most of them, and we have never had any problems. Now, obviously, in the mostly caucasian population, we would run into more category VI's than anything else except everybody forgets about category VII. Target cells are very common, but it's only a point mutation in the D protein, and I think there is eight reported cases of target-positive people with anti-D, and all of them were weakening, they were of no consequence.

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MS. ROSSMAN: Hi. I am Patti Rossman with the American Red Cross. Delores alluded to some point that I hope will not get lost in this discussion, and that is we have just recently completed an evaluation of some monoclonal reagents using our automated blood grouper, and we found that some of these monoclonal blends work very well with an automated system, but some do not because of background noise.

So, whatever we decide about the specificity of our monoclonal anti-D, we would like to suggest that it is very important to us, as the end user, to have reagents whose formulation is such that it can be used with our automated equipment.

MR. CASE: The background noise, I think has to do with something in the formulation other than the monoclonal antibody, doesn't it? I presume.

MS. WEILAND: Debbie Weiland, Ortho Diagnostics. I just wanted to bring up a point with respect to our discussions on the European approach to the D testing, and that is, to remind us all that many of them do pay attention to K-typing, Rh phenotyping, and will transfuse K-negative individuals, K-negative blood, et cetera.

So, I think there is really a different level of emphasis on some of the more unusual or rare occurrences

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that the Europeans tend to be mindful of in light of what they are doing.

I also seem to remember lo those many years ago, some discussions at AABB about whether we should treat DU-positive people -- this would be the early seventies -- as Rh-negative when they were patients and Rh-positive when they were donors. So, it is a philosophy that had some discussion within this country many years ago.

I think we have tended to move away from that discussion in more recent years, but it was something that we went back and forth about as to what we should do. I think the overriding concern was what are we going to do with a lack of Rh-negative blood if we classified these D weak individuals as Rh-negative as recipients - are we going to impact Rh-negative blood supplies negatively.

MR. CASE: That is a concept that goes back to the old days when it was considered very, very important that you did not give C-positive/D-negative blood to D-negative recipients, et cetera, which we have kind of discovered wasn't really important at all.

It is very interesting actually that years ago I remember somebody at the Munich ISBT meeting, somebody from Germany presented a paper in which they described how they had adopted the policy of giving Rh phenotype-matched blood

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to everybody and K-negative blood to any K-negative recipients, which, in fact, it produces a logistical problem of enormous proportions, you can't keep it up. That is the bottom line.

I remember Marcella Contreras castigated this fellow for proposing such a preposterous, ridiculous idea, and yet the same Marcella Contreras is behind this idea that it is very, very important to give D-negative blood to D-VI recipients, and I just don't follow the logic. Just because it is D it is important, but because it is little c and little e, it doesn't matter, so I don't know.

MS. COGHLAN: I think a lot of it is because we make the call, we say this person is Rh-negative or Rh-positive, and if you don't test for K, if you give a K-positive unit to a prenatal patient, I mean there is certainly lots of very serious cases of hemolytic disease of the newborn because of anti-K.

But, fortunately, if you do have a woman with anti-K from a transfusion, in our population there is about I guess it is 10 percent chance that her husband is K, and then he is going to be a heterozygote. But, otherwise, I mean those types of things would be serious problems.

MR. BECK: John's comments have reminded me of another European approach to the partial D problem, that at

at least are seen suggested. I am not sure whether several are being put in operation. That is the fact that a category D will nearly always be traveling with big C or big E, why not test all apparently Rh-negatives with anti-big CE and any positives would then be considered as captured also, the partial D's.

MR. CASE: It will find most of them. In fact, category VI's are almost invariably R1's. There are R2's, and so if you test with anti-C and E, or CDE, you know, you can catch them.

The problem there is that if you are talking monoclonals, you are talking about a mixture, a blend of monoclonal antibodies that are expensive, and you know, if you want a monoclonal anti-CDE, which is a blend of anti-C and anti-D and anti-E, it is going to cost you some, because it is not easy to produce these at low cost.

MR. BYRNE: Peter Byrne, American Red Cross.

In the late eighties, in England, there was a great debate going on as to whether to label r-prime, big C, positive D, negative units as Rh-positive or Rh-negative, and, in fact, I remember getting r-prime units from different regions in England that were labeled differently. Some were labeled Rh-positive, some were labeled Rh-negative, and that goes along with the whole European

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paranoia of transfusing antigen-positive units to antigen-negative people, I suppose.

MR. CASE: The question of RoHar, we haven't discussed up until now, and perhaps we should bring it up now. RoHar in the old days with polyclonal reagents, you could get a positive, quote, unquote, "DU" test, but it was an extremely feeble one, and I suspect that in the majority of instances, with the majority of polyclonal anti-D's at the Coombs phase, it was missed.

So, these people got classified as Rh-negative, and to the best of my knowledge, there is no evidence that any Rh-negative recipient of one of those units of blood ever produced anti-D.

On the other hand, I think there are two cases in the Netherlands of RoHar recipients that have produced anti-D. Sheryl asked the question in her presentation, should we, in fact, be concerned about this, should we wait and see if it's a problem or should we be proactive and start thinking about some way to avoid this.

Now, my view of that is that we have no choice but to wait and see, because the number of monoclonal IgM's that do not detect RoHar is very few, just as there are very few monoclonal IgM's that do detect category VI, there are very few that don't detect RoHar as D-positive.

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Even NELP-3, which was the monoclonal source in our original monoclonal-polyclonal blend, if it is formulated at a particular concentration, will detect RoHar in the hands of people that don't shake too hard.

Now, the newer one, the one that contains the Gamma-401 anti-D, it is gangbusters, it is 4-plus at immediate spin. So, you are going to find RoHar's as D-positive with the modern monoclonal anti-D reagents.

The MAD-2, which was the monoclonal IgM component of Ortho's product, does not detect RoHar, but it is one of the very few monoclonal IgM's that don't. Incidently, when we are talking about does detect and doesn't detect, there are degrees of all of this.

I remember years ago I was giving a talk on this subject in London at the North London Regional Blood Transfusion Center to an audience slightly bigger than this in a rather smaller room, so it looked crowded, and I remember I threw up a slide showing the various categories and how the different monoclonal anti-D's reacted with them.

I was disconcerted to note that right in the middle of the audience was Patricia Tippett, the very person that devised this system of classifying partial D's, and she was laughing, and so I was, to say the least, rather disconcerted, and so I stopped in my stride and I said why

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is Dr. Tippett laughing, and she said, well, she said, just as there is variation in the expression of D on cells that have a normal D antigen, there is variation in expression of the particular epitopes that are present on these, and you can't generalize, in other words, you can't say your monoclonal always reacts with category III or it always gives 4-plus reactions with category III, you know, sometimes you may get a weaker reaction, sometimes it may not react at all, and I think that is a reality that we have to face.

MS. COGHLAN: I was just going to add something, John. When I was looking through our files about RoHar, actually, when you look at single donor anti-D's, the IgM in those will pick up an RoHar, some of them, so we have sort of made the assumption that, if anything, people with that phenotype used to be called the weak TR and Rh-negative depending on what your source was for your anti-D. We may have been calling them -- well, we probably would have recognized that it was a bit altered.

MR. CASE: Well, you may not have. I remember years ago there was a particular lot of Dade's anti-D. Actually, we got a product complaint because our anti-D didn't react with this D-positive cell, and when we investigated, we tried umpteen lots of umpteen sources of

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anti-D and were unable to get a reaction, and so we asked for a sample from this particular Dade lot that had reacted, and it was giving a good, strong reaction, and we managed to discover that, in fact, somewhere in that blend of anti-D was something that was reacting with Rh33-positive cells.

So, every now and again, even among blends, there were ones that did react with RoHar cells, but they were relatively uncommon.

MS. MALLORY: I would just like to make a couple of points, and I am afraid one of them is the same thing all over again, but it doesn't appear to me that there is a huge risk in typing a partial D woman as a D-positive and giving that person D-positive blood.

I would really think that it is going to be very expensive if we try to go to several different kinds of D, training on the different kinds of D, different SOP's that we have to deal with.

All of these things are going to cause more problems for us I think, and we can't, I don't think, get to the point as we have, I think with the infectious disease testing where every test has to be done because of the emotional impact that it has on the public.

But one of the things I would like to stress is that I hope that the manufacturers will not make this happen

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by just saying I have got an anti-D that has to be used for patients, it is better than my competitors' anti-D, therefore, we are going to go do it anyway.

I would hope that maybe out of this meeting, that we can reach some sort of a consensus and that that sort of thing isn't going to happen as a way of selling one anti-D over another one.

MR. BECK: But I think we shouldn't forget the impact on the donors, and Sheryl touched on the topic of what happens when a donor becomes a patient, and apparently the Rh types change, and then you are in the embarrassing position of having to explain that.

We see that already with ABO typing. We have several people we thought were group O's in the past and until they are retyped by a hospital with a stronger monoclonal anti-A and find they are weak subgroups of A, and then you have the situation of having to tell the donors.

I suppose the ultimate in schizophrenia is going to be the autologous D-VI donor, who gets typed as both a positive and a negative.

MS. MALLORY: I would like just to remind those who are not maybe as old as John Case and I, but we went through this for years with donors who were typed by the military. It isn't a big thing to talk to a donor and tell

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them what has happened. I just can't see that explaining to a donor what has happened with their blood type is going to be any major problem.

MR. BECK: It's not a big deal. It just takes time.

MR. CASE: I have got a number of thoughts in my mind here, one of which I put in the background as in response to something you said when you are talking from this microphone, but it occurs to me that the dividing line between "normal D," quote, unquote, "normal D," whatever that is in this context, and weak D, or what we used to call normal, has always been a movable feast, hasn't it, because it has depended -- I always tell the story. Years ago, some lab up in the northeastern part of this country returned to us 110 vials of our high protein anti-D on the grounds that it was too strong. You know, the imagination boggles, what on earth is too strong.

Well, it turns out that during the night some lady had been admitted to the hospital after a road accident or something, and the night tech had typed her with our anti-D and had got her to be Rh-positive, and he had cross-matched two units of Rh-positive blood accordingly which had been administered to the poor lady.

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So, the following day they typed her. Now, whether they used our anti-D or somebody else's, I don't know, but another movable feast is immediate spin, which people don't quite realize that, you know, if there is two minutes delay before you spin it, you get an entirely different answer.

Anyhow, when the day staff came on at this hospital, they retyped this patient's pre-transfusion sample, and behold, they made it Rh-negative. Then, they proceeded to do a DU test by incubating the test for 15 minutes and then taking it straight through to the Coombs phase without looking at it again, and behold, it came out 4-plus at the Coombs phase.

So this, in the parlance of this particular lab, was a DU. And, oh, my goodness, as a result of using Gamma's too strong anti-D in the night, they had misclassified a DU patient as D-positive, and had improperly given D-positive blood to this allegedly DU patient, which was terrible and therefore sent this stuff back to Gamma because it's too strong.

This has always been a problem, hasn't it, and as I see it, you know, the monoclonals have introduced a different problem, but in many respects, it's the same. It

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is confusing confusion in the minds of people that really don't understand the subject properly.

Now, one of the things that you said over here had to do with manufacturers' claims, and the thought that crossed my mind, when we had our last anti-D approved by the FDA for licensure, one of the things we were asked was to say in the package insert how the product behaves with the different categories of D, and I felt that was improper on the grounds that Patricia Tippett raised when she ridiculed me in public, that, in fact, you cannot make a blanket statement in this regard and that if the feds allow manufacturers to make such claims, then, they are inviting misbranding. That is my view.

Now, the same applies to anti-little e monoclonals, do they react with HRS-negative or HRB-negative cells? Well, you tell me. If you take anybody's collection of HRS-negative and HRB-negative cells, there is so much variation within those groups that the whole thing is meaningless.

So, in fact, we should not be asked to make claims in this regard. Indeed, we should be forbidden to make such claims. Such is my view.

MS. KOCHMAN: It is interesting that you bring that up, John, because I was going to ask Dr. Garratty if we

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do encourage manufacturers to come up with all the different monoclonal anti-D's with all their different tricks, how would he propose they be labeled, what would he want to see in a package insert, so that he can make the risk assessments himself, what information is important to him.

DR. GARRATTY: I certainly couldn't come up with wording, but I am sure that people sitting here for the manufacturers, having read all the wording for the check cells -- I am going to be talking about later -- would have the intelligence enough to write the sort of things we have been discussing in their package insert on the attributes of these antisera.

I mean they would be able to say about what it does regarding category VI or category whatever in the package insert and give advice on its application to donors or pregnant women or recipients. I doubt if you would come up with standard wording.

MS. KOCHMAN: But, see, that contradicts what John Case has just said, that he feels the manufacturers should make as few claims as possible, but the users want as much information as possible. How does the FDA reach a happy medium in this area?

DR. GARRATTY: I have no idea how you can reach a happy medium. I can't see -- John, you wouldn't be averse

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to writing some educational blurb in your package insert that would give indication, because you would add the sort of write as you just said.

MR. CASE: I would not be averse to saying that we have tested three, category VI cells and find them to react, but I would want to caution that that may not mean that there are not category VI cells out there that don't react.

DR. GARRATTY: Absolutely, but you would cover that in your educational part of your --

MR. CASE: Absolutely.

DR. GARRATTY: The trouble is, as everybody knows, who does read the package inserts.

MR. CASE: I am not sure whether the average user could care less about this. I mean one of the things Susan said to me today was that, you know, in the old days we always had this intense curiosity, that we were excited when we found something that was a little bit odd.

Nowadays, they don't want to know about that. The level or the standard of education is lower, and people don't care. I mean they took full professional use off the label, for goodness sake.

MS. JAKWAY: Jan Jakway from Ortho. I also would agree with John. I think it is going to be hard for us to say we can detect D-VI. Right now we know there is at least

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three different types of D-VI and probably a lot more. We can say it maybe reacts with three out of three that we have tested, but there is an availability problem, where do we find these cells and to have enough to be able to be, you know, to be assured that we are detecting exactly what the customers want.

I know in Europe, they use a panel of weak D cells, you know, something like that may be considered, but, you know, to say that our reagent is going to detect every D-VI cell, I mean I don't think we can ever make that kind of claim.

DR. GARRATTY: But haven't we already agreed, I thought most of us agreed, we will never reach perfection in this quest. I think everybody in this room would agree on that. That would be a compromise I could see that you would have to say this in your package insert. Then, it would be an education process.

MR. CASE: There are some pie-in-the-sky ideas that you can reach perfection, and I think the panel you are referring to is the panel of 39, or whatever the number is, cells that Dr. van Rhenen has in Rotterdam, and he makes pronouncements about this anti-D is good because it finds 67 percent of my cells, and this particular anti-D is no good because it finds only 44 percent, and the whole thing is

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meaningless, it's an exercise in futility in my judgment, because he doesn't know what these are, they are mostly quantitatively weak D's, some of them are category VI's, he doesn't know which ones are which, and the whole thing, in my view, is, as I said, meaningless.

If Dr. Van Rhenen were in the audience, I still wouldn't be afraid to say that because I have told him to his face.

MS. COGHLAN: I think one of the problems, too, is that you can be a partial D, but also have a low copy number of the partial D on your red cells, so you can be either or you can be both, and everybody within a category doesn't have the same expression, because there is a tremendous range in the copy number.

MR. CASE: That is exactly why Patricia was laughing.

MR. BECK: I think another element that we haven't even touched on yet is going to emerge when there is no polyclonal anti-D to make Rh-immune globulin, and then therapeutic anti-D will have to be formulated from monoclonal anti-D.

Then, there is a very interesting problem with partial D's, isn't there, because you need an anti-D that will not react with the partial D of the mother, but will

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react with the fetal cells. I wish I hadn't brought it up now.

MS. COGHLAN: Well, we have category VI women are Rh-immune globulin, and it doesn't do them any harm.

MR. BECK: Maybe we should do the same thing with donors and patients.

MR. CASE: But does it do any good if the antibody goes on to the mother's own cells?

MS. COGHLAN: Well, that is the question, and that is the debate. You can never really do controlled experiments unless you have a big number of prenatal patients that have a particular phenotype.

The only reason we treat the category VI's is because they -- well, and now we know they have very few epitopes of D -- and there are some experiments to show, for example, if you absorb your Rh-immune globulin with the category VI cell, it virtually --

MR. CASE: It washes it out.

MS. COGHLAN: No, it doesn't actually. I suppose eventually, you might get rid of the D, but you don't really absorb out much, nothing compared to a normal D cell.

The other thing, someone -- Marian actually did, and this was years ago, and I don't know how they quite managed to do this, but they had given an Rh-negative woman

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Rh-immune globulin, and her baby was category VI, so they actually measured the level of the passive anti-D that had gotten into the baby's system, and although the baby's cells were category VI, the passive anti-D persisted in its system for a long time.

DR. GARRATTY: If anybody is interested, I happen to have the absorption paper in my bag here from Le Banco and Marcella Contreras in the British Journal of Hematology. I have got it here if anybody hasn't seen that or wants to see it, where they do suggest that it would be effective if you gave it to a category VI based on absorption experiments, and there was a lot of anti-D left.

MS. COGHLAN: Actually, he says, if anything, you should give a higher dose to someone that is a category VI, which we don't do.

You know, you could do that experiment, I suppose, although we have never done it, but if you could get someone of a particular partial phenotype, a prenatal patient, say, they were GOa-positive, your category IV-A, and give them Rh-immune globulin, one dose isn't going to hurt them, and follow in their serum how long the passive anti-D is there.

MR. BECK: But the problem becomes acute with the monoclonal anti-D therapeutic being very epitope-specific.

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MS. COGHLAN: Right. That is definitely going to have to be a blend of a lot -- I would think probably maybe even more than our typing reagents.

MS. KOCHMAN: There is some literature out now about some therapeutic anti-D's from monoclonal source that people are looking at, particularly in Europe, and this is a concern to us because we foresee the same thing happening in the United States. They are blends of just a few clones, and they tend not to cover all of the epitopes.

So, how much of a problem is that? And do we need to think about that issue when we are thinking about how should the anti-D reagent be working? If we know that someday the therapeutic anti-D is going to be monoclonal, do we have to think about that now and where we are going with anti-D reagents now?

MS. COGHLAN: Well, when you think about it, it only makes sense that the therapeutic monoclonal anti-D would have to be a blend. You might argue that you only need to have something that will react with certain epitopes, but the human source is not only polyclonal, but it's a blend of I don't know how many different women, as well. So, it is very effective, but it also covers virtually, I am sure, every epitope on D, it must.

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DR. GARRATTY: When we were having our discussion a little while back when Sheryl asked about the package inserts, most of the concentration was on the fact that you wouldn't be happy putting in your insert about that this anti-D may not detect all category VI.

Now, that part doesn't concern me so much because, as I mentioned earlier, I am really not concerned about donors, I am really not too worried about that, but if I had to pick one category that I would be worried about, it would be the pregnant women, where I could accept that perhaps it would be nice to use an anti-D that did not detect category VI, or they would be called Rh-negative.

Now, would you be happy there, John, with a package insert of having the monoclonal say this anti-D will not detect category VI?

MR. CASE: Oh, yes.

DR. GARRATTY: I mean that is the category I would be most concerned about and wanting people perhaps to concentrate on, because they are the ones that perhaps you could argue from a legal point of view, as well, that you wouldn't want to miss them, and you would like to give them Rh-immune globulin.

MR. CASE: Not detect is a different kettle of fish from will detect.

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DR. GARRATTY: Yes. That is why I think in that category and treating that group of women, you could make that more definitive statement.

MR. CASE: Absolutely. I am quite happy to say this product will not detect because you have categorized, you have got your monoclonal, you have had the opportunity probably to test dozens of category VI's and say it doesn't react.

The problem is where your particular formulation may or may not react with category VI.

DR. GARRATTY: But that would cover you for an anti-D that could be used for recipients and pregnant women.

MR. CASE: Right.

DR. GARRATTY: And the other one is just for blood donors. We have already discussed that -- I really don't care because it is so rare that they would ever make an anti-D if they were labeled as D-positive to the recipient.

MR. CASE: Unless you have the viewpoint that exists in Europe where it is terribly important that category VI's are classified as D-positive, and -- and you really prefer not to do the testing by the antiglobulin test, you know, which is what they are saying.

MS. COGHLAN: I was just going to make the point, it seems kind of odd to me in England that they are having

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this debate because since 1976, we have always given antenatal Rh-immune globulin, and they are just discussing now in Britain whether they should do that.

Well, when we were just doing postpartum treatment, 2 percent of Rh-negative women were at risk of making anti-D, and with the antenatal treatment, it dropped to about 0.25.

Well, if you don't want any prenatal patients to have anti-D, you should be treating them antenatally before you worry about the odd phenotypes.

MR. CASE: In other words, you save more people from producing anti-D if you give a dose at 28 weeks than you do worrying about whether or not you give it to category VI's.

MS. COGHLAN: By far.

MR. CASE: But this is the sort of irrational thinking that goes on in Europe. See, there is another thing in Europe - it is very, very important, terribly important that your screening cells include the CW antigen, and, you know, everybody here gets screened without screening cells that possess the CW antigen, and we do not hear about people falling about with hemolytic transfusion reactions due to anti-CW.

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So, where is this concept that is terribly important? I have a friend in the Czech Republic that says it is very important for them because they have 6 percent CW in the population, and I said to him that's not what is important, is it. How often do you detect immune anti-CW when you are screening for it? He doesn't know.

MR. BECK: That is why he wants CW-positive cells.

MS. WEILAND: I would just like to put in another thing that maybe we want to consider, and we are talking about such marked differences between Europe and the U.S., and yet some of the things we are doing in other regulations are trying with, in particular, the bar codes, is to harmonize what we are doing across the globe, and do we, if we decide to take a totally different track from what is being proposed for Europe, do we need to consider how that affects the transfer of blood across the ocean given that we might be screening or typing our blood quite differently.

MR. CASE: That is a good point. I nearly mentioned before this obsession in Europe with how important it is not to give K-positive blood to K-negative recipients, results in the fact that much of the Euro blood that comes into this country is K-positive.

In other words, they get rid of their K-positive blood by sending it here, because we don't care.

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MS. KOCHMAN: We have run past the time that we were supposed to take a break. Can we reconvene at 10:45, and resume our discussion? You can all talk amongst yourselves during the break, and maybe we will have some answers or maybe we will just have more questions.

[Recess.]

MS. KOCHMAN: I think that we are ready to resume the discussion followed up by a summary of what has gone on.

During the break, a few people voiced some comments and concerns, so I would like for those people to come up whenever they feel like it.

MS. MONTANDON: Carol Montandon from Ortho. I just had kind of a comment on a practical nature for manufacturers, that it is nice to have all these discussions about what we want in the U.S. and what would be nice, but as a practical aspect, manufacturers are forced to go pretty much whatever the country the most conservative is.

We only want to make one reagent. It is cost effective to only make one reagent, and as long as the U.S. is not objecting to what these European countries are proposing as far as their reagents, that is pretty much where the trend is going to go.

It is very difficult from a cost perspective to manufacture reagents, but it also gets very difficult. You

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want to provide a reagent to the United States, you want to provide a reagent to the European countries, but there is also the regulatory aspect with having, you know, the long regulatory approvals in the U.S. often forces two reagents for periods of time, and I know at Ortho, we struggle with finding what is the reagent we can manufacture that would be globally accepted in all the different markets.

MR. BECK: Doesn't that also apply to the issue of standards, if the FDA standards are minimal and the European standards are more stringent, then, you have got to comply, the manufacturers, that is, with the European standards, haven't you? And then you will satisfy FDA, too. Isn't that true?

MS. KOCHMAN: Yes.

MR. CASE: That is more or less the case. There is a distinction. I guess the folk from Ortho would testify to the fact that they have to make a special antiglobulin reagent for France, because France has a much higher standard for the anticomplement component. That is correct, isn't it? I remember that, I think it was Sandy Ellisor said that at a meeting of the expert panel in Japan last year, and Phillipe Rouge sat there and denied it, denied it, but I know that to be a fact.

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But in regard to the different kinds of reagent, one of the things, there is a perception, for example, in Australia, that this problem that I mentioned of competition for sites between the IgM and the IgG component of a blend, is such that they want a pure IgM reagent, and, in fact, that was more or less what fueled our decision to make a pure IgM reagent, but it is like a drug on the market, you know, nobody wants to buy it except the people that have this concept.

In fact, I think it would be largely a waste of time to try and promote the IgM reagent in this country because even though there are people that are testing almost exclusively recipients for which the IgM is ideal, there are occasions when they need to do a test for weak D by the Coombs test, and they don't want a second reagent. They want everything to be with one reagent.

In fact, you know, a compromise is always unsatisfactory in some way or another, but that is the way the cookie crumbles. You know, you have got to make an IgM to satisfy some eccentric view overseas, but there you go.

MS. ROLIH: Susan Rolih with Immucor. I realize that we all are manufacturing for different markets and many times in order to conserve our production capabilities, we will manufacture to the most conservative market.

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However, I would feel uncomfortable if we set up our standards in this country to match market standards for which I don't necessarily think there is a good basis for science all the time, so I would like to plead with this group that if we come up with standards in the United States, it should be based on what we think is science.

You know, if we have to tool our reagents to meet multiple standards, so be it. I would still like the flexibility that if I chose to ignore a market with undue pressures, that I could still manufacture more cost effective reagent based on science for the United States.

MR. CASE: I agree with that sentiment, but the only problem is it is difficult to make different products, and I don't know whether you have seen the latest version of the in vitro diagnostics directive that has come out in Europe where they classify blood grouping reagents as, quote, unquote, "high risk" and quote, unquote, "highest risk."

If you have ever heard of anything so preposterous in all your life as the idea that you have to submit your product to this, whatever the body is that you are supposed to submit each lot to, to get it approved, I don't know where this idea came from, but I suspect it came from

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France, which is the very nation in Europe that has this preposterously high standard for anti-C-III activity.

MS. COGHLAN: I was just going to say basing things on some scientific evidence, I have never seen, and maybe others have, anyone give the actual frequency of people in Europe, or maybe in England it would be fairly easy to do, people with partial D phenotypes that make anti-D. They say it happens, but I have never seen anybody give numbers.

I don't know if they don't know or nobody has ever actually thought about how many people we have ever tested and how many of those have been a problem.

MS. ROLIH: That is a very good point that you make, and I think it goes even beyond the anti-D with some of the other issues that John has alluded to. There are requests for screening cells carrying a particular antigen that we don't consider that immunogenic in the United States.

As a manufacturer, that is what makes it very difficult for you to argue with those marketplaces, and I think that is why they don't always give us the data is because you can argue with facts, you can't argue when you don't have the facts in front of you.

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So, you are sometimes shooting at a moving target with that, but there isn't always that data that is available.

MS. COGHLAN: Well, I know, or I would imagine anyway, in the States, that it might not be that easy to put together some numbers, but like in Canada or in Britain, where there is a social medicine program, the testing isn't as widespread, it's more centralized, so you should be able to come up with those numbers.

Actually, when I looked at our own data, I was surprised at how few cases we had. But, you know, what happens, too, is a case turns up and it goes from the local lab that found it, to the next one up the chain as far as the referent lab goes, and then it goes -- so, you know, we may often be thinking of cases, but we are all thinking of the same people.

I think you do have to make some attempt to get some figure for actually how many times has this caused a problem.

MR. CASE: I agree. Like I said, I think most of this feeling in Europe has to do with theoretical possibility, and some armchair serologist with influence sits down and decides that, well, there is a possibility. I mean I happened to see one time, he might say, a category VI

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person that made anti-D, I know of one actually that is near to us, and some of our consultation lab people were involved in the publication of a report of this case in which the lady lost her baby, as a matter of fact, from severe hemolytic disease due to anti-D in a category VI person.

But one swallow doesn't make a summer, that Gail has seen one example of this thing, many people go through their entire working lives without seeing one, but this does exist as a possibility - category VI people, transfused with D-positive, normal D-positive cells can make anti-D, and therefore it's a potential problem, and therefore it is very important that, you know, so-and-so, and so-and-so.

But like I said, I am having some difficulty in seeing it as a problem, as a menace to the public health. I mean if we can accept that it's okay to transfuse somebody on the basis of a negative screening test without a cross-match, without even an immediate spin cross-match, with so-called computer cross-match, if we can accept that, surely, we can accept those rare instances where a category VI person makes anti-D as being just the way the cookie crumbles.

MR. BECK: When we do for everything else. I mean I don't know how many JKA-negative people, given JKA-

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positive blood will make anti-JKA, but I am sure that it is much greater than category VI.

MS. KOCHMAN: So, it seems to me the consensus of the group would be that the Voak and Scott paper is interesting, but very emotional and not necessarily appropriate for North America.

Do I have any people who feel otherwise?

[No response.]

MS. KOCHMAN: The next problem we are going to have then is with the labeling, and that is, you know, what information does the laboratorian need to be able to make these informed decisions about how they are going to handle category VI's or RoHars if they find out they do indeed have one.

That, I know, we are not going to come to any conclusions here today on that, but it is a feat just to come to the conclusion that we are probably on the right track as it is.

MR. CASE: Probably those of us that are not trained in the healing arts ought not to make any pronouncements on this subject, but I guess some kind of guidance is required.

I wonder whether your average consumer would understand if you tried to explain it in the package insert.

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One of the things I came to recently was with the realization that anti-U is not always anti-U, you know, just to get off anti-D for a moment. Anti-U is not always anti-U, and we occasionally get a complaint of your anti-U reacted with this known U-negative cell.

I think the reason for that is that our anti-U is not really anti-U in the sense that it is directed specifically at the U antigen. I think it is directed at the glycophorin B, and the cell that is known U-negative according to the customer, in fact, has a fragment of glycophorin B left, and the antibody is detecting that.

I sat down and thought, well, now can we put this into the package insert, so that it would be understood, and I sat down and I spent hours trying to think of some way to put it in a nutshell for the average consumer, eventually gave it up because there is no way that your average consumer is going to understand what you are trying to say, and I think the same applies to the situation with the various forms of D.

I mean they are confused. There is no question that if they have got a patient who has historically always been Rh-negative and suddenly it gives a 4-plus positive reaction, then, plainly, your monoclonal anti-D is nonspecific, but how can a monoclonal anti-D be nonspecific?

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I mean in the sense that it is directed as an epitope of the D antigen, there has got to be something D-like on the cells.

Then, they say, well, what are we supposed to do about this? Upon your head be it. You will just have to decide what to do, because we are not in the business of practicing medicine, and neither are the feds.

MS. COGHLAN: Well, there is no good agreement now, there never has been as far as what should be done, whether these people should be called Rh-negative or positive. I think that has always been sort of an individual decision.

MR. BECK: But we were never trained that way, you see. We were all trained to believe that the world was cleanly divided into Rh-positives and Rh-negatives, and A's and B's, and U-positives and U-negatives.

Until people grasp the idea that it is all a continuum, and different reagents make a cut at a different point, you will never get over this problem.

And that is all that has happened, there has been a change of reagents. Polyclonal reagents made the cut on a point we understood. Now, we have happened to move that a bit. Unfortunately, monoclonal reagents don't come to the

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same sharp focus that polyclonal reagents did; depending on the clone, they are going to move up and down I think.

MS. KOCHMAN: I think that unless John has something else to say --

MR. CASE: No, I don't have anything else.

MS. KOCHMAN: We have probably covered everything we can on this.

MR. BECK: I have got one more thing.

MS. KOCHMAN: Okay.

MR. BECK: I want to know to whom the FDA reports their labeling errors.

[Laughter.]

MS. KOCHMAN: That is a very good comment.

With that, let's break for lunch. I know it is a little bit earlier than we had intended, but since we have gained a little bit of time here, our afternoon session is particularly tight, so if you could all come back 15 minutes earlier than the program suggests, so be back at 12:45 and we will start the afternoon session.

Someone suggested that 12:30 would be better, so we will reconvene at 12:30.

Thank you.

[Whereupon, at 11:15 a.m., the proceedings were recessed, to be resumed at 12:30 p.m.]

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AFTERNOON PROCEEDINGS

[12:30 p.m.]

Performance Standards for Antiglobulin Control Cells

MR. WILSON: I would like to welcome you back from hopefully a pleasant lunch. We would like to pick up the afternoon session by beginning with an issue regarding antiglobulin control cells performance standards. At issue here is the potency of the cells and how they could potentially cause some problems in getting accurate answers.

The first presentation will be by Dr. George Garratty. He will attack this issue from the user perspective.

User's Perspective

DR. GARRATTY: Thank you. When Sheryl first called me up to talk about this, I don't think I had thought about this subject for at least 15 years, and I think the last time I thought seriously about it, it was about when John Case and I were on the AABB Standards Committee, and they were debating whether they should be mandated as a control. It was probably around that time, wasn't it, John? It probably was 15 years ago.

And that is true, I really haven't thought about it other than to accept the fact of what has already been mentioned, that most customers -- I know I am supposed to be

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representing consumers here -- but most consumers really don't know what they want, and they put demands on the company that aren't the proper demands, and I think you are all aware of that, that the cells out there perhaps are too strong because the consumers complain if they are too weak, and they don't know that it is better to have a weak control cell.

I would like to just address some of those issues. It was rather interesting thinking about this again, and it did stimulate us to do one or two days' work, and there is a lot more work that could be done on it.

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This is like preaching to the choir here, just to put it in perspective then. I am going to be calling them control cells for the antiglobulin test, not because I feel emotional about that, it is just what I happen to call it on these slides.

IgG-sensitized cells of course are added to cells that have already been washed, that have yielded a negative antiglobulin test, and therefore, if the antiglobulin serum reacts with these control IgG-sensitized cells, it is supposed to indicate that your antiglobulin serum is still active, and the advantage of this particular control is that it does control every tube rather than just controlling that

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your washing machine is working, which is being advocated in Europe at this moment or for some years, that this test is the only one I know of that indicates that every tube perhaps has active antiglobulin serum in it.

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Now, it is interesting when you look at what these are called. If you look at the commercial ones, they tend to use Coombs control cells, which is interesting as we are trying to teach people for some 20 years now not to use the word Coombs, and use antiglobulin test, but many of the reagents are called Coombs control cells.

I think check cells is very commonly used out there in the real world, but I was surprised to find that I think it was only one company actually uses that, but I know check cells are used as a term in the hospital a lot, and I find myself using that term a lot.

When I looked in the AABB technical manual, they called them IgG-coated cells or IgG-coated red cells throughout the book in different places, and the AABB standards similarly use IgG-sensitized cells or IgG-sensitized red cells, so any of those terms, and I am sure many more, are used throughout the blood transfusion medicine community, but those are the ones I found mainly in print.

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As I say, I am not picking for any particular reason to use the word control cells. I don't think it is any better than any of the others. I guess if I had to do away with one, it would be the Coombs one only that we have been trying to persuade people not to use that term.

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It is interesting to talk about the factors that affect the ability of control cells to indicate that the antiglobulin serum is no longer capable of detecting a weak antibody, and that is, the antibody that you are trying to detect in the cross-match or the antibody screen.

The strength and volume of the antiglobulin serum certainly is a major factor that affect how good this system is, and I guess by strength and volume, one of the most practical aspects is where we use one volume or two volumes of the antiglobulin serum.

The strength of the control cell IgG sensitization, which I guess is one of the major reasons -- and I am only just guessing about why FDA is perhaps interested in this -- and we have already been through this problem that theoretically, and what I have always taught, certainly is that if you want to pick up partial inhibition of the antiglobulin serum, that maybe you have just got

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small amounts of plasma contaminating, that you just get partial inhibition.

If you want to demonstrate that partial inhibition, you will not be able to demonstrate it by a very strongly sensitized check cell or control cell. You need to have a weakly sensitized cell.

Having said that, I realize the practical limitations that it is difficult to provide a company to provide a stable, weakly sensitized cell. We know that because we make one, and I have made one myself for 30 years, have made one and kept in my own lab, and know the difficulties of this, of keeping it at that low strength, and we still do use a weak one, I will show you in a moment the sort of cell we use.

But, of course, the customer will tell you if you sell them a 1-plus cell that they don't want to buy this reagent because some competitor gives a 3- or 4-plus. So, they are absolutely thinking the wrong way, and that is an education process that needs to be done on the customers I guess to educate them that they are thinking the wrong way, but I understand the problems that the companies have had over the years and why these cells are probably stronger than theoretically they should be.

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Then, I think something that in my mind now has turned out to be very important, and that is the endpoint that you are looking for. Are you looking for a negative test when you add your control cells or are you looking for a weaker than expected result?

Now, I have already biased you in the way that I think and that I have always trained people over the years that one should do the latter. One should be looking for any innovation of the antiglobulin serum, any contamination makes that antiglobulin serum less than optimal, and that is what I have always trained my staff to do.

It is interesting that I did an informal review of the Los Angeles technologists in several hospitals, and I also asked some friends of mine, some colleagues, John Judd in the University of Michigan/Ann Arbor, to ask his technologists, and I asked Estre Culotta in New Orleans to ask her technologists.

The amazing thing was almost 100 percent of them came back to me saying that their technologists were trained to look for an endpoint of a negative test, not a weakening of the reactivity, which is very interesting and you will see why in a moment.

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These are some questions that we thought would be interesting to pose after looking at those factors that affect the test. How much IgG, that is, plasma or serum, is needed to inhibit currently available AGS, antiglobulin serum, particularly anti-IgG?

There are data in the literature, of course, and certainly I relate when I have been teaching my lectures, I tend to throw out a figure of how many parts of plasma you need to inhibit an antiglobulin serum completely, but I realize antiglobulin serum have changed since I used to make them myself, in England certainly, and in the earlier years here, and that we all have monoclonal products now, and that maybe they are a lot more powerful and maybe they are a lot harder to inhibit. So that was one question we thought would be interesting to look at.

Do, or perhaps should, after what I just said, should users look for complete or partial inhibition? It appears to me that many of the technologists in not just our area are looking for total inhibition, not just partial inhibition.

Will strongly -- or I put currently available and most of them I think in my mind are strong -- sensitized cells detect this partial inhibition if that is what you want to look for?

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The final question, which is perhaps the thing you really come to, is will partially inhibited antiglobulins that are missed clinically significant antibodies, does it matter? Does it matter whether we look for complete inhibition or partial inhibition?

We tried in a few days of experiments -- that is all we did, just a couple of days, but we have got interested now and perhaps we will do some more -- we just did a few experiments, only a couple of which I have got time to show you here to try to answer some of these questions. Obviously, number four will take a lot longer to answer, and I can't even address that today, but we did try to address some of those other questions and show you a small amount of data.

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The first thing is what do the package inserts say. I said all those techs said that they were looking for complete inhibition, they weren't looking for partial inhibition. Now, interestingly enough, when I look at all the package inserts, only one company says that -- and I have just lifted verbatim from package inserts the part of what you are supposed to be looking for, a negative test or partially inhibited.

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Only one of the companies actually goes for looking for total inhibition, Ortho, which says no agglutination indicates that the antiglobulin was inactive neutralized or was not added to the test tube. All the others have a variation of the theme of suggesting to the customer you should be looking for partial inhibition.

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Dade interpretation, negative or questionable, cells not agglutinated or cells agglutinated less than 1-plus.

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BCA negative or weakly positive reactions indicate tests on those tubes that should be repeated. I don't know how you repeat a tube, John.

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Immucor package insert, it says agglutination of less than 1-plus in each previously negative antiglobulin test indicates result obtained may be invalid and that these tests should be repeated.

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Gamma, I think Gamma I will compliment them on their package insert. It is a lot more educational and it has more in it than what I put here, and it does refer to a buff which gives quite a lot more educational information.

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No agglutination indicates that active anti-IgG is not present in the test system as noted above, where they have given some more educational notes. Weaker agglutination than is normally observed may be indicative of partial neutralization of anti-IgG. As you say, I am biased towards this sort of verbiage. I think this describes the way my mind would think.

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What we did on the next couple of experiments, I will show you three pieces of data on the slides following this. What we did was try to mimic then inhibition that might occur, that these check cells are used to show you if inhibition has occurred.

We took group 0, 1 little r cells, and we sensitized them with anti-D, and we actually used a 1 in 10,000 of our Rh-immune globulin to give us a 2-plus antiglobulin test, so they were moderately sensitized cells, and we ran a control of non-sensitized cells.

Then, we took both of those lots of cells and we washed them with diluted pooled plasma, diluted 1 in 100, 1 in 500, 1 in 1,000, 1 in 2,000, so that we would have less the amount of residual wash solution that you normally would have left, but it would have this amount of plasma in it.

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Then, after adding the anti-IgG and reading the test, the check cells were added, so the first lot of data I want to show you then is how much did we have to add to inhibit completely the current available antiglobulin sera.

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These are antiglobulin sera we tried, although I understand from my technologists that they no longer make anti-IgG, but when they phoned up to get some more, the FDA would know more about it than me.

But interestingly enough, they fell into three groups, and Dade was very resistant to neutralization. It inhibited it completely, 1 in 100 parts of plasma to inhibit it completely, and then you started getting partial inhibition of 1 in 500 and 1 in 1,000.

The other two, American Red Cross and Ortho, were the next resistant. You moved over into this area to get complete inhibition. We went down a step, and then BCA, Immucor, Gamma fell into this area, complete inhibition a step down here. So, these were perhaps more resistant to inhibition than these.

I am not saying it is very important, I am just pointing it out there are differences, and that elaborates on that factor, that one of the differences when you test sensitized cells, if you are going to test them, is that the

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lots of the company's antiglobulin serum you are testing when you are evaluating these, or customers.

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Now, there is a lot on this. This is my main piece of data here. The only other piece of data I have got is on flow cytometry, I will show you after this, and I could use this to illustrate a lot of things, but I am just going to reduce it to a few points, because of time limitations.

What these figures here indicate on this side of the slash are the expected result of the control cells here. If the system was working properly, this is what you would get.

Now, this is L.A. Red Cross. The ARC you saw before was the antiglobulin serum made by a national, by Roger Collins. This is our own homemade Los Angeles Red Cross control red cell where we used just a 1-plus result.

Interestingly enough, our technologists recorded all these completely unbiased, and you see that they all appeared about the same. She recorded them all as about 3-plus, and these are after adding to washed red cells, didn't seem to be very much different although this one was marked weak and this one was not weak.

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I am going to be showing you the amount of anti-D that really is on these cells by flow cytometry in a moment, a much better index than the antiglobulin test on how these are relative to each other and the amount of anti-D that is on there.

So what we are really looking for, then, is remember that these are ones that what we have done here is to wash these cells with a diluted plasma that would either inhibit the antiglobulin serum completely, which it did in most of these ones, these three antiglobulin serum there.

I deliberately removed the company names because it isn't relevant to the particular points I want to make, but I just want to make the point, though, that these two antiglobulin serum using that dilution of plasma were not completely inhibited, they were partially inhibited, which was good because it showed us some things here.

Now, you will see that if you were looking for total inhibition, which is what a lot of technologists it seems to me are doing -- although the package inserts you have to remember, only one company tells them to do that, all the other companies tell them to look for partial inhibition -- but if they were looking for total inhibition, our cells work nicely all the way across, with all five

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commercial antiglobulin serum they did give the expected results. They were negative. They showed the inhibition.

Only the BCA weak one gave total inhibition for them all. They were followed closely by the Gamma weak and perhaps the Immucor one may be next. But you will see that I should go across this way with the different cells. There were differences when you get into this area where some of these were partially inhibited.

Now, if you were looking for partial inhibition, as all the package inserts except the Ortho one say, that, in fact, they all did what they were supposed to do, because you will see that every one of them did show a difference in reactivity here.

If you go down this way, the only other thing I would point out here is if you look at the different antiglobulin serum, it is interesting that the Gamma cell, which is the Gamma strong cell, which is very strong, was the only one here that actually came up would have given a false result if you were looking for complete inhibition, but its package insert says that any diminution in strength is an indication.

So, you can look at this in different ways. I have just looked across here with the cells, but I think there is other data we could pull out of there, but I was

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interested that these all look the same to me, these are a little bit stronger, we tend to grade in halves, and it gave that general picture.

It brought out to me the major point of whether we are looking for complete inhibition, whether we are looking for partial inhibition, and whose antiglobulin serum you are using will depend on that because some are more resistant to inhibition than others.

What we thought then would be interesting was just to look at all the commercially available cells, and including our own cells, just to have a look by flow cytometry, how much anti-D do they have on them.

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This is just relative strength, then, looking by flow cytometry. Here is the negative control here. These are cells, non-sensitized cells, just to give you an idea. Here is the median fluorescence then. It is a fluorescent labeled anti-IgG that is being used to say how much IgG is on these cells.

I want to point out, though, it is very important you realize that this is a log scale here, so these cells here, here is our own, L.A. Red Cross, that gave a 1-plus antiglobulin test. Immucor are the next. That has three

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times the amount of IgG that our cells have on them. This is a log scale.

In fact, there is a 7-fold difference between this and this, between the different commercial cells, there is a 7-fold difference in the amount of anti-D even though they all looked the same, even though they had given a 3-plus antiglobulin test, the Gamma strong cells have far more anti-D on them than any of these, and the amount is strong.

So, the interesting thing is the one that is marked weak is about the same as all these that are not marked weak, so they have about the same amount of anti-D on them. Now, I have no idea how this will vary, and if I tested batches or lots of pure sensitized cells, I have no idea how good your in-house control is, and how this would change each time I checked a lot of sensitized cells. We will maybe do that in the future.

But the other interesting thing is with flow, you see different patterns. Obviously, I was rather fascinated, this Gamma weak here -- and John may have an explanation of this -- appears to have two populations of cells.

I mean this could happen if it was an R1 cell and an R2 cell mixed, and the R2 cell was slightly more sensitized than the R1 cell, but there are different patterns here in these very sharp peaks here compared to

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these broader peaks and showing heterogeneity amongst the cell populations perhaps. That is about all the data I have for you.

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These are just very personal. I don't feel very emotional about these, and I had these the other way around. First of all, this is the theoretical, and I decided to put this as my top thing, that really it seems to me from these small amount of data that we should be educating users to react to any change in reactivity, i.e., if the control cells are 2- to 3-plus when added to a true negative test, then, 1- to 2-plus indicates a possible problem.

If control red cells are 1-plus, then, a negative result may be an acceptable endpoint, so that if you were using like an RO reference if we are using a 1-plus cell, I wouldn't mind if people are just looking for a negative.

Using weakly sensitized control cells, 1- to 2-plus reaction after adding to the washed red cells, I would say would be ideal. We have a small amount of data, but not a lot of data that you just saw. It did appear that the weakly sensitized cells were slightly more efficient, but not perhaps as much as I thought in telling you, you have got a problem.

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But if you go by the package inserts, I am sure the company would argue that they did what they were supposed to do.

I will leave it there and leave it for discussion after John's presentation.

[Applause.]

MR. WILSON: We would like to continue with the Manufacturer's Perspective from Dr. John Case from Gamma Biologicals.

Manufacturer's Perspective

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MR. CASE: Again, it is the cross that I have to bear that I follow behind somebody who has already said most of what I want to say. However, I do represent the manufacturer's point of view, and I may be the one that raised this issue the last time there was a workshop here, because it did seem to me at the time, on the spur of the moment, so to speak, that there would probably be some virtue in having some kind of standard for Coombs control cells. I have since given it some thought, and I am not sure that there is too much virtue in it, but you will see why as I proceed.

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Like I said, it is my cross to bear, I saw it as my role to review the whole subject here, so this boring slide just gives you a historical perspective. You will notice I put rather more on the slide than is generally recommended, but I adopted the principle that supposing I were called to my eternal reward before I finished my lecture, there would be a possibility that you could go on and examine my slides and therefore be appropriately educated.

The bottom line here, though, is that unfortunately, the test, as it is most commonly applied, is insensitive to partial neutralization. Now, one of the things that has always bothered me a little it is that not only do people not look for weaker reactions, they are inclined to accept even a microscopic positive reaction at the Coombs control stage to be indicative of a test that has passed with flying colors.

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Unfortunately, what happens, and perhaps that is why our cells come out being coated the strongest, although I suspect that maybe it has to do with the fact that we are now coating them with a monoclonal source of anti-D rather than a polyclonal source, which for some reason or another, it probably has to do with the affinity of the antibody we

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are using stays on the cells instead of coming off and going on all the time.

If you take the majority of Coombs control cells that are out there, and take the supernate, you will find there is anti-D in it, whereas, with ours, there is little or no anti-D in it because the anti-D is staying on the cells. So, that may be part of the reason for George's results.

It was interesting to me that the Coombs control weak cells that we offer evidently are not all that much weaker, in fact, there was half the amount if you noticed of anti-D on the cells as there was on the strong ones. I do think there is a mixture of cells. I think each time it is not a contrived mixture. Sometimes they may be all R1 little r's, sometimes they may include R2 little r's, so there will be variability from time to time.

So, as I said, the manufacturer's interests are best served by coating the cells as heavily as possible, because now you avoid the competitive element where the customer's best interests appear to be that they want to get strong positive reactions, they are really don't want to know if their Coombs test needs to be repeated.

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It is a fact that cells coated strongly with IgG outsell those coated weakly by approximately 3 to 1, and as I said just now, consumers often will bend over backwards to avoid recognizing that their Coombs control test has indicated what they need to know.

Thirdly, manufacturers who are tempted to apply scientific correctness in their labeling are inhibited thereby because they lose market share if their product is not perceived as meeting the consumer's expectations.

One of the things, incidently, that I was thinking of while George was talking, is that there is a difference in reactivity, and this is what is rather awkward when it comes to assessing whether or not you have partial neutralization.

If the cells in the original negative test are Rh-negative, they tend not to participate in the end result at all because they are Rh-negative. If, on the other, they are Rh-positive, any antibody that is in the supernate or comes off the cells before you actually spin, bring the original cells into the reaction and so you get a stronger reaction if the original cells were Rh-positive than if they are Rh-negative, and it is very hard to take account of that if you are looking for differences in the strength of agglutination at the end.

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Not, the apparent remedy, and the remedy I suggested the last time we had a workshop up here, was that the FDA should consider establishing performance standards for control cells and enforcing them uniformly, so that we could all adopt what is scientifically correct without penalty.

I have given some thought to various ways in which you might measure the strength of antibody that is coated to the cells assuming you don't have the luxury of being able to do it with a flow cell cytometer.

Firstly, you could titrate your coated cells with a standard anti-IgG preparation. Secondly, you could use selected dilutions of human serum in neutralization experiments, as Douglas Voak has suggested in print, or you could use both methods in combination.

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The foreseeable difficulties there are that you are not going to get straightforward results for several reasons. One is that the reactivity on top of a negative test is not the same as an antiglobulin test in which there are no unagglutinated cells.

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Secondly, as I just said, the strength of the control reaction is influenced to some extent by the D antigen status of the cells in the original test.

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Thirdly, attaining the same strength of reaction in the hands of all bench workers is, for all practical purposes, impossible, and that has to do, not only with the shaking, but also with the brand of antiglobulin they use because, as George points out, there is considerable difference in resistance or susceptibility to being neutralized by the presence of human serum, which presumably is a function of the anti-IgG potency of the product in the first place, which is always measured by a rather empirical method.

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As always, I am finishing with a quotation. "With willing hearts and skillful hands, the difficult we do it once, the impossible takes a bit longer."

That's it.

[Applause.]

MR. WILSON: Thank you.

Summary

MR. WILSON: What I would like to do is just take a moment and try to capture what I thought I heard. Again,

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I would like to reemphasize to the audience that we are here to hear comments, both good and bad, where we should go, maybe where we shouldn't go on this issue.

I would just like to begin by saying that Coombs control -- oops, I said it, sorry, I am an old serologist from in excess of 20 years ago -- antiglobulin control cells are regulated as Class II medical devices, and as such, those products would be eligible for performance standards.

FDA's current thinking at the moment is that executing performance standards are best met in the form of a guidance document. So, that is really what we have consideration, at least from FDA's point of view, at this point, and the effort here was to gather some information, pros and cons, strengths and weaknesses, so to speak, of where we would like to go if we were to be able to develop a useful, practically applicable standard.

But I think what at least I heard was that there are a number of variables here that we have to deal with. First and foremost was I think the last point made, individual classical manual serologic technique plays a confounding role with individuals who shake too hard or shake too lightly. So, that is one area where at least manual testing certainly has a degree of confounding to it.

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Secondly is the potency of the cells. The coating of the IgG seems to be at various strengths, and the word "weak" seems to be relative.

Thirdly, interpretation of results, is a clean negative indicative of a high level of assurance that you have got the answer that is correct as opposed to some measure of partial inhibition.

That being said, and I hope I captured all of it as best I could, if FDA were to develop some performance standards in terms of starting at the simplest place, potency of the coating levels of the IgG, do we have any comments about how that might be best standardized?

I might add, in many of the blood-borne pathogen test kits, HIV, hepatitis B surface antigen, et cetera, we had run into this problem and asked the manufacturers to bring the positive controls down to what we call the medically meaningful decision point, i.e., the cut-off. That is when a control reagent is supposed to tell individuals when something is going wrong, and I think that is what we are trying to get at here.

In that case, what we had said to manufacturers is to place the potency of the positive control at a nominal 3-standard deviations above the cut-off. The idea there would be that if the control failed to operate, the control would

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predictably fail to operate positively, the positive control, approximately 3 in 1,000 times.

If it failed to react properly in excess of that, you had a system problem, you needed to upgrade your QC or whatever.

But I would be open for discussion or for any proposals on whether or not that is a practical approach, tighter endpoints and going for nominal 3-standard deviations above the cut-off.

Any takers?

Open Discussion and Proposals

DR. GARRATTY: I would just like to pose the other question to the audience, and this might sound funny after what I have just reported, but is any harm being done using them as they are used now? I mean I don't know if people have any comments about are any changes needed, because if they followed the package inserts, they were doing the job.

MR. COLLINS: Just a comment, I guess, to back onto that. I don't know, I have not heard myself, and I would be interested to hear what significance there is and how big a problem neutralization has been in most laboratories.

I certainly don't hear a great deal about people having problems with check cells not working because of a

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neutralization problem. I tended to think, or at least I thought part of the reason for the check cells was to detect whether the antiglobulin serum was even added at all, which it seems would be more important as to whether the product was neutralized or not.

MR. CASE: One does hear instances of the control test failing in cases where people are using the polyethylene glycol procedure as an additive, and in particular where they are using plasma with polyethylene glycol as an additive, because polyethylene glycol tends to precipitate high molecular weight proteins, and it tends to produce particles of IgG that get entrapped with the red cells and remain there if you happen to be washing the cells with a cell washer that doesn't resuspend the cells adequately each time.

So, there are instances where they are being told what they need to know even by the strongest coated cells. So, I think there is some evidence.

What there is no evidence for, as I understand it, is that we are not hearing of people falling about with hemolytic -- everybody laughs when I say "falling about," but I always say it because it makes them laugh -- we don't hear of instances of people falling about with hemolytic

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transfusion reactions that results from the failure of an antiglobulin test because of partial neutralization.

So, I think probably George's proposition is perfectly true. The only thing is that what you have got to remember is the vast majority of antiglobulin tests done really doesn't matter because they are negative anyhow, and it really doesn't matter. It is only where there is a clinically significant antibody in the patient's serum and there has been partial neutralization, such that that antibody is not detected, that you are at risk, so that reduces the incidence of this phenomenon.

DR. GARRATTY: John, I am sure you would probably agree if I pushed you, that our standard should not be to prevent people falling over with transfusion reactions, because if you use that as your standard, then, each one of your companies' potentiators wouldn't be selling because, you know, you could use the same argument there, that most of the antibodies they detect may be shortened red cell survivors, weak Kidds, Duffy's, and Kell's but people aren't going to fall over with hemolytic transfusion reactions even with those.

MR. CASE: This is absolutely true. In fact, there must be -- you have only got to look at the results of any serology survey, proficiency testing survey, to be aware

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that clinically significant antibodies are being missed every day of the week.

I mean we all get oh, oh, I might miss a weak anti-JKA, or I might miss a weak anti-Duffy-b. Absolutely, you might well miss it, but those antibodies are being missed somewhere every day of the week, and people are not falling about with hemolytic transfusion reactions as a result.

MR. WILSON: One of the things I might question is that -- again, there is a lot of parallels to blood-borne pathogen testing -- most situations where you are trying to detect an antibody, the potency of the antibody in the specimen is usually fairly strong, so you are not -- you know, you are going to be able to pick it up by other means -- it is only when you get to that cut-off area, the weak area, that is when it becomes a problem.

MR. CASE: That is true.

MR. BECK: Roger suggested that there might be two applications for the use of control cells. One is to detect partial neutralization, the other was to detect whether the antiglobulin serum had been added at all.

I think it is to demonstrate partial neutralizations. There is better ways to detect whether the

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antiglobulin serum has been added at all, and that is the use of dyes and things. Isn't that right?

MR. CASE: Yes.

MS. ROLIH: Susan Rolih, Immucor. I think that it is difficult for us to determine whether or not there have been delayed serologic reactions or even delayed hemolytic reactions as a consequence of a failure to control a test properly or to determine that the test was not performed properly through the control, because I don't think there is really a way of us determining after the fact whether that was at issue, because there are just so many other things that come up when you do an investigation, was it just technical error, did the tech not do something correctly, was something not added to the test unrelated to the antiglobulin reagent, so I don't think we have ever done a purposeful look to see whether or not there have been clinical consequences that would have been predicated by failure of the control, because it is just difficult for us to trace that.

Now, personally, I think that there is a lot of incidences of neutralization going in the field. We are a manufacturer of a Coombs control cell that a large percentage of our newly acquired customers, who have been purchasing from other manufacturers, call us continuously

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about the fact that our antiglobulin cells or control cells are not working, and when we pursue the issue with them, it is because, for cost containment purposes, they have cut their centrifuge cell washing devices down to two or three washes.

If we have them increase the washing up to four washes, our antiglobulin control cells work perfectly well, but in lieu of using our reagent, and for cost containment purpose, they then will punt and go back to their manufacturer of choice in order to get the reagent to work.

So, I think it is happening a lot. Whether or not the control is relevant, we have some reason to suspect that perhaps doing a sink test would just be as effective in determining whether or not you have valid antiglobulin results as throwing in an antiglobulin control cell.

I think that maybe that would be another area to look at is even for those instances that we do pick up if they are not clinically relevant, are we perhaps spending money on a reagent that we don't need to use at all.

MS. WEILAND: I would support what Susan has said. It is not an uncommon occurrence to have customers, in the same type of scenario that she has related, call and complain because their Coombs controls or antiglobulin control cell is not working, and in working through this

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with those customers, it is obvious that it is, in fact, working exactly like it was supposed to be working and alerting them to their inappropriate washing system, either because they are hand washing or automated washing systems are not functioning.

So, I think we probably are seeing instances where neutralization is occurring on a routine basis. Whether or not that is significant, as Susan has said, I don't know. I think there is an argument for that approach, as well.

To speak to the point about patterning the system, if you will, after so many infectious disease assays in the positive control, one of the difficulties that will occur because of that, for the very reason that George mentioned, is that antiglobulin cells from one manufacturer may be used with an antiglobulin reagent from another manufacturer, so you will have some variations as to the level of neutralization that would occur, and that's not the case in the infectious disease setting, where you are using the control within the confines of the test system itself.

MR. WILSON: In fact, that problem does occur when external controls are used for blood-borne pathogen testing because the controls are not "matched" to the test kit.

DR. GARRATTY: Is anybody else as disturbed as I am that people are not doing what the package inserts say?

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I mean maybe it's just in the people I have talked to, but I was amazed, it was almost totally that they all were looking for complete inhibition, and as you saw there, only one company's cells would have done the job.

If they had been using anybody else's red cells, they would have missed that 2-plus antibody, they would have called it a negative antiglobulin test when that was a 2-plus reaction. It could have been a 2-plus anti-JKA. They would have said everything was okay and gone ahead and used it, if they looked for complete inhibition.

Does anybody have any feeling on that in your areas of what your techs are doing? No? Go back and ask them.

MR. CASE: I think that is a very good point. Clearly, that is what is important here. I don't know, Debbie remarked about the washing. I think cell washing centrifuges, when all is said and done, like any other automated or semi-automated equipment, are prone to fail from time to time, and if you have a partially blocked spigot, for example, that is probably not delivering enough saline to a particular tube, then you could very well have poorly washed cells in that tube.

I don't think people really care. They really want to be assured that their Coombs test was okay. It is

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inconvenient if they have to repeat anything, and so they are looking for cells coated as strongly as possible.

I thought it was rather interesting when Susan was talking, and she mentioned how they get calls saying your control cells are not working. Now I understand why we get salesmen's reports saying that such and such a customer just switched to Gamma's Coombs controls. Incidently, I understand the word Coombs test embarrasses Robin Coombs. He really doesn't like the test named after him, but somehow we have all slipped into the habit, and I confess I find it hard to give it up.

But now I understand why our salesmen report from time to time that such and such a customer has just switched to Gamma's Coombs control cells because Immucor's are no good. I mean the thing is you do what is right, and you suffer the penalty. The road to the bankruptcy court leads in this direction.

DR. GARRATTY: Because you were at the two opposite extremes. Immucor has the weaker sensitized cell out there, and you have the two strongest.

MS. MALLORY: Again, maybe I am being too simplistic, but if we did as Roger suggested, and put a dye in the Coombs, so we knew that there was antiglobulin reagent in the tube, and that was out of the way, we didn't

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have to worry about that, then, you decided to make your control cells a weak control cell, so that what you had to deal with was one of two things, technique on the technologist's part or partial neutralization, you would have only -- I think you would have those two things really to deal with, and when you got a call or when you dealt with your customers, you would have to -- you would only have that to deal with, which is basically what you are dealing with now, but at least you would know that if the test was positive, that there was no partial neutralization.

Does that make sense? I am trying to get it down to a little more simplistic area. Dealing with the strongly coated cell, to me seems to be self-defeating.

MS. ROLIH: Delores, I agree with you 100 percent of what you said, and it would work out really well. I am always horrified, and I was horrified again today, when George put up his slide on all the different reactivities and I saw that our Coombs control cells were giving a 3-plus or a 3.5, and it just, you know, like it just doesn't sit well.

I have to tell you I would think if went to a weak cell, 50 percent of our customers would take such an extensive amount of retraining. We have a large percent of our customers with our cells, which I think are pretty

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strong, probably never see more than a 1-plus on a final test.

DR. GARRATTY: I am sure.

MS. ROLIH: You are getting a 3-plus, which is marvelous. I mean that is what we would get in our lab, but I don't think a large percentage of our customers do, so I think that while most of the manufacturers, if not all of us, would agree that we would like to have a reagent that is more meaningful, I think that we also have to face the facts that there is going to take a considerable amount of education of our customer base, and it is going to be a difficult hurdle for them to overcome to retrain their people how to shake properly, how to standardize their centrifuges properly in order to get the appropriate test results.

DR. GARRATTY: Of course, if we had the Europeans here, they would say we don't have a problem, we use gel tests, and we don't have to have control cells.

MR. WILSON: Your remark about the training of the individuals, I think is important because if you have got a culture out there that needs to be readdressed, in other words, that there is a problem in interpretation, as well as it's a manual technique albeit with difficulty standardizing, but better standardization is one place to

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start with eliminating the variables, in other words, taking a dye and putting it in, but moreover, standardizing all the reagents to nominal 3 standard deviations above the cut-off, so you only get false negatives 3 times out of 1,000.

Maybe that's a place to start. I don't think we are going to change the world overnight, as you kind of described, but I think a gradual step-by-step approach is at least progress.

MR. BYRNE: Questions concerning the actual standards or guidelines for these products. It is very difficult to compare an agglutination test with an infectious disease ZIA test, and the 3 standard deviations is not something that you can fit easily into an agglutination test.

In addition to that, traditionally, an FDA standard is run along the lines of you titrate this antibody. If your product exceeds that antibody or equals or exceeds that titration strength, then your product is okay.

In this situation where we are considering perhaps using an anti-IgG, titrate in the anti-IgG, test your cells against that, what we are saying here is exceeding the FDA standard is a bad thing, because potentially, your cells are too strong.

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MR. CASE: Peter, you are talking about the potency of anti-IgG, aren't you, which does present --

MR. BYRNE: Using the anti-IgG as an indicator of strength of your control cells.

MR. WILSON: I think you have two variables here. One is the dosage of the IgG on the solid phase, which is the red cell, and then the potency of the anti-IgG. You have got to freeze one to be able to measure the other variable.

MR. CASE: Absolutely.

MR. BYRNE: That is what I am saying, is if you are using a standard anti-IgG that the FDA supplies, then the FDA are going to have to supply a standard control cell, as well, so that you can then test yours in parallel with our standard control cell.

I can't see the FDA making lots of anti-IgG control cells to send out to manufacturers.

MR. WILSON: I don't see that that is practical, particularly in this downsizing environment, but I think that there are ways of addressing standardizing it. I think clearly, one is going to have a difficult time standardizing a reagent red cell antiglobulin-coated cells, and not look at the antibodies that are going to be used to detect them.

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So, I think that it will need to go hand in hand, but I would reflect back on your point. I mean we have wrestled with how do you standardize such less than quantitative systems, and I use the comparison of blood-borne pathogen tests because they are not quantitative for the most part. They are what they call binary tests, positive or negative. However, in all instances, every manufacturer uses highly quantitative, semi-quantitative, but certainly not qualitative techniques to manufacture the kits.

So, from this point of view, just looking at it from the angle of where do you start in terms of trying to quantitate the potency of IgG, well, you do it by twofold serial dilutions since 1900, and the way you can do that is if you look at some math and some distribution curves, you would be surprised how you might be able to find some performance characteristics that can fit standard deviations with frequency.

Now, this is not going to be rocket science type analytical results, however, I think it is a step forward. That is why I brought it up.

MR. BYRNE: As an aside, investigators and inspectors, if institutions make their own control cells, will an FDA inspector investigate, they will be looking to

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make sure those cells are controlled in the same way that manufacturers control their cells?

MR. WILSON: If the question is if there is a home-brew control cell made by a laboratory, what would the FDA inspectors do about it?

MR. BYRNE: Yes. Would they be looking for the same standards?

MR. WILSON: Is the product in commercial distribution, are you distributing it, you know, those kinds of things.

MR. BYRNE: No, you wouldn't be, no.

MR. WILSON: You know, you would make the first several passes on those types of issues, and then I think there is clear components that enter into it. I don't think we could address it here. At least my initial thought is that FDA does not prohibit home-brew controls.

In our blood-borne pathogen invalidation guideline, where we talk about use of external controls, we clearly state if you want to manufacture it yourself, and use it yourself, we don't have any objection to that.

MR. BYRNE: Okay.

MS. MALLORY: I think my comments are a little bit in the same line as Peter's in that I had suspected if, for example, we went to a weaker control cell, that many

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institutions would start making their own, and that they would make them very strong, and what tends to happen, and has even happened with some of the commercially prepared cells is that they spontaneously agglutinate.

I think there is a lot of problems that are going to be associated with home-brewed preparations, that I think would have to be addressed by either FDA saying this is the method you would have to use or somehow looking into it, because I know from years and years back when we did make our own, everybody again made very strong cells very often, and if you had a very potent anti-D, the cells tended to spontaneously agglutinate.

I wonder what effect having a monoclonal anti-D would have on those cells.

MR. CASE: Well, it is a fact that monoclonal, or at least the monoclonals, we tried it with two different monoclonal IgG reagents anti-D's, and with both of them there is less anti-D coming off into the supernate than there is with polyclonal, and that might explain to some extent why there is an illusion that we have got at least twice as much IgG on our strongly coated cells than anybody else has on their cells.

DR. GARRATTY: It's not an illusion.

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MR. CASE: I know. What I am trying to say is that in the case of polyclonal reagents, much of the antibody is in the supernate, whereas, with the Gamma Coombs control cells, the antibody is all on the cells.

You see what I mean? There is a difference whether you make it from monoclonal or polyclonal reagent. I don't know what the answer to this thing is because, like I said, there are so many variables, and I don't think -- I mean it is all very well to make your own. George can make his own for his own institution. According to his own philosophy, it ought to be giving a 1-plus reaction. You can do that, and you can train your people to do it.

DR. GARRATTY: They are used to reading those.

MR. CASE: And they are used to reading that. If you are a commercial manufacturer that has to make pearls to cast before swine, with all due respect, you unfortunately have constraints that he is not under.

DR. GARRATTY: I understand.

MR. CASE: I think it is quite understandable that people might make their own, but if they make their own, it's a little unjust if they are not held to good manufacturing practices the same as we are.

DR. GARRATTY: I agree with everything you say, John. I didn't come here to preach anything to you, but

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just to show you the data if you do use a 1-plus cell, that it is not just theoretically, it does seem to evaluate it better.

MR. WILSON: Just to come to your last point about the good manufacturing practices on a home brew, I think the position -- we would have to look at the situations on a case-by-case basis, but I think that there are elements of GMP that do apply, and certainly the 606's would apply, as well as CLIA regulations, because there is a requirement to be able to validate under CLIA, so there are compensatory mechanisms, but each case might be slightly different, so I would be hesitant to try to articulate it entirely here.

But the thing that is most important is that if you are going to put a control reagent on line, regardless, it has to be validated, it has to be shown to work, reliable, stable, et cetera. It is not just something that you would want to throw together and put on line before it is thoroughly validated.

MS. WILLIAMS: Candace Williams from Olympus. Listening to you talk, I have to think about other assays that we do, the blood-borne pathogens that you have mentioned, and other blood grouping antisera, and to my knowledge, there is not another assay out there in which every test is controlled.

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On a blood-borne pathogen, you have controls on your microplate, but every well is not controlled in that assay. There is nothing there that assures that every well is washed with every test adequately.

The same with blood grouping antisera, you know, you run your controls with each vial or at the beginning of the day or the beginning of the shift, but you don't have any kind of control on every test that is run, and I do have to wonder about the level of concern for doing this historical procedure. I don't know that there is a whole lot of significance there.

Listening to you talk, I have also come to the conclusion that there is a lot of problems with the current methodology that is being done with manufacturer's cells and Coombs sera not being matched, no requirement to match those, test procedures not being done correctly, manufacturer's inserts not being followed, so it seems like we have got a fairly ineffective control system going on right now.

If we are going to continue it, I think that standards are needed to make sure that it is of some value. If you do impose the standards, though, I worry about cost of implementing those standards. Certainly it is going to be more work for the manufacturers, more work for the FDA to

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develop these standards and impose them, and those costs ultimately are going to get transferred along to the consumers, and are we really, you know, much ado about nothing here.

DR. GARRATTY: I think John and I would agree with that because at the time of the Standards Committee, when that standard was put in, that was just the sort of discussion that went on, and I don't think it has been so. In fact, you have stimulated me to think that one piece of important data that nobody has -- and yet we have all got it sitting there -- is how many times do we get contamination.

Now, I have just realized in our own lab, we are using a 1-plus sensitized cell, which is a very sensitive system. We also use peg routinely, which tends to give, as John said, false negatives. I need to go back, I haven't done that. I have got it sitting there in the books. If I go back and look over the last year, how many times did they have to repeat a test, it would be some good information in our own institution, and I don't know that.

It would answer that question, are we doing all this for nothing, because we do have a very sensitive system, and we are using peg, which makes it even a better system to evaluate.

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MR. WILSON: Maybe I could wrap it up saying that the effort to develop a standard would be in accordance with our good guidance practices. It would be an opportunity for comment from industry or any other interested person.

The purpose of this meeting here is to elicit these types of comments to see where we are at. Certainly, if there are issues that will absolutely derail a high quality guidance that is so narrowly focused that it doesn't address some of the other issues, then, it may not be worth it. We will just have to take that just one step at a time.

The last question?

MR. BECK: Just a comment really. I am moved to ponder a while, what is the purpose of the antiglobulin control cell. Now, I am wondering is it to detect a defect in a specific test in that test tube, or is to indicate that practice, as you suggested.

If it really is to indicate general bad practice, maybe efforts should be better expended in proficiency and competency testing, and maybe it could be mandated that this include a demonstration of very weakly reactive antibodies by the antiglobulin test.

MR. CASE: It is interesting, Malcolm, that you should say that, because Douglas Voak, whose name has been invoked here numerous times since we began this morning, his

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position is that the Coombs control test is useless and that it is much better to train people to read the test better, and that this is done by means of a standard anti-D, that they are expected to replicate testing, as George says, and there may be something in that argument.

DR. GARRATTY: And, in fact, we agree with Doug Voak on that. In fact, we do that in our own lab. We believe that that replicate testing is good for the reason that you said, Malcolm, and to look at your washing machines and that they are working adequately, but it still leaves me the question that it is not controlling each tube. Whether it is necessary or not, I don't know.

MR. WILSON: Thank you.

Let's move on to the next subject.

Performance Standards for Saline

MS. WORST: Our next topic is Performance Standards for Saline, and I am happy to introduce our first speaker, Roger Collins from the American Red Cross.

User's Perspective

MR. COLLINS: Thank you. I should probably dare suggest that if the FDA regulates and strictly enforce the pronunciation of "saline" instead of "saleen" we probably wouldn't have any problems with this particular subject.

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Judging from the number of accents on the podium today, that would not be a problem.

[Overhead.]

I should probably explain that although I am putting forward a user's perspective, I am not strictly a user within the definition of a user within the American Red Cross.

We do manufacture reagents within the American Red Cross, and as such, I provide a service, I guess, for want of a better term, to our regions in terms of handling some of the problems that they either can't handle themselves or have problems in handling, saline being one of these.

A number of problems get referred to us because we do have some of the equipment and ability to analyze these problems that they don't have in the regions. So, from that perspective, I am sort of looking at this as a user and also as a manufacturer that handles problems.

So, what you are going to be seeing today is more of problem aspect than a performance review.

As you can see, we have got quite a number of titles for what is typically termed saline. Essentially, they all mean the same thing except for maybe this last column. Basic ingredient, either 9 grams per liter or 8.5

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grams per liter of sodium chloride. Why the difference, I have not been able to explain.

Isotonicity is defined as the osmotic pressure compared to human blood. I don't know whether one person read his results at sea level and another person read theirs up a mountain, and got disparating results, but I have not been able to explain why one isotonic solution is 9 grams per liter, why the other one would be 8.5, but in terms of serological application, it really doesn't matter.

This last group, immunohematological blood bank, certified blood bank, is basically a manufacturer's definition. They have done a little bit more and defined it for actual application in blood bank use. The previous one, saline normal isotonic physiologic are basically general purpose solutions, normally have applications in the blood bank or they are being used in the blood bank activities.

The blood bank and blood bank certified are basically labeled by the manufacturers as being more or less specific for blood bank use, specific inasmuch as they have either defined that they may meet the NCCLS standards for saline.

One manufacturer claims that their product is virtually sterile until opened, whatever that means.

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Buffered saline. Two levels, one buffered isotonic, which presumably is taking an isotonic solution, keeping it isotonic, and buffering it at the same time. Buffered saline can basically mean any isotonicity or hypertonicity, taking your standard salt concentration, add in some buffers as sodium phosphate or potassium phosphate, and getting a desire pH, sometimes without reference to whether it is isotonic or not.

[Overhead.]

The FDA defines saline as basically a general purpose reagent. If it is not labeled or otherwise represented as being sterile, then, it is exempt from good manufacturing practices. Essentially, this means that anybody can make saline and label it as they want to. As long as they don't claim that it is sterile, they don't have to meet any strict regulatory requirements.

[Overhead.]

There have been a number of problems attributed to saline - false negatives, false positives, grainy reactions, crenation, rouleaux, hemolysis. The bulk of the complaints or problems have generally fallen in these first three areas. Low pH has been a common problem especially with the S and Kidd blood group systems where saline has gone into a

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low pH. Sometimes these have been missed, creating a false negative reaction.

False positives, generally, and grainy reactions, are caused by contamination in some form. Bacterial and fungal contamination generally isn't a major issue unless it is severely contaminated. Saline isn't a great grower of bugs. They will grow in saline if left long enough and sitting around long enough in a stale condition in sufficient quantity, but even a small quantity of bacteria that is present generally won't affect a serological reaction.

Where it does affect it is if the saline is being used as a diluent to prepare the reagents for automated systems or microplate systems where that saline is now going to sit in a nice growing media like serum for maybe a week or so while those dilutions are being used up.

Particulate contamination can come from a number of sources, primarily in the handling of the equipment that the users maintain. Cell washers, if they are not adequately maintained, then build up material, not necessarily bacterial, can start to cause false positive reactions.

Crenation, rouleaux, and hemolysis generally are caused by formulation and isotonicity problems. Hemolysis

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generally in a lower salt concentration has been diluted with saltwater or been contaminated in some way with another solution. Rouleaux and crenation generally are thought caused by a hypertonic solution, that is, a greater salt concentration.

[Overhead.]

The areas for potential problems to arrive from -- let's look at the manufacturer, and I am including in the manufacturer, locally made saline, too. A lot of people still do make their own saline.

Obvious errors in formulation, that is, the salt wasn't weighed out correctly or the ingredients weren't weighed out correctly, a basic error. There are also errors in formulation where the formulation has been changed, but not controlled. That is, somebody has decided to add a preservative or put in what a lot of manufacturers called a proprietary ingredient or a proprietary method to either extend the stability or stabilize the pH, and they really haven't controlled what they have been doing with it.

Contamination again from the manufacturer. This can occur primarily, what we have seen at least, or been able to deduce, is chemical types of contamination where their equipment maybe has not been cleaned out. Generally, at least -- and we are making some assumptions here because

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we are not able to determine exactly what goes on in the saline to cause some of these problems, some I will discuss a little bit later -- the contamination from I think -- and I will get into some of the problems that we have had in examining these issues -- from Clorox.

Clorox is used extensively, one, to flush the deionized water systems on their regular maintenance programs, and also from what I can gather, they flush their equipment occasionally, which is not either sterilizable by steam or other methods, where Clorox is a favorite to flush through, sterilize, or at least sanitize, and then wash the remaining Clorox out. If there is any left behind, it gets in the saline.

Labeling issues. Generally not a major problem, but in clarifying what is in the product exactly what it is, and especially when they start getting into proprietary type of issues, you don't really know what is in the product, to extreme issues where one good company had a beautiful label that was on the side of the box, but when you flipped the box over, the Cubitainer, to get the saline out, the label was underneath the box.

Control testing. A lot of the manufacturers do or do not do testing specifically for blood bank activities. Those that do claim it as a blood bank saline may make

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statements that they do an antiglobulin control test, generally a pH, and that's about it.

For the user, potential problem areas are storage of materials. If they are a fairly large user, the material is going to be used up pretty quickly. Storage in warehousing or in a non-controlled area can be a problem if it is exposed to great heat or other problems where there is fluctuation. Again, saline is fairly hardy. It is only a solution of salt, it doesn't necessarily break down at all. If there is deterioration, it is either in pH or in contamination.

Stock rotation. If the stock isn't rotated efficiently, that is, new lots that come in get put on last, and the old not used up, then, you can get a variation there.

Non-dating and expiration of open containers. When the container is opened, generally, the standard practice in the Red Cross is that it gets discarded after a month, but if that is not done, and you have got a Cubitainer sitting around for a long length of time, the pH can change considerably and it is open for other contamination.

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Contamination again generally either chemically or from the equipment that it is being used on. Dirty equipment correlates with that.

Incorrect application. In the old days, where we just had tube testing to worry about, and the anti-human globulin test, you filled up your squeeze bottle maybe three times a day, it wasn't a problem. Now we have got to deal with automated systems, microplate systems, monoclonals, that are all affected in some way by possibly the saline that is used. So, some of these systems need to be clarified as to what is the appropriate saline to be using with a particular system.

Lack of daily or routine QC. Most people are at least doing a pH now to QC their saline. Others are doing an actual serological test, whether it be an anti-human globulin procedure or a serological procedure. Lack of QC can lead to potential problems.

[Overhead.]

Problem-solving is the most frustrating. As with any problem that occurs, whether it be the reagent or the saline, trying to dig up the information to try to find out and make a value judgment of what is going on can be extremely difficult both dealing with the manufacturer and with the user.

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With the manufacturer, you get into this proprietary information. Trying to glean out what are the things are in that saline that may be causing the problem that we are seeing just is like pulling teeth.

The manufacturer generally has the inability to really investigate the problem. Most of the problems that have occurred, occurred on automated machines. They don't have the machine to be able to duplicate what they are seeing, and a lot of times it is almost impossible to duplicate. "It is not my problem," is not being really facetious, sometimes it isn't their problem.

If the user is having a pH problem, unless they feel that the initial product that they purchased or if it is a buffered saline that was labeled as being a specific pH, then it is not their problem, it is something that the user is doing wrong that has caused the problem to occur.

From the user's point of view, the initial assessment can be wrong. Usually, when something goes wrong, they blame the reagent, not the saline. If something is not working, if they are getting a false negative result where they are expecting a positive, it is the reagent that was wrong, and when they spend a lot of time determining that it wasn't the anti-S or it was or wasn't the anti-S that was causing it, but the pH. This has occurred with us

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specifically on a problem that was saline related, with the bromelin that we manufacture for the automated machines. The initial reaction was the bromelin was bad, and we probably spent a week analyzing the bromelin to determine that it wasn't the bromelin after all, but the saline that was being used.

Most users don't have the adequate resources to really test completely. Most do have at least the pH meter, an osmometer to evaluate the isotonicity or the salt concentration, ability to do sterility testing if sterility testing is an issue. Other analytical machinery just isn't generally available.

A lot of saline problems yield inconsistent results. Sometimes they see them, sometimes they don't, especially when it comes down to the sort of what I call the "grainees." These are the false positives across the board, all of the negatives just appear of that sort of grainy type of reaction. You give them to somebody else to repeat, and they go away.

Instrument variability for automation and on the microplate systems, someone sees it, someone doesn't, and it can vary from one container to another.

Generally, the conclusions on what is wrong is based on an assumption. There is no real hard facts. It's

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a matter of going through what you have done, what you have eliminated, and come up with the fact that you have eliminated everything except the saline, so it must be the saline. So, to some degree, saline gets the hard knock when you can't pinpoint it. Even if you can pinpoint the saline, you don't really know what is in it that went wrong, whether it is Clorox or some other ingredient.

[Overhead.]

The problems. Some are manufacturer related, most of them are user. Generally, it is in the hands of the users where the saline has deteriorated in some way or the user has applied some measure of instability, and this is starting to show itself in the testing.

Are they clinically significant or a safety issue? I don't know. I have not been aware that there is any clinically significant error that has occurred because the saline failed to detect or caused some incorrect reaction.

Obviously, if saline is causing a problem that could cause a misinterpretation of results, there is a safety issue.

[Overhead.]

Greater regulation. I don't know whether greater regulation is a requirement at this point. Certainly, if we do, then, we have to redefine the classification at least

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for blood bank use. There is obviously need for saline in other areas of the laboratory, clinical chemistries and testing, et cetera, that don't need the definition that is needed for blood bank use.

Given where we are going today with the testing, with automation, monoclonals, I don't know that one blood bank saline is going to fit the bill and that we need several. Maybe this should be left up to the user in terms of knowing and having better awareness of the saline limitations, and the application of the saline.

Better handling and application practices, daily control parameters, and vendor orders, the person that you are buying from, which is a standard isopractice now, and I know a good GMP in terms of auditing the purchases and determining just what their manufacturing capabilities are and whether they meet your GMP requirements.

Maybe we should expect a little bit more from the reagent manufacturer. I know John Case will love this. If there are issues that pertain to the saline or the quality of the saline, the pH of the saline that is used for the product, then, that should be defined by the manufacturer. I think that is more critical in the automation area.

For the machine manufacturers or instrument manufacturers, system manufacturers, they, I think should be

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held a little bit more accountable to define the support solution criteria, so that we don't get caught into these situations, along with a description of any interfering parameters.

Okay. Thank you.

[Applause.]

MS. WORST: Thank you. Our next speaker is Susan Rolih from Immucor. She is going to give us the Manufacturer's Perspective.

Manufacturer's Perspective

MS. ROLIH: I am going to try to give you an overview of some of the problems that we have experienced, that we feel that we have been able to trace to saline in use in performing tests.

[Overhead.]

Some of this is going to support what Roger said, and certainly we have become aware of the fact that when we do encounter product complaints, one of the components within our complaint investigation now involves a look at the type of saline solutions that are used in the serological testing.

[Overhead.]

The next transparency will show you some of the undesirable effects that we have been able to trace to the

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types of saline in use in the laboratory, one of which is decreased test sensitivity.

We certainly weren't the first to publish data in this area. Martin Bruce and his coworkers out of Scotland in 1986 published information in Transfusion that showed that some proficiency samples that had been sent out to hospitals within the United Kingdom were not performing as expected, and when they evaluated the problem, it seemed to be traceable to the type of saline that was used in the laboratory.

In particular, there was an anti-big S that went out into the field, and those hospitals that were using a more acidic type of pH seemed to have difficulty, if at all, detecting this antibody.

In 1994, we published similar findings on decreased test sensitivity, more appropriately for our testing was related to solid phase technology, and again we found that there were some cases of antibody misses or very reduced reactivity of antibodies in the case of using salines.

So, we have seen decrease test sensitivity in antiglobulin phase tests, whether it be hemagglutination or solid phase, and I might add -- we will come back to this later -- that we also have seen failures in hemagglutination

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with an anti-big S. It just happens to be our reagent anti-big S that has been on the marketplace.

We have also seen a failure in direct agglutination tests if cells are used that have been suspended in hypertonic saline, that has been unknowingly prepared at a higher salt concentration either by the user or the manufacturer and then substituted in test systems where self-suspensions are made.

We have also reported increased nonspecificity at the antiglobulin phase with certain types of salines, most notably those with acidic pH in hemagglutination and in solid phase tests, although I might say that some of the problems that you see with saline reagents seem to be exacerbated when you go to microplate systems, and it probably is one of the reasons why, when you get into ELISA base test systems, you are usually always using buffers as wash solutions as opposed to unbuffered reagents, such as the salines used in hemagglutination assays or have been used in the past in solid phase assays.

[Overhead.]

Some of the other undesirable effects that we have seen is that red cells that have been suspended in saline of an inappropriate pH have a failure to bind the coupling agent used in the first-generation solid phase tests, which

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makes it very difficult for you to set up your initial monolayer with which to support your antigen-antibody interactions in subsequent test steps, so it can cause spurious and false positive results to occur in cross-match tests or selected cell panels, which are the type of assays currently for which the first-generation test has been used.

We have also run into situations where we have noticed false positive results of a weak, spurious nature in laboratories that are using saline that contains phenoxyethanol as a preservative, and this has been more a problem for us in accounts that have been using our solid phase based infectious disease tests as opposed to those for the detection of red cell or platelet antibodies, and I don't know if that is just coincidental that the labs that are doing the infectious disease tests, also happen to be closest to this type of saline or if there is a preference on infectious disease labs for using this type of saline over standard blood bank.

[Overhead.]

I would just like to review for you the types of saline in use and by telling you that as I get to the end of this list, we found all of these types in active use in laboratories, in blood bank laboratories throughout the

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United States and so Europe and Canada, as we have done some of our investigations.

The types we found included isotonic saline without inhibitors or preservatives, either user made or purchased from a manufacturer; isotonic saline that just contains azide as a preservative; isotonic saline that is azide-free, that is pH controlled, usually buffered to a pH of 6.5 plus or minus 0.5, although the range may shift upwards slightly depending on the manufacturer; isotonic saline that is azide-free with phenoxyethanol as a preservative, and this is a reagent that is more frequently sold for use with Coulter counters.

[Overhead.]

Buffered isotonic saline that is made with a phosphate buffer and has been designed for use in a blood bank environment; also, buffered isotonic saline that has phenoxyethanol as opposed to one that has azide or is without azide.

An isotonic saline with preservative in a gelatinous substance that has been manufactured for use in the blood bank that allows easier resuspension of red cells because of the addition of the protein.

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Lastly, there are still a number of laboratories that are substituting saline that has been designed for irrigation or injection, and using that in the laboratory.

[Overhead.]

Our concern as we have been investigating some reagent problems that have been reported to us -- and, of course, the user initially assumes is the problem of our reagent, and not something that they are using with our reagent, or something that they are doing with our reagent in the laboratory, which we naturally assume our reagents are the last things that are failing because we know we have taken so much time to manufacture them -- one of our concerns is that laboratory personnel and particularly in this day and age, since purchasing agents have more power to determine what is and is not going to be used in the laboratory, is that they consider all isotonic salines equal.

In fact, we have run into other situations where the laboratory director or the purchasing agent has told us that they have made a decision to buy a saline not necessarily labeled for blood bank use, for use in the laboratory because they cost less than standard blood bank saline or phosphate-buffered salines, and that is where we sometimes run into the issue where the hematology lab and

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the blood bank labs are now using the same saline, and that sometimes does contain preservatives, such like phenoxyethanol or other compounds that allow the automated instrumentation to work appropriately and to keep the cells that are counted or analyzed from clumping.

There are no professional guidelines that exist for salines being employed in serological testing with the exception of the NCCLS guidance documents or guidelines or standard AS-1, that talks about how to make isotonic saline for general laboratory use.

There are no recommendations that are made by organizations, such as the AABB or the American College of American Pathologists, et cetera, that give guidance to people as to what they should be using. Therefore, it probably is understandable why everybody thinks all isotonic salines are equal.

Another concern that we have is that manufacturers of saline products may add substances or might treat those salines in such a way that they feel that additional treatment method or that additional additive eventually decomposes to the point where it is no longer active in the product, and therefore, they are not liable to have that included on the labeling.

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What we are finding is that that treatment or those substances, sometimes if you don't know about them in the product, you don't realize that they can have an adverse effect on the serological test.

So, when you look at a carton or a carboy of saline, and you look at the label, just because it has certain things on the label doesn't mean that there is not other compounds in there.

[Overhead.]

Now, some of the issues that have been reported before and I have mentioned, have to do with pH, and this is one of the areas where we have had the biggest problem particularly with the advent of our solid phase assay.

I do have to say that there is a typo on here. This should be 1,000 ml instead of 100 ml, because at 9 grams at 100 ml, it would probably be virtually assured of never having a positive serologic result in that test system, and, in fact, might be considered by some of our customers to be the appropriate saline for use, because then everything would be negative, and it would be short work day.

But if we look at the USP requirements for saline, which is to make a point, 9 percent solution of saline with no additives, and then look at their requirements for sodium

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chloride for injection or irrigation. It is just an extension of their requirements for a technical reagent of saline.

Now, what needs to be cognizant of the fact is if you just make saline to the USP standard, you have quite a wide pH range, and, in fact, the pH range drops into an area that we all know is not conducive to supporting agglutination or sensitization by a lot of antibodies.

This is one of the concerns that I have with laboratories that use sodium chloride for injection or irrigation, is that if you look at the manufacturer's specifications or claims for the product, they give this range, but in many cases they say that the target final product is 5.5 pH, and I don't think very many of us would feel comfortable knowing that we are using what appears to be a consistently acidic reagent.

Now, acidity is not always a problem with all antibodies, and, in fact, may enhance to some extent the reactivities of certain selected examples of antibodies, but I think overall, if you using an acidic pH, certainly by our studies we have been able to show that there has been a consistent decrease in antibody reactivity.

I think if you are using a saline that tends to compromise the ultimate reaction strength that you can get

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in a test system, and you are working with a test system that might already be compromised because you don't have your cell washer standardized appropriately, or you don't have your shaking technique up to optimum, all those factors in combination tend to allow you to have a test system that is not terribly efficient at doing what you want it to do.

Now, the laboratory salines that you can buy for use come either unbuffered, as I mentioned before, that can also be slightly acidic, some of these laboratory salines may make claims that they meet NCCLS criteria. In fact, several of those that are labeled specifically for blood bank use do say they meet NCCLS standards, however, if they are not buffered, the pH, if it is close to 7 at the time that you open the carboy, by the time it is left standing, exposed to air, where it can absorb carbon dioxide from the air, will be come acidic with time.

We do have a number of customers that have been purchasing pH-adjusted versus buffered saline, and I do want to caution them that a pH-adjusted saline is usually one that is manufactured, and at the end of the manufacturing process, is adjusted either with HCL or NaOH to bring it up to a pH of 7, but it is not the same thing as a buffered solution, and once opened, it is open to the effects of absorption of carbon dioxide, and the best possible product,

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in order to maintain the pH throughout the usable life span of an open bottle or carboy of saline is one that is buffered.

[Overhead.]

Osmolality issues have presented fewer problems for us, but still on an infrequent basis have reared their ugly head.

Preservatives. As I mentioned before, there are salines that can be purchased without preservatives, however, we have become much more appreciative of those products that are labeled that they are produced by proprietary production methods to reduce the microbial burden and, hence, extend the shelf life or maintain virtual sterility until they are opened.

We have had some of our blood grouping reagents become virtually nonreactive in certain lots of these products from the manufacturers, and because they are proprietary, the manufacturer will not always readily relinquish the information to you as to what they are doing.

Now, with twisting some arms, we have gotten information out of one company that they are treating the product with ozone. Within three days, the ozone is supposed to break down, therefore, they consider it

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inactive, and we have a concern that this oxidant is seriously compromising some serological tests.

We have been able to work with the manufacturer, and we assume have received from them certain lots that have been treated or charged with different levels of ozone, and, in fact, they have gotten back just to say that there is a difference in the performance with selected antibodies that we have sent with them.

But because this becomes inactive at least by their chemical tests after three days, it does not appear on the label, so it sometimes causes a considerable amount of sleuthing on our part although now we know that there are certain manufacturers that, when we hear their names, we know exactly what to call them with as far as questions on particular lots of saline.

This treatment is not necessarily consistent from lot to lot as far as results come in a serologic-based test, and we have had one manufacturer that has gone so far as to label carboys of their saline with a label that says, "found to be compatible with Immucor products," because they know that the residual that is left in there is of such a level that it no longer interferes with some of our blood grouping reagents.

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So, yes, these types of production methods that are proprietary may have a negative effect on your test systems. Saline with phenoxyethanol, again I just want to caution laboratory workers to something that is designed for hematology equipment, and it definitely causes false positive results in the solid phase assays, and by taking the phenoxyethanol out of the saline, you could show that you can get rid of those spurious weak unwanted or very difficult to interpret reactions, and get to the definitive break point between a positive and a negative result.

[Overhead.]

Osmolality issues are a smaller problem for us, but we have seen it happen. With user-prepared salines, where salines were made that were hypertonic, and, in fact, caused our saline anti-big E reagent to become totally nonreactive.

Some sleuthing did show us in this particular case, the customer was making saline in a 25-liter carboy, and did not completely mix the salt crystals to total dissolution before using the saline in a test system, and as they went to this new carboy of saline and used the spigot at the bottom to put the saline in their saline bottle, they suddenly lost activity with antibodies when they made the red cells up in the cell suspension.

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All we had to have them do is open the carboy and look at the bottom of the container, and did they, in fact, still see undissolved saline crystals, which, of course, they did, and then we got the aliquots back in and saw that the top half of the carboy, of course, had virtually little sodium chloride in it because it hadn't been mixed yet, and the bottom of the carboy was very hypertonic, which, in fact, killed the reagent.

When we had them go back and make appropriately mixed, thoroughly dissolved saline, then, the reactivity of the antibody came back in their laboratory.

We have also run into situations, again, it is of more frequency when people make their own salt solutions. It seems that this is a valuable learning experience for med tech students or SBB students when they come into a laboratory, if the laboratory is preparing their own saline, they give it to the students, and not all students understand how to make percent solutions, or not all of them can use a calculator and figure out how to use it appropriately to get the right concentration, so that they have ended up with hypertonic solutions, and in this case, has caused us to receive complaints because the reagent red cells were not particularly robust and kept disappearing in

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their cell washers when they were washing the cells as part of the end of the antiglobulin test.

In come cases, it appeared to cause an increase in false positive results, when they, in fact, could get cells left following the washing steps, so that they had something to test.

[Overhead.]

In conclusion, it is certainly our feeling that the type of saline that is used in serological testing can influence the test outcomes and that perhaps we would see an improvement in test outcomes if the formulation and treatment of serological saline for blood bank test is standardized.

Thank you.

[Applause.]

Summary

[Overhead.]

The quality of saline used in immunohematologic testing may be affected by such things as variations in pH and osmolality, contamination, whether it is bacterial, fungal, or chemical, formulation differences, preservatives, and micromethods and automated blood bank procedures may be more sensitive to the saline quality fluctuations.

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Hopefully, during the open discussion, we can attempt to arrive at a consensus as to the minimum requirements for blood bank saline. I would like to begin the open discussion for performance standards for blood bank saline.

Open Discussion and Proposals

MS. WORST: At FDA, we have been discussing some possibilities of changing and reclassifying saline used for serologic testing to a Class II device, and have some special controls for that developed in a guidance document.

How would people feel about that? Or we could get John Case riled up and put it all on the manufacturers to have their package inserts state exactly what performance standards the saline used with that product could be.

MR. CASE: Perish the thought. Perish the thought. I think there probably is some justification for having some kind of standard. I remember well the instance that Susan mentioned when there was a particular saline out there that was sterilized with ozone that was destroying the S antigen, and, in fact, it was made to look as if it was our anti-S, and then some other manufacturer's anti-S that was at fault, and it had to do I think with the fact that somehow or other there was residual ozone in the saline

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which was causing the S antigen to be destroyed on red cells.

I am very much in favor of setting standards. To what extent those standards should be defined, I really don't know, I mean whether it should be buffered or unbuffered saline. Again, in Europe, there is a perception that no saline is suitable for serological use unless it's buffered, whereas, all my working life I have used unbuffered saline.

I remember one, talking about my working life, if you go back donkey's years to when I was a young lad, I remember I worked in a lab in London where a large aspirating jar came into my possession that had a brass tap on the front of it, and I thought, oh, that would be lovely to keep my saline in, and I soon discovered that the passage of the saline through this brass tap on the bottom was causing heavy metal ions to be left in the saline, and, in fact, it was creating aggregation of the red cells. The more you wash the cells, the more they aggregated spontaneously, so that is an anecdote that illustrates how the presence of some unknown or unsuspected substance in saline can cause problems.

So, I don't know, it is not for me to say, but I certainly would hate to think that manufacturers would have

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to address this issue in their package inserts. That is to say manufacturers of reagents, not of saline.

MS. ROSSMAN: Patti Rossman with the American Red Cross.

We have seen in our National Testing Laboratories, some problems with saline, particularly I believe hypertonic saline, where we bought buffered saline without paying much attention to the isotonicity of the saline. Like most people, we are not standardized across the board with our saline.

We are in discussion presently, though, about the fact that we feel like we need to standardize our saline within our individual sites, and we need to validate our testing processes with the standardized saline, so I believe that we believe that saline needs to be standardized. I am not sure that we have a position, particularly on how that needs to be standardized, but certainly we feel that the end user or a guidance from the FDA would be appropriate.

DR. GARRATTY: This time I do agree with the Europeans, John, and for years we have been using buffered isotonic saline in our lab. We make it ourselves, and I have been thinking that most self-respecting red cell immunohematology labs always did use buffered saline.

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We did run into problems of sensitivity some years ago now, and when we looked at commercial salines at that time, we were horrified at the pH's. Just regular saline that was being sold that people were using, that some of them were way below 5, they were very acidic, and this is what drove us some years ago into making our own and using buffered isotonic saline, and I feel much more comfortable using that routinely.

I would hate to go back to just relying on just buying saline without knowing anything about the pH, because, as it has been well documented, you reduce your sensitivity, red cell antigen antibody reactions when you get down to that low.

MR. BYRNE: Again, my European indoctrination said that you always have to use buffered saline, particularly in antiglobulin tests where the particular worry was that a very acidic saline would actually elute antibody from your red cells. As we all know, the most common elution method is acid elution.

MS. KOCHMAN: I have to admit that when this topic first came up, I automatically thought that the answer was going to be performance standards on the manufacturers of the saline, but in retrospect, I can't help but think that the manufacturers of the reagents have little control over

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the purchasing agents in a facility, and the only way that they can perhaps exert some control is to describe more descriptively than simply saying isotonic saline, what kind of saline we are talking about.

As much as I don't want to anger John Case, I do think that perhaps there needs to be clearer indications of what kind of saline is acceptable for use in the package insert, and as I said, just a little more descriptive than isotonic.

MR. CASE: Can you just imagine what manufacturers are now going to have to do to satisfy the feds, because every time you put in a lot of particular reagent, you are going to have to test it with all the different kinds of saline that are available. It ain't possible. I mean give me a break.

[Applause.]

MS. WEILAND: I would like to add to that comment because I think poor Susan has been through an odyssey with the investigations that they have performed and the sleuthing that they have had to do with respect to finding out what is in the various types of, quote, unquote, "saline."

When you layer that on top of all of the different reagents that must be qualified, there must be some happy

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medium between standards that the saline manufacturers need to comply with, as well as perhaps some instruction on the part of the manufacturers of the blood grouping reagents.

MS. KOCHMAN: It just occurred to me that maybe what we need is an intermediary here, and that intermediary being the AABB. Perhaps if they could describe what saline is appropriate for use in a blood bank, that would allow purchasing agents to have a list of things they could purchase from, but it would also give them a little more specific details about which salines are not appropriate and which salines are more likely to be appropriate.

MS. WILLIAMS: I was listening to yours and thinking of an answer to that. Part of the problem with having the AABB come in as an intermediary is that is going to force the AABB to take a look at all the test systems out there - solid phase, microplate, and our microplate is a bit different than the normal microplate, and they are also going to have to look at the results interpretation systems on the instruments, because we have actually found with the Olympus instruments, that some of the salines interfere with the settling patterns and the results interpretation. It may not have anything to do with the antigen-antibody reaction, but actually interfere with the photometer, so I

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don't know how the AABB as an intermediary can come in and say this one will work for all blood bank testing.

The other thing is if the manufacturers put in their package inserts that saline used in this test system or with these reagents must meet this criteria. That now is going to force every customer out there to go through what Susan Rolih has tried to do with the saline manufacturers to find out what they are putting in there and, you know, what the preservatives are, how they are sterilizing, how they are buffering, and that information is not easy to come by if the manufacturers don't share it, the saline manufacturers don't share it.

DR. GARRATTY: I was going to second what Sheryl said, but now after listening to Candi, I just wanted to ask her a question. Are you saying that if the -- I was going to say that this one of the first meetings I have attended with manufacturers and the FDA, I don't usually attend these meetings, and it has been interesting for me to hear this dialogue because many of the things going on, seem to me that they do relate to educational functions like the AABB technical manual. For instance, they don't mention anything about the check cell problem in it, they just say add them, but they don't go into any rationale or any of the things we have been discussing.

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I think there is a place there for educating them how they should be appropriately used, so I was agreeing with you, but are you saying, then, that if you used pH-neutral buffered saline, you would have problems with the Olympus, or are you saying that some of these other salines that we talked about, uncontrolled ones, give you a problem? I am surprised that if you used a buffered saline, neutral pH, that it wouldn't work for most of the things that we do.

MS. WILLIAMS: We have just observed problems with some salines and some accounts where there has been other material in there. Maybe it's the gelatinous material that Susan mentioned some of them have in there, where it actually causes some problems in how the button is formed when you are doing a settling assay, and therefore, when you are doing an image analysis of that, it can interfere with it.

DR. GARRATTY: This is again addressed that we will never reach perfection, so it can be something wrong from some manufacturer, but I just think as a general philosophy, it is something they could say in most serological procedures, it would be a good idea to use a buffered neutral pH saline, and just warn them that a low pH might affect sensitivity. There is going to be all these

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other things that could happen with an individual batch of saline from a company.

MR. COLLINS: George, I think that sort of still gets back to the standardization and definition question because buffered saline and all buffered salines are not the same. When you refer to buffered saline, you are saying that it is saline that has been buffered in a way that has stabilized the pH, but I mean that can vary considerably in the ingredients that are used to buffer the solution, whether it is sodium or potassium phosphates, and the concentration.

DR. GARRATTY: I have never seen anything to suggest that matters at all. We have used over the years, I mean Sorensen's buffer and Hendry's buffer, and I have not really noticed any serological difference as long as you have a stable buffer at a pH you selected, which to me would be anywhere around neutral, you know, anywhere around that range I would accept. You could come up with a buffer range.

MR. COLLINS: And that is appropriate, but it comes down to whether it is isotonic or not, and the biggest problem that Patti alluded to, and that we have seen at the Red Cross, was this issue where somebody labeled their product isotonic buffered saline, and their way of

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manufacturing that was to take their isotonic buffered saline, presumably with 9 grams per liter salt in it and add potassium and sodium phosphate salts to it.

What it did, it raised it to a hypertonicity saline, and that started to cause problems on the Olympus machine specifically for us, and it just threw that particular buffered saline out of what we would define as isotonic buffered saline.

So, I am agreeing with you, buffered saline is appropriate, but there needs to be what is buffered saline in terms of its relative salt concentrations, what types of salts make a good buffer specifically for serological applications, because I don't know that, like Hendry's or other buffers, may be appropriate in other situations, and it comes down to that point is what we are looking at is probably a variety of saline solutions that fit the bill for different applications.

I don't think we can come up with two anisotonic regular saline with or without preservative and a buffered saline with or without preservative that is fully applicable for everything that is being done today.

DR. GARRATTY: I think we could. I think it would be relatively easy. I just think that you are going to have a manufacturer that will have some of these others, but I

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would think that you could come up with a relatively easily good buffered saline that would be generally applicable to everything

MS. ROLIH: I think some of the issues on saline could be readily addressed if it was a requirement of the manufacturers to stipulate in their labeling what the pH was, that there is no such thing as a proprietary treatment method, that you have to let the user know how it is treated in case they are using it by a method other than by which the manufacturer is assessing whether or not that proprietary treatment method really becomes inactive or not, and if you had all that in the labeling, then, the user could make a much more informed decision, but as it stands, you can't make an informed decision.

I think, secondly, as a manufacturer of a buffer or concentrate that had to go through a 510(k) approval process, I think that it is an unfair application of the law that some of us are required to verify in clinical trials and through stability studies that a product does what it does, when the manufacturers of the salines, whether they are labeled for blood bank use or not, are Class I devices and don't go through the approval process, so the burden of proof is at an entirely different level, so that we are not treating everybody with similar products or where the end

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results are similar the same way as far as regulatory issues are concerned.

MR. WILSON: I would like to chime in just a little bit. I think that from at least my perspective, having manufactured blood typing antisera 20 years ago, no one really thought terribly much about the physiochemical characteristics of water and saline the way we do in the 1990's.

As the technology and the refinement and the unbridled horsepower of these terrific reagents now that can pick needles out of haystacks, and the like, now we are beginning to see where some of these subtle kind of lost in the baseline physiochemical attributes of some of the reagents, now all of a sudden become an issue.

I think where the line is drawn, at least from what I can see, is that we have got a situation where you can get a stone-cold false negative. What I hear is anti-S can be falsely negative, and I think when we get to that point, we have to stop and say look, we have got to take a look at what we have been doing in the past, and possibly reevaluate for the future, and I think we are all sensitive to trying to get to doing this in the most expeditious way.

One of the things that I think we all want to keep in mind is who owns what. Reagent manufacturers are

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obligated essentially to provide adequate instructions for use. They are also obligated to define the instructions for use. If they say it is supposed to be used under these circumstances, that is more or less the way it is supposed to be handled.

If someone else takes that reagent and uses it in a different circumstance, then, they are obligated to redefine to ensure that the performance characteristics of that product, in fact, are meeting the intended use labeling claims.

Some of these lines are not drawn absolutely clearly, and as I can say, not only in the area of blood banking, but also in the area of infectious disease testing and other biologics, where manufacturers are now using other components, some manufacturers use other final product components to manufacture new products, it becomes more and more complex as to how to resolve problems and who owns what measure of the problem.

My sense here is that the place to start is clearly defined, at least from the beginning, what the reagent, i.e., the antisera manufacturers, how they develop the performance characteristics in their clinical trials, what were the characteristics of the saline that got these

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types of performance characteristics, and then it becomes an issue of the free market, so to speak.

If the manufacturer of the reagents elects to check out other salines to come to the idea that the manufacturer wants to broaden its market, then, he can do those types of studies. That, I think, you know, in a very, very simplistic approach, would be elective on the part of the manufacturer of the reagents.

If there is instrumentation manufacturers, if they are taking a finished reagent, and they are using a different technology than the original reagent manufacturer intended, to get to the answer, then, I think that that instrument manufacturer is obligated to perform some measure of testing to ensure that certain types of -- has to describe in their package insert, so to speak, what criteria is to be met for the saline on that side.

The best thing I can offer, at least, you know, after saying all that, is it is really a good idea for everybody to get together, talk to each other, and work these things out, because I think that what I am hearing is that there is an array of situations where if the industry could come together and sort out which is the most promising saline formulation buffered, which buffer osmolality, et

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cetera, and try to work a consensus up from there, then, maybe FDA would then take it from there.

Sheryl, maybe you could comment on that.

MS. KOCHMAN: The one comment I had was that as long as there is saline available that is for general purpose use, and it remains a Class I device, we at CBER have no or little control over that. The best way to get a handle on the situation might be for reagent manufacturers to stipulate in their package insert that the saline must be one approved for use in immunohematologic testing, and then we could have Class II salines that CBER does get involved in reviewing.

So, there is a little more burden on the reagent manufacturer and a lot more burden on the manufacturers who want to sell their saline to people who are doing blood grouping, but that way at least both reagents are in the same camp, they are both going to be at CBER for us to work with you in getting them working together.

MR. WILSON: I think the bottom line is that the information about what saline works with which reagent, or which system, is what people need as information. How they get it or by which camp or route, I am not sure. We will try to do it the easiest way, and, of course, the easy way to some might be not so easy for others.

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MR. COLLINS: Just an issue to take the devil's advocate position is that as soon as this goes to a Class II, and we put definitive requirements on the manufacturers, obviously, the price is going to go up.

For most people that is probably going to have a significant effect. I know the American Red Cross probably uses close to 1,000 liters a day systemwide. That would have a significant financial impact at least on the American Red Cross or other large users that rely on Cubitainer or at least commercial supplied product depending on the increase, and I really would be very surprised if at least the resident manufacturers would not increase their price if they were held to any strict standards.

The other point is that isotonic saline is isotonic saline and regardless, if the manufacturer is not able to stabilize it in some way as soon as the cork is popped, it is back to the user to control and maintain that product.

So, you can make the best isotonic saline in the world, if it doesn't contain any additives, you can make it sterile in packages, ship it out, so that it will arrive at your facility sterile, but as soon as you pop the cork, it's what you do with that is going to dictate how long that is

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going to last and any potential problems that you might have for that saline.

Buffered saline only gives you that measure of security from the manufacturer's point of view that the pH is going to remain stable, and it may be sterile, and whether he labels it sterile or doesn't label it sterile, most of the saline packaged today are sterile, they are put through a 0.22 filter to reduce the particulates and generally, if you do the sterility test on the saline that arrives in your facility, you will probably find there is not a bug in it, but they don't label it sterile because it throws it into that category of having to maintain the paperwork, to maintain controls under FDA guidelines.

So, I am not sure if standardized definitions are made, what they are going to tell the manufacturer or what is going to hold the manufacturer to do in terms of creating stabilizing agents to maintain that product when you open it.

MR. CONNELLY: Hi. Marc Connelly from Ortho Diagnostics.

As a developer of reagents, as we go along in the development process, part of what we have to do is characterize what are the critical performance parameters. We look at pH, oftentimes look at tonicity, and most of the

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cases, as George Garratty points out, the saline is not necessarily a major critical performance parameter. It is, as in the case of the Olympus system, it can critically affect the performance.

When you know it can critically affect the performance, then, you really should spec it out in some way, so that errors don't occur. When it is not a critical performance parameter, I don't know that there is a great deal of value added in being very, very specific about precisely what reagent you use when all the evidence indicates it doesn't make much of a difference.

Where it becomes particularly a problem, a big problem, is in the example you have illustrated, where as a manufacturer, I may spec out very precisely the tonicity, osmolarity, pH, et cetera, et cetera, but it could be the antimicrobial system that somebody uses that completely destroys any reactivities, and then to have to spec out which antimicrobial system you use, and if you don't use it, how many days, and how it is handled at the site, these become quite an effort around that and quite a burden to put on a manufacturer who has to manufacture dozens or hundreds of different reagents.

MS. ZERGER: Raya Zerger, Olympus.

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One of the experiences that we have had is that in trouble-shooting these types of problems -- and we were having a similar problem at the time Susan was experiencing her problems -- in dealing with the manufacturers, is that we found that it may be labeled from a particular company, which is typically a distributor, and when you do investigation and try to find out who manufactures it, it may or may not be on the Cubitainer, and one particular instance, we had multiple manufacturers under one labeling, so it wasn't just -- as a matter of fact, in none of the instances that we dealt with was the label or the brand, so to speak, on the container the actual manufacturer.

They were sourcing this from another person or company, and it was either there or it wasn't there on their Cubitainer, but you couldn't just say saline from company X was made by company Y. It may have actually been several companies that they were sourcing saline from depending on what region of the country they were purchasing this from.

So, it makes it even harder to -- and this was labeled isotonic buffered saline and very clearly was not, and it caused a false negative, overt negative test with anti-D in our system on our analyzers.

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We found that it was very difficult to nail the details down because of the variety of individuals involved in putting saline out for one of these particular companies.

In addition, we found in trying to address this issue of can we give you specifications, I mean I can tell you from working on the instruments for years, that if it changes the shape of the cells, you are probably going to have problems.

Well, what changes the shape of the cells? Well, we know, particularly with the isotonicity, that is an issue, pH, we know dramatically affects the way antigen and antibodies come together. So those are the kind of the no-brainer things that some of us that are into the hard-core aspects of blood bank think about.

The problem is the first time I ever saw a problem with saline -- this was years and years ago, and I was in an account for a week trying to solve the false positive problem they were having in reverse ABO testing -- and we think we have it solved over the phone, and then two days later it would come back.

So, I finally went out there and spent a week, and it is to Ed Steen's credit, who I suffered through all of his antigen antibody lectures, that I figured out this problem. I sat there and I asked myself what causes cells

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to come together other than antigens and antibodies, and I just started thinking through could these cells be aggregating rather that agglutinating.

Sure enough as I began to look at the false positives versus the true positives under the scope, I found that the saline was causing rouleaux.

The point of my story is then in further investigation in this blood center, they had five lots of this particular distributor's saline, three of the lots caused the problem, two of the lots did not, and they did all the classic testing that we would think to do. They cultured it, they pH'd it, they did osmolality studies.

Everything with those five lots came out very similarly. Now, there were no significant differences among these five lots. Yet, what caused my horrific problem I was having, I will never know, and I told them, I said perhaps there could be mode-release compound they use for the plastic liner in their Cubitainer, you know, some other contaminant, something that they are using in their production environment that is not a part of their labeling, and why three lots and not the other two, we don't know, but I just think it would be difficult outside of these standard measuring parameters, whatever we want to use in talking

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about saline specs, to nail down some of these problems that certainly we have seen.

MS. ROLIH: I am glad that you said that because that has been my one concern, particularly when you go out and you do your clinical trials and you try to establish the performance characteristics of your product, it's a crap shoot.

You could have people use all the salines from all the different manufacturers, and since this is not a regular occurring problem on a lot-to-lot basis, they either grab the right saline that is going to tell you what you want to know or they are going to have one, and they have no problems with it, and so you are totally unprepared.

MS. KOCHMAN: We at FDA really don't have any control over a crap shoot, so I am not sure what kind of answers you would want from that other than maybe the burden falls on the user themselves to validate through some sort of product acceptance testing, each and every lot of saline that they receive, because they will use it in the system they intend to use, and if an appropriate protocol were developed, incorporating some of the known problems, like, for example, make sure that it doesn't cause your anti-S to go negative or your anti-D to go negative, you know,

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actually have a protocol where the user would validate it in a preliminary use test before they accepted it.

Otherwise, I can't see how FDA is going to pull off these different things together. We are not going to be able to do it alone.

MR. COLLINS: I would agree with that although I am never one to put more burden on the poor hardworking tech in the system, but we basically have quality control systems set up routinely now. Nearly all institutions are leaning more and more to quality systems, ISO-9000, GMPs, it is thrown at us every day where we are improving our quality system in the way that we work.

I do feel, and I think I have sort of said it before, that I don't know that controlling the manufacturers to the nth degree is the way to go in this particular issue. There is enough variability in the salines and in the application of the salines that most of the users can make educated decisions as to what type of saline needs to be used for their application, do the particular evaluations and validations, and come up with maybe, where there is a standardized quality control test or at least some sort of assay that is done on a regular basis, not necessarily does lot by lot cover the bases.

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We have seen instances where one Cubitainer within a lot, or several Cubitainers within the same lot, have shown variable results. Why, we don't know; what, we don't know, but it is there. I think it is a matter of making sure that the user is educated and having the fallback controls to be able to catch those, so that it doesn't become a clinical issue.

MR. WILSON: I have one more point. In the labeling for in vitro devices in the regulations 809.10, it states that known interfering substances should be described in the package insert, and that is not elective on the part of the manufacturers, that is an obligation.

I think that what we would view is that when complaint files have such information in it, from a manufacturer, for example, if the manufacturer's package insert says use isotonic saline, period, and then they get a report where a user used isotonic saline to that same definition, but found out that the pH was, I don't know, 4 or 3 or something like that, then, I would view that the manufacturer of the reagent then would be obligated to revise their package insert to state that either there has been a report of false results in the reagent at pH 5, or they would set specifications for the saline of an operating range.

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This is why it is so important when the manufacturer of the reagent conducts their clinical trials, to define all these parameters as best as possible, to, you know, avoid these problems. Most of these seem like they are relatively easy to avoid.

MR. COLLINS: If I am not mistaken, don't some of the manufacturers have disclaimers as far as at least the S, that pH may affect the reactivity or sensitivity, have I not seen that in somebody's --

MS. ROLIH: Yes, you have, plus all of our solid phase inserts now have a restriction on the type of saline that you could use. I just wish people read that part of the direction search.

MR. COLLINS: I agree with John, there is no way to know that the manufacturer is going to bubble ozone through their system or add some gelatinous material or perform some other manipulation of the material, that the reagent manufacturer just has no knowledge of what that might be.

MS. RAY: At the risk of making everybody angry, I think it puts a lot of responsibility on the end user, number one, to have to validate every cubicle of saline to make sure that it meets the manufacturer's specifications for each lot, and also to ensure that each cubicle of saline

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is compatible with the specific reagent and methodology that they are using for all of their testing.

MR. COLLINS: I would probably add that we basically do that anyway with every reagent that we use. We basically do that anyway with every reagent that we use every day, we have to validate it some form or other, so validating saline is really just one more to add to the list.

MS. RAY: Back when I was in the blood bank, the extent of what we did pretty much was we would do the pH, we would not go through further testing to ensure that it didn't have other additives that might interfere with the different test systems, whether it was potentially solid phase, the Olympus, tube testing, et cetera, and if different manufacturers, in qualifying their supplier or having difficulty in getting the information they need to make those determinations, the end user on the bench doesn't have the time or the resources to try to do that either.

MR. COLLINS: I think my point was that we basically are doing that with other -- I mean all of the antisera that we use on the Olympus machine, every new lot is tested to make sure that that particular lot works within the parameters, so adding saline to do the same type of characteristic is really not another big deal.

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Certainly, it is another test that you have to perform, and we don't want to do that, but that is a lot easier from my perspective.

MS. RAY: If it were that easy to do, then, why wouldn't it be easy for the manufacturer of the saline to do as a whole lot and standardize it?

MR. COLLINS: Because the bulk of the errors or the problems that are occurring, are occurring at the user's end. When it leaves the manufacturer, generally, the reagent, unless it contains one of these abstract proprietary ingredients, is in good condition.

They may have adjusted the pH with sodium hydroxide or acid and achieved a pH of 6.8 when it leaves their factory or when it leaves their facility, but as soon as the user takes the top off that, and sits it on their bench, sticks a tube in it for their cell washer, or whatever they are doing with that saline, then, the variable changes, and that is when it is up to the user to verify that over that period of usage, is it still doing the job that it is supposed to do.

MS. WORST: In the interest of time, can we take the last two comments, and we are going to have to move on because have a really tight schedule this afternoon.

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MS. MALLORY: I would just like to support her in a way, and not really oppose my colleague up there, but there is more than just testing on automated equipment, and when you get into a reference laboratory situation, it can be a great deal more tedious to have to do a lot more work, and I would caution that if we can make whatever validation that needs to be made extremely simple, I think that we need to keep that in mind.

I wonder also, again, how big a problem this is. I agree with Susan that we need to have I think labeling that indicates what is in the package, but I wonder do we have any idea how much of a problem saline is in our industry.

MS. ROSSMAN; I would also like to support the end user. I feel like if we are left with all the validation, that it will be a great burden, because I think if there is no guidance and we have no indication of what kind of saline we need to be using, then, it is our responsibility to test all the parameters, the pH, the osmolality, bacterial contamination, all of these factors that we have listed, whereas, if there is some guidance and we have it narrowed down as to the type of saline that we should be using, then, our validation is more of a performance validation, which is

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what Roger is talking about, the performance validation that we always do, which is no problem.

MS. KOCHMAN: I have one last comment.

MS. WORST: I won't cut you off.

MS. KOCHMAN: Delores asked if we know how much of a problem this is, and I have the unique position of being someone who has gone into almost every reagent manufacturer and had an opportunity to look at their complaint files, and I can say that I have seen it everywhere I go, so it is a problem, it's part of the reason it was put on the reason it was put on the agenda today.

I don't know that we know the scope of the problem, but we do know that it is a problem, I have seen it at every reagent manufacturer.

MS. WORST: We are going to have a 10-minute break now and reconvene at 10 after 3:00.

[Recess.]

MS. KOCHMAN: This talk is going to be User Interpretation of Labeling Information.

Ironically, as I was preparing some of the materials for this talk, this Dilbert cartoon appeared in the Post.

In case you can't read it out there, Dilbert says, "Ken, may we have a word with you?"

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The other guy says, "We heard it. You gave an interesting presentation at the sales staff meeting."

Ken says, "Thanks."

"You told them our new product kills mold and mildew."

"Well, you mean it won't?"

"We make software."

"Well, haven't you ever heard of the placebo effect," et cetera.

With that, I would like Harry Malyska to come up and share some of his experience with us.

User Interpretation of Labeling Information

One Manufacturer's Experiences

MR. MALYSKA: After you hear all these great speakers, I am sort of embarrassed. This is not my primary trade and I am not that good at it, but I will give it my best shot.

[Slide.

Micro Typing first would like to thank the FDA for inviting me to speak to you today. I also would like to make it clear that I have the greatest respect for all other reagent manufacturers and hopefully, my comments today reflect the type of situation that most, if not all, reagent manufacturers are facing.

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The FDA references, I thought, for any of the people that don't know, are for instruction circulars, can be found in CFR 21-606 and 801, and all manufacturers of diagnostics sold in the United States comply with these laws and all products manufacturing facilities subject to FDA approval, registration, and audits.

The reason I point that out is more and more we have people that are reading their CLIA regulations and I guess all other regulations that are out there, and most manufacturers quite often now get phoned to verify that they are licensed and many other questions that probably lead to their misunderstanding that we could be in business and not be licensed.

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Most current package inserts are laid out to assist the reader. You should be able to quickly determine the product name, intended use, major limitations, preservatives, cautions, storage conditions, and at least in the blood bank trade, these tend to be the types of headings, the headings that I will be focused on are obviously the ones of limitation and also how to use the test method.

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There seems to be a dilemma, that often a dilemma is created of the direction inserts for use in the Specific Performance section, and the paradox that while some customers still fail to read or follow the package inserts, others over-analyze every word, comma, period due to fear of the FDA, CLIA, ISO, or litigation concerns.

Thus, in order to continue to remain flexible with their test procedure -- I am talking about now the end user -- they would prefer very wide ranges of test limitations in the package insert, as well the manufacturers guarantee that they will detect all clinically significant antibodies no matter how they perform the tests.

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Thus, when the manufacturer uses their best efforts to clarify these instructions, to give the consumer additional information which could be helpful in the use of the product, the FDA may interpret this additional statement as a cause for concern if this information has not been used previously in other similar licensed manufacturing products.

This, in turn, generates the need for additional data and sometimes delays the approval to market times lines.

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The consumer would like to use the product again with few limitations on time, temperature, interpretation even when they academically know the need for such limits.

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The competitor can often use additions to the package insert to cause doubt in the consumer's mind or to make inappropriate claims of superiority.

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I thought a specific example might be interesting. Micro Typing Systems is a relatively young diagnostic manufacturer who makes just a single product called a gel test and the reagents to go with it.

When we started out, we tried to use 20-plus years experience to add statements that we knew were universally true to help the consumer. One of those statements on our IgG gel card package insert was, "Use of enzyme-treated cells with anti-IgG cards may detect many clinically insignificant antibodies."

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As I said, this a well-known fact and many nuisance antibodies will be detected with these enzyme-treated cells. This would be a true statement for any IgG reagent that I know of, whether it was the tube or other test method.

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Again, it was MTS's intent to supply the consumer with additional information to help them understand and interpret results should they choose to use enzyme-treated calls, and then the famous thing is what happened.

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A competitor used this additional statement to wilfully cause doubt in the consumer's mind about the quality and usability of the anti-IgG card. One competitor actually began to distribute a list of misinformation including the statement that you could not use anti-IgG gel cards with enzyme-treated cells.

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The consumer, many consultation labs have historically use enzyme-treated cells in the antiglobulin phase for additional testing, and they are normally well equipped to understand the risks they have in finding antibodies that are not going to be useful to their clinical work.

Some of them overread these instructions to mean that they could not use enzyme-treated red cells in the card.

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Many of these individuals were under the assumption that their current tube procedure and the anti-IgG that goes with it were recommended and approved for this test. The fact, however, was that the anti-IgG tube reagents were never licensed for use with enzyme-treated cells, nor was it in their package insert.

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I believe maybe in this discussion, we are at a point where we might want to discuss leveling the playing field.

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I believe perhaps manufacturers should be subject to a consistent standard for some of these universal statements that aren't limited to one reagent over another. Perhaps we could have similar verbiage for well-known serological facts, such as the fact that no one incubation time or temperature is ideal for every antibody.

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The marketing departments of all companies, including mine, it would be nice if we could alter the rationale that package insert is primarily a marketing tool and the practice of trying to avoid issues or inappropriately causing doubt in regard to a competitor product was changed.

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Perhaps the FDA, during their review of manufacturing labeling could bring those historical inserts up to the new standard, so that there wasn't sort of a competitive advantage for those who have not included some of these universal statements.

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The user of the diagnostic needs to develop confidence I think in the testability and have the courage to face their auditors with facts and validation. To the best of my ability and knowledge, there are certainly things that each manufacturer can do with their package insert to assist the user, however, the package insert will not replace good common sense, in-house education, quality procedures, or documentation.

Thank you.

[Applause.]

MS. KOCHMAN: Once again, I would like to have John Case came up, and he is going to present some of the problems that he is concerned about in the labeling area.

More Manufacturer's Experiences

MR. CASE: Thank you, Sheryl.

To tell you the truth, this is not any more my forte than it is Harry's, and again I am in the awkward

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position of coming after him and having to repeat some of the things that he has said. In a way, I think most of what I have to say is just to present more examples of consumers of misconstrued labeling.

Let me tell you how I first got involved in this or how my interest in this subject caught Sheryl's notice. It sprang from a request that was put upon us by CBER the last time they were about to approve a monoclonal reagent of ours.

They wanted us to consider putting in the labeling a caution similar to the caution that exists in all reagents made from human source material now, which was to the effect that monoclonal reagents can be a source of disease agents, and therefore, need to be handled in the same way as human source reagents. They asked would we consider putting that in the package insert, and we said no.

Now, the reason we said no was as follows: that if it appears in our package insert, there are going to be people out there that interpret the package insert so literally that they are going to assume that this caution applies only to our reagent, and not to those of our competitors.

Let me emphasize that we are not opposed, as a company, to putting that in the package insert. What we are

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opposed to is being asked verbally to put it in a given package insert when this is not a direction from CBER to the effect that all manufacturers, to do this with all their monoclonal reagents, at the next printing of their package insert.

If they would do that, and dictate exactly how that wording is to be, then, we would gladly comply, because I think it is appropriate, but that we should be asked to do it when other reagent manufacturers are not being asked to do it, places us in the awkward position that Harry complains about where one or more of our competitors may, in fact, come out with a document that says are you aware that when you use Gamma's anti-e monoclonal that you could catch some sort of dreadful disease from it. I mean, really, let's get real here.

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Here is my first slide, which is just a title slide.

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Now, one of the things that I think is a problem nowadays is that common sense is actively discouraged, and there may be some reason for that in that common sense is a commodity in short supply, and it may be true, in fact, that

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if you are going to apply requirements to reagents, they need to be applied in a consistent and uniform manner.

But I think it is a shame that we were required some years ago to remove the expression For Professional Use Only from our package inserts, because, in my view, they are, these reagents, in vitro diagnostic reagents are for professional use.

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As I said, I am going to give you some examples, and one is that incubation at 37 degrees centigrade, which for donkey's years, as far back as anybody can remember, was always taken to mean incubated approximately the temperature of the human bode.

Nowadays, what happens is that if your incubation is at 36.9 degrees centigrade, or you haven't proven that your incubator is not, in fact, occasionally going up to 37.1, you have a problem in terms of the validity of your test result.

It is interesting that nobody has ever really considered the fact that when you put your tests into the water bath, it takes them a short while to warm up. In fact, the length of time it takes is a variable that depends upon the number of tubes you put into the water bath. So,

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somehow or another, there is a inconsistency in this that I have never quite understood.

Secondly, we ran into a difficulty with a customer that was cited by an inspector because he kept his reagents in the fridge along with his blood, and the AABB recommends that blood should be stored between 1 and 6 degrees centigrade, and we, as the manufacturer of the reagents, were recommending that the reagents should be kept between 2 and 8 degrees centigrade.

So, I asked the customer is your blood bank refrigerator where you keep both your blood and your reagents ever between 1 and 2 degrees, and he said no, so, in fact, this was a non-problem, nor should it even have been a problem if it was between 1 and 2 degrees centigrade, but we have been forced in sheer self-defense to change the storage range both for reagents and for the samples that will be tested with them from 2 to 8 degrees to 1 to 8 degrees, so that we cover this range adequately.

So, we put this proposition to the FDA in getting our package inserts reviewed, these changed package inserts, and one FDA person -- who was not Sheryl I hasten to say, nor Helen, nor anybody here present -- said have you proved that your reagents are stable down to 1 degree centigrade, whereupon, I gasped a little and said something impolite.

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Now, there is an even sillier literal interpretation. When you do a test for weak D, it is my view, in order to prevent -- those of you that were here this morning and heard my story about the lab that refused or sent back 110 vials of anti-D because it was too strong would appreciate this -- in that it is perfectly proper when you are doing a test for weak D, that after the incubation phase, you should look and see if you have got direct agglutination before you go to the Coombs phase, because if you don't, you are wasting Coombs reagent and you risk classifying as weak D a cell that really has a normal D.

So, it is perfectly proper for the manufacturer's package insert to suggest that after the incubation, after 15 minutes at 37 degrees, you should spin and read the test, and if it's positive, the patient is positive, and if it is negative, then, go to the Coombs phase.

But, in fact, some of our competitors don't actually say so, and by so doing, gain an advantage in the minds of those people that don't want to do it, and the only reason that I say it is perfectly proper to read after incubation is that an immediate spin test is itself a variable depending upon how many tests are being set up at

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given time, how much delay there is before you actually do the spin.

Now, I think it is well accepted nowadays, although I am astonished sometimes to look at how much 22 percent bovine albumin is still being sold, but I think it is generally accepted nowadays that it is a concentration of 22 percent. Bovine albumin does little, if anything at all, to improve the sensitivity of an antibody detection test, and so incubation for 30 minutes is obviously better than incubation for 15 minutes.

However, manufacturers are driven by competitive forces in the market to recommend 15 minutes incubation, and if, in fact, you do what Immucor does, which is to recommend 30 minutes as being the minimum, you risk being shut out of the market by people who feel that although you may be using the same bovine albumin as the source material, people will say, well, I can't use your bovine albumin because your package inserts says to incubate for 30 minutes, whereas, your competitor says 15, and I only want to use 15.

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We had a debate with a lab at one point in time over whether or not it was necessary to wash the cells from donor segments before using them in the cross match, and I think everybody knows that depending upon how much plasma

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you put into the cell suspension, you may or may not get a fibrin clot. It is always better to wash the cells to make sure there is not plasma in the test system that you are going to be using with humane serum.

But to stipulate that you have to wash the cells means that you lose a customer that is determined not to wash the cells even though it will be obvious that the same stricture applies equally to any additive.

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So, having given those examples to add to the ones that Harry already mentioned to you, I have a further quotation from the literature on human failings.

"For the American people are a very generous people and will forgive almost any weakness with the possible exception of stupidity." So said Will Rogers.

Thank you.

[Applause.]

MS. KOCHMAN: John made a comment in his talk that common sense appeared to be a commodity that was in short supply, and I think Dr. Linden might have some examples to show us that that could be the case in some institutions.

Dr. Linden.

An Inspector's Findings

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DR. LINDEN: My presentation is definitely going to be very different from the other ones you have heard today.

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The New York State Health Department operates a laboratory licensure program that is state-exempt under CLIA, and some 355 laboratories either hold or have applied a license in the category of immunohematology. I would like to clarify -- much of what I am saying is on my handout -- I would like to clarify, though, that there is a typographical error in the cover sheet. I am not affiliated with any manufacturer.

In 1997, we identified numerous errors and deviations from manufacturers' package inserts at several facilities, and I am going to relate five case histories. These all occurred at clinical laboratories that were not associated with blood banks, and these facilities all perform immunohematology testing primarily on pregnant women, and these results are the basis for decisions regarding administration of Rh-immune globulin. Again, these are all recent cases.

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Facility A is a moderate-sized independent laboratory. In the interests of time, I am not going to

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read all of the results of our findings. I think they are readable on the slides, and also these data do appear in my handout.

But as example of about 800 ABO and Rh tests that we reviewed, of the most concern, we identified three patients reported as Rh-positive despite negative D and weak D reactions, as well as some unresolved ABO discrepancies, and some patients reported negative on antibody screening despite one or more positive screening cells.

The Rh negativity rate in this laboratory varied from 1.5 percent in one month, literally to 25 percent the next month. We also noted that gel serum separator tubes, which are not suitable for immunohematology testing according to the tube manufacturer's package insert, were accepted and constituted about 20 percent of patient specimens at this facility.

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Facility B is a laboratory associated with a large clinic, and we reviewed a little over 5,000 ABO and Rh test results. We identified four patients that were identified as Rh-positive despite negative reactions in anti-D testing, and 12 patients interpreted as Rh-negative despite positive reactions in anti-D testing.

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We also noted that all reactions were exactly 3-plus, never anything different from that. At this facility, gel serum separator tubes, since these patients were actually clinic patients, were used for all of their immunohematology specimens.

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Facility C is a small independent laboratory which initially actually was operating unlicensed, and then we told them to stop doing that, and they started operating only in parallel with a reference laboratory.

Of about 300 ABO and Rh tests that we reviewed, three were identified as Rh-positive by this laboratory, although the reference laboratory reported Rh-negative. There was one, 3-plus anti-D reaction interpreted as Rh-negative, and this report also misspelled a patient's last name. There were two ABO interpretations that didn't agree with the reactions.

We also noted that the QC SOP stated that for the anti-D negative control, that staff would add two drops of anti-D, one drop of rr cell, and two drops of 22 percent albumin contrary to both the manufacturer's package insert and the facility's SOP for anti-D testing.

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Facility D is a laboratory associated with a large clinic, and although this doesn't relate to immunohematology, we were very struck by the fact that anticipated results in chemistry were recorded prior to sample accessioning as much as a day before, they anticipated what the results were going to be the next day, and then when they did the test, they changed the result with correction fluid if the result didn't turn out as they expected or if the line turned out to be used for a control of a patient who didn't undergo testing for those particular analytes. Tubes for immunohematology testing weren't labeled at all, increasing the likelihood of mixup.

For ABO testing, reverse grouping appeared first on the worksheet, then forward grouping, although a separate log sheet that they used only for Rh-negative patients used the standard format. ABO interpretations were made and recorded based on forward grouping alone.

We noted that a variable number of drops of both reagents and patient specimens were used.

In conformance with the facility's SOP, Rh interpretations were made and reported based on anti-D alone, and then if the weak D testing proved positive, they made a phone call to the clinic, but there was no evidence that there was ever any sort of amended report.

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Out of about 900 patient records, there was one patient positive on anti-D testing reported as negative, some ABO errors where the interpretation was the opposite of the reactions, that is, an O was called an AB, and A was called a B, and so forth, and eight unresolved ABO discrepancies.

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Facility E is a laboratory associated with a large clinic. Worksheets were not in use at this site when we went. Immunohematology results were reported directly onto the patient report forms, then later transcribed onto the worksheet. For a time, on the worksheet, the reverse grouping appeared before the forward grouping, but the test results were reported in the standard format, so that they didn't agree with the headings that were on the worksheet.

Out of about 2,800 ABO and Rh tests that were reviewed, there were nine patients with negative anti-D reactions reported as Rh-positive, and there were 16 erroneous ABO interpretations and 18 ABO unresolved discrepancies.

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These errors may be very clinically significant since this patient population is almost exclusively pregnant

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women, and Rh-negative patients may not receive Rh-immune globulin if they are falsely reported as Rh-positive.

Many of the errors that we identified were essentially user-operator error, involved staff deviating from established SOPs, but some SOPs identified were not in conformance with the manufacturer's package insert.

We have also noted in the course of inspecting laboratories under our purview some cases in which laboratories changed from one reagent to another without modifying their SOP accordingly.

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In conclusion, in the real world where there are some laboratories where people are not concerned about identifying anti-vel [phonetic], but are concerned about the blue stuff and the yellow stuff, and we have actually heard people say that, there are some facilities in the field that continue to make really egregious errors in basic testing and lack knowledge of basic proper techniques, and while some of the problems involve staff deviating from SOPs, further direction to users might be helpful in improving testing, although certainly the basic problem is certainly lack of education and training on the part of the staff.

It is certainly important to make sure that the SOPs conform with the package inserts, and it is possible

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that more clear basic and explicit package inserts might facilitate this in some of these laboratories.

Thank you.

[Applause.]

Summary

MS. KOCHMAN: As you can see, this section kind of covered a variety of labeling problems. I have tried to summarize the things that were going to be brought up.

First, there is the failure to read the package insert. Second, is the failure to follow the package insert directions. There appears to be a misunderstanding of package insert information, in some cases misinterpretation of package insert information, and in the cases that Harry and John described, misrepresentation of package insert information, and we also have a problem of package inserts being either too general and/or inspectors being too specific in their translation of the package insert.

As we said at the start, we came here with lots of questions, we didn't necessarily come here with answers. One of the things that John Case commented on was that he is perfectly willing to include some of the statements that CBER recommends as long as all manufacturers are required to do the same.

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So, I am going to use that as a springboard to start the conversation. Right now there are general guidelines for what is to be included in the package inserts for the in vitro diagnostic reagents, and primarily we get most of that from 809.10.

Since John brought up the point of having FDA describe the language that should be included, I am wondering if he is recommending or suggesting that CBER prepare some guidance documents including specific kinds of labeling statements that manufacturers can and should use, and we can go from there.

Open Discussion and Proposals

MR. CASE: That is for me, I suppose. I think there are certain things, certain aspects of what should be in the package insert that CBER needs to dictate, just as they dictate precise wording of the caution that has to appear in connection with the possible presence of infectious agents in human source material.

That is laid down. We all say exactly the same thing, and my view is that we should exactly the same thing in connection with the possibility that monoclonals might be a source of infection.

When it comes to other matters, I think maybe more broad, general recommendations are appropriate, but like I

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said this morning, I don't think that manufacturers should be required, for instance, to state which forms of partial D their monoclonal anti-D can be guaranteed to react with.

Indeed, as I also said this morning, I don't think they should be allowed to make such claims unless they can demonstrate that they have tested all the various forms of those particular variant forms of D and shown that they do consistently react.

So, I guess I would suggest that they need to consider it both ways. There are some things that should be left to the manufacturers, perhaps with them approving what is said, and there are some things, as the disease caution, for example, that should be dictated by them to make sure that there isn't variability among manufacturers that can, in fact, lead to a competitive advantage or disadvantage.

DR. STIFANO: Toni Stifano.

If you find that competitors are, in fact, abusing your information to promote their products, you can, in fact, if you can get your hands on your information, send it to us, and so we can evaluate it to determine if they are, in fact, making inappropriate claims. So, that is one way to address a level playing field.

Another question I have to pose to you, as industry, is it is not with precedent for us to institute

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class labeling changes for therapeutic products. I don't see why it is not something we couldn't look into the possibility of doing with your products, as well, with that help. That is one good way to start with a level playing field is to look at everyone's reagents.

Another thing, too, is that if you have data to demonstrate that you can support a range of incubation temperatures and times, then, perhaps it is not such a bad idea to include those in the labeling, so you are not cited by an inspector, and again, those are the kind of changes, too, that would not require prior approval by us. They could be, in fact, implemented with the notification of changes being effected type changes to your inserts.

How do those things sound? I guess I need feedback from you, too.

MR. CASE: I can't say that we have had a whole lot of success at notifying the feds that some competitor is violating.

DR. STIFANO: To whom are you sending the information?

MR. CASE: Well, to CBER.

DR. STIFANO: To whom in CBER?

MR. CASE: I presume that we -- I mean we are going back a few years now -- nowadays, we write to the

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directors of the divisions, but we used to write to the director of CBER, and nothing would happen.

In fact, I got so frustrated at one point with writing to the director of CBER that I wrote to her with her HFM number, whatever it is, 1, isn't it?

DR. STIFANO: Yes.

MR. CASE: And I still got no response. So, you know, it is not always as simple as it sounds, is it?

DR. STIFANO: Well, no, but there is a staff in place now that is there to answer those kinds of questions, and to try and look into it.

We have even committed to responding to the complainant to let you know if your complaint has merit or not, so that you will know right upfront, if you send in a complaint, can we do anything about it or not. So, yes, there is a mechanism in place.

MR. CASE: Well, that is ever so reassuring because, like I said, it has been very frustrating in the past because we get no response.

The other thing is that changing a package insert is not an easy matter.

DR. STIFANO: I know that.

MR. CASE: That is something else that used to happen in the old days. You submit your package insert for

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review and about three years later you still haven't had it back. The fact of the matter is we were forced into the position where we had to print revised package inserts without getting any approval from CBER. I know that is a mortal sin, but we had absolutely no alternative because we were not getting them back.

We are not getting them back now in a timely manner. The problem is that if you want to change your package insert, to change 2 degrees centigrade as the bottom storage level to 1 degree, you have still got to get it reviewed, and you want to do it in such a timely manner that you can now go to the printer, you are ready to reprint the package insert, you have got to go to the printer, you have got to wait for the feds to respond with their review, and three months goes by, and you still haven't had it back, I mean what is a poor manufacturer to do.

DR. STIFANO: I have a partial answer to that, John, and that is that there has been some recent changes in what kinds of changes need prior approval, and some of the labeling issues will be much easier to deal with in the future.

MR. CASE: When will we see the final rule in the Federal Register, so that we can implement that?

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DR. STIFANO: It has published. The 601.12 changes to an approved application has published, and it is, in fact, in place. If you look for some specific guidance, I believe because this is classified as an in vitro diagnostic or an in vitro device, whatever, and you follow 809, and you look at some of the changes that are allowable under the device regs, clearly, anything that would enhance the use of that product is a change that can be implemented prior to receiving approval from us.

So, since most of the guidance that has been written for the products that are covered by biologics are therapeutic products, a lot of the guidance is geared towards traditional therapeutic products, but I don't think it is unreasonable to look to the device regs to see, under their changes to be reported, the nature of the changes that can be implemented for your products.

MR. CASE: I don't think that is unreasonable either, but, you know, up until now, any change has had to be reviewed.

MS. KOCHMAN: Toni, do you have additional information that manufacturers could include on any information they want to submit in terms of having promotional claims investigated, like the HFM?

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DR. STIFANO: Sure. It's the Advertising and Promotional Labeling Staff. It is HFM 202, to the attention of Mr. William V. Purvis, who is also in the audience. If you can't get him, you can yell at me.

Again, if you find that manufacturers are inappropriately using your materials to promote their products, it is important that we have actual copies of this. Otherwise, we don't have the evidence to go on. So, if you just call up and say this representative was in there saying this to that person, there is absolutely nothing we can do.

However, if you find this list that inappropriately lists information, and we do find basis we can, in fact, do something about it. Okay?

MR. CASE: That is good.

DR. STIFANO: And we will do it quickly. You will hear from us within 30 days if your complaint has merit or not.

MR. MALYSKA: I think there is also another issue, though. Some of these things get into -- I don't know a polite word for it -- sort of a contest. Obviously, somebody that says refrigerate, and that needs further definition, and in John's case it was 2 to 8 degrees, does that mean now that some other person is going to write 1 to

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8 degrees, and the next guy is going to run 1 to 9 degrees, or incubation temperatures where it is clearly that serology, there is no ideal one temperature, we are misinforming the consumer, and I think that there needs to be some understanding from the inspector's point of view that if somebody is incubating for 15 minutes, if that is what is recommended, and he only does it for 14, that he is reasonably in compliance, and that if there was a failure at 14 minutes, certainly, the manufacturer would need to have all kinds of warnings below that, because otherwise, we are getting into a situation that if you take low ionic strength reagents, they are all incubated, and it wouldn't make any difference whether they are gel test, tube test, or any other test I am aware of, the serology has remained the same, but the claims are all over the map.

DR. STIFANO: I understand this. Are you speaking of CLIA inspectors, the HCFA people?

MR. MALYSKA: I believe -- somebody help me -- I believe a lot of labs are getting inspected more than they ever had before. They are very concerned of being out of compliance, and some of these inspectors, if they are CLIA or AABB, are way beyond common sense. They are into absolutes.

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So, is 1.96 degrees, 2 degrees, or is below 2 degrees.

MR. CASE: I think CLIA inspections are being done often by state authorities, aren't they? Some of the abuses that I am familiar with have been with AABB inspectors -- or they don't call them inspectors now, I don't think, but whatever they are, they are people representing the AABB. That is the same AABB that is recommending or was recommending its members that they need to qualify all their vendors including those that are licensed by the FDA. Can you believe that?

DR. STIFANO: You leave me speechless, and that is a rare thing.

MR. CASE: I think it is an outrage.

DR. STIFANO: I honestly don't know how to respond to that, because I have not experienced it firsthand.

Again, I offer, you know, if you think it would help to look at package inserts uniformly across the class, please let us know. The other piece, too, is that it is not without precedent for us to work with the state agencies. In fact, in a different task force, CBER and CDER are working with HCFA trying to get information out, so that everyone knows who is on what page, and we all end up on the

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same page specifically having to do with information in the managed care area.

So, you know, it is not doable to educate state inspectors, if you would, by providing guidance.

MR. WILSON: Two quick points, one to take a step past what Toni had said.

What you said in terms of enhancements, one can go ahead and make the enhancement and go ahead and distribute that to the package insert, I think before you do that, you may want to check with us, because some people may have a very, very broad view of what an enhancement is.

The intent there is to clarify the use or to clean up some ambiguity in a procedural step or something. It is not in any way, shape, or form to allow for a new claim or add additional performance data which implies a new claim, et cetera. So, in that regard, I would say if you have any questions, pick up the phone and give us a call, and we could talk to you about it.

I think that there is some relief relative to our new regulation that you heard, where we can review these things in a little bit more timely manner.

I would like to address one of the other points that was made regarding this 37 degrees versus 36.5. About four or five years ago, Ann Hoppe, who was the director of

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the Office of Blood, or whatever we were at that time, acting director, had come to many of the groups and said, look, the industry has a problem here, we have got these package inserts where manufacturers have developed their products with very, very restrictive and unrealistic temperature, timing, and centrifuge speeds, et cetera, that are absolutely draconian, and from a regulatory point of view -- which that is what the FDA unfortunately has to work with, and if you haven't noticed, we don't have standard deviations on the regulations -- they are written, we have to deal with them the way they written, and it says, you know, follow the package insert.

Well, if the package insert says 37 degrees, an inspector walks in, while I grant to you there is some overriding consideration of, well, is 36.5 going to be a problem, the reality is that I think we all have to look at the way products are developed in the late 1990s.

The notion of developing a product with only one narrow parameter is not the way to go. What Ann Hoppe had asked us to do -- I know she asked me, I am sure she asked Sheryl -- to talk these manufacturers into broadening their studies a little bit, so we don't get into this kind or problem.

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We can only approve the product based on the data that we are given, and we would ask those reviewers to encourage the manufacturers to broaden these things out.

Now, let me go a step further. In the course of some of the discussions, I learned a new term, BMPs, Bad Manufacturing Practices. Some manufacturers proposed specs that you could drive a comet through and claimed that their product worked under any circumstances, and we said, no, no, wait a minute, hold on a second, what you need to do -- and we have been basically encouraging this all along -- is that when you develop any part of this manufacturing specs of your product, take the worst case scenario and the best case scenario for your raw materials, timing, process steps, et cetera -- what I am basically doing is paraphrasing the 1987 process validation guideline -- and develop your product with those parameters, and then run your clinical trials, the three lots that we asked, at the minimum and the maximum, so that you have actually got a little bit of a broader range to work in.

Then, when the data comes in to us, that will allow us to say, here, we will give you those specifications that allow a lot more flexibility, but we can't do the experiments for the manufacturers, and I understand the

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problem, the in vitro diagnostic field has a very, very fast turnover of getting products to the marketplace.

The objective of doing studies that would take more time is absolutely not desirable, and the objective is to get out there before the other guy does. The bottom line is that we have limitations, and that is what I am trying to express, and the industry would help themselves and help us and help the blood industry users of these tests to have a little bit more flexibility that has some validation behind it.

MR. CASE: All I can say on that question of incubation temperature is that I believe in the literature somewhere, there is something to the effect that anywhere from 32 up to 40 degrees centigrade, it doesn't make any difference with most reagents.

The other thing I would say is that in this document that has still been in the draft stage since 1992, namely, recommended methods for blood grouping reagents evaluation -- I repeat has been in the draft stage since 1992 -- it talks of incubation at 37 degrees without stating a range.

So, you know, we are being encouraged to do the same thing.

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MR. WILSON: Thank you for pointing that out. That again was written in 1992. That was our best effort at the time. What we would certainly do is view that as a guidance, and if a manufacturer came in to us with data that said 30 to 42 degrees, and they had some reasonable data at those extremes, I think we would be hard pressed not to approve it.

So, I agree with you that looking back at 1992, and looking at the 37 degrees plus or minus zero, you know, that indicts FDA in a sense for that guidance. I will take that on my shoulders and say, okay, it is 1997, we all have this problem, let's try to work through it.

My earlier remarks stand, and I would hope that the industry would get together and develop amongst themselves some of these broad-bands, so to speak, because you are looking at the new FDA that is reformed with minimum resources to spend time writing the guidance documents.

We are hampered drastically with our ability to do this. I know that industry is hampered drastically because of market pressures, but something has got to give, and the best thing we can do is say, look, work together as best as you can, you know, broadening some of the bands on the standards, provide us the data, we will be able to do it.

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As a matter of fact, if you would like to, if you would like to offer rewriting that guidance document in light of this conversation, we would love to look at a draft from industry.

MR. CASE: I practically did. I spent several hours in 1992 making suggestions, and no result. My suggestions were never even acknowledged.

MR. WILSON: Have they been added to the docket? In other words, you have to put them to the docket to have go into the guidance system. Regardless, whether they are in the docket or not, if you have good suggestions, please, get them to me, get them to Sheryl.

MR. CASE: Well, we were obviously told to adopt that document even though it was still a draft, we were told verbally to adopt it, which is not acceptable, no direction from CBER is ever given verbally, it is to be in writing or it's not valid. So, in fact, the valid recommended method is the one that was published in 1986.

MS. KOCHMAN: I think that you will find that under the new Good Guidance Practices, you will be seeing things and having much better opportunity to comment on them, which brings me back to one of Toni's comments, that being a class review of the labeling for these products.

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I haven't heard any other suggestion as to how to deal with getting the package inserts to contain the information that we need them to contain. Is there any objection to some sort of an across-the-board review and then a guidance document on how they should be revised and implemented?

MR. CASE: No, I think that would be a very good idea. I mean I think it is something that we are all hoping for, because there is a lack of uniformity as things stand at the moment.

One of the problems we haven't actually touched on here is the problem of labeling to meet requirements in countries that don't have English as their mother tongue. I noticed that one or two of our competitors now have package inserts in six languages, and it looks like a broad sheet newspaper, and, you know, we are already criticized because our print is too small, and I am really not quite sure how we are eventually going to handle this stuff especially if we are going to be required to put more material into the package insert.

I mean one of the things we have always tried to do at Gamma is to make our package inserts instructional, you know, educational, and I guess that the problem now is that most people out there don't want to be educated, they

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just want to come to work at 9 o'clock and go home at 5:00. They are not interested in learning anything.

So, maybe what we need is advice from the feds as to how we can reduce the content of our package insert, so that we can write them in French and German and Portuguese, Spanish, and you name it

DR. STIFANO: Again, as part of a class labeling review, it is not just what has to go in there. It is looking at what is in there, what needs to be in there, what can be eliminated, and the like.

I mean so it is not just that we are going to tell you to put more and more and more. When you do a class labeling review, you go top to bottom, what is necessary, what is not, what is a point of confusion, you know, who has got this and who doesn't have that, so you literally do level the playing field.

Will that help?

MS. WEILAND: I think it would. One of the questions that I had for Dr. Linden and for the users in the audience is if you listen to what has been presented, there is such a wide spectrum. Some people want more information out of the direction circulars, and yet, in other instances, it sounds like we are giving too much information and perhaps the information isn't structured in a way that is

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useful given the situation that Dr. Linden was talking about.

MS. YASKANIN: Dania Yaskanin. I am also from Ortho.

Actually, you just touched upon my point, because I have been a part of several discussions at Ortho about the length and size and the difficult readability of some of the package inserts that we have, although I would like to say in defense of the technologists out there, I am not quite so sure that they don't want to learn these days. I think they are suffering from the pressures of downsizing themselves and having to cover more than one department, and being forced to do so under situations where they are not allowed to have adequate training.

I do think under those circumstances, it is time to address whether or not our package inserts need to be conducive to people at that level of experience and training, because those are the people that are doing the work today.

MS. KOCHMAN: We are going to have to make this the last comment, I think.

MR. CONNELLY: Part of what I am kind of hearing ss sort of a theme in several of these talks is you can lead a horse to water, but you can't make him drink. A lot of

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what we put into the direction circular, as we have heard today, is coming under increased scrutiny and requirements to back that up with scientific studies, clinical trial data, so to that extent, there is a tremendous pressure to minimalize the amount of information that is in there, maximize its impact, but try not to put things into the direction circular because if you put it in there, it becomes part of the product's claims and part of the product labeling, and you had better have all the inherent data, validation, et cetera, to back that up, because that is what you will be held accountable to.

That tends to mitigate against putting in a lot of instructional teaching type information because then that can come up to a lot of subjectivity in the course of reviews, and things like that, so it mitigates against that.

The other side of the coin, though, is part of what we are hearing is in many cases, with the saline issue, with a lot of your sites, clearly, the package insert says don't do this, and it is done anyway. So, tightening up and being extremely precise on what goes into the package insert may clearly build in a lot more better science, it may clearly build in a lot more time, review time and cost, but it may not improve the problem in the hands of the end user,

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because they are getting the water in many cases, but they are not drinking it.

MR. WILSON: Just a quick remark. Number one, the labeling regulations have been in place since 1976. They were developed when the Medical Device regulations were formulated. That list of items in 809.10 were developed with CDC, as well as open public comment.

I am sure the docket is still open. If people want to change them, it's a free country, go ahead and make your comments, number one. Number two, unfortunately, we think they have stood the test of time, notwithstanding the concern about readability, and the like, which CDRH has, in fact, published guidance on how to write instructions, so that they are better readable.

The problem is that we have had a number of situations where manufacturers of package inserts have truncated, in other words, they have the small version on the back panel of exactly how to do the test, and I will just leave it that that resulted in a very large blood bank losing its license and hundreds of thousands of units of blood being recalled.

So, the more you truncate it, the more risk you have of having problems on that side. I don't think that there is a perfect answer to this. I think the next step is

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readability, but when you start to pull information out of the package insert, you may be fixing one thing, but possibly breaking another. It is a very, very slippery slope.

MR. CASE: I think you are right. One of the things that struck me is that during the session this afternoon, we have actually seen both sides of the coin, haven't we? We have seen instances where people read the package insert so literally that they form the wrong conclusion, and then Jeanne presented a whole slew of cases where it was obvious they didn't read the package insert at all, or if they did read it, they deliberately ignored what was recommended there.

MS. KOCHMAN: I think at this point we need to move on, but I do think that the consensus we need to take a closer look at what is going on here with labeling and I will see that we do what we can with that.

Validation and Use of Blood Grouping Instrumentation

MR. WILSON: For this last session for today, we are going to be discussing the validation and use of blood grouping instrumentation.

For openers, we are going to get a historical perspective on dedicated equipment by Debbie Weiland.

Historical Perspective, Dedicated Equipment

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MS. WEILAND: Thank you.

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When Sheryl asked me to provide a historical perspective on this topic, and told me that I had 10 minutes to talk, I was rather daunted, because it is a subject, as we have discussed throughout the day, for many of our subjects, that could go on extensively.

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In looking at the transition of validation and use of dedicated blood grouping instrumentation, I think that we will see that there has been an evolution of technology and regulation, and that the validation and the extent of which user implementation has grown, has transitioned through that evolution.

There has been a transition, as well, from a laboratory environment where scientific curiosity and the desire to put their own stamp on test methods has changed into a manufacturing environment where strict process control is the name of the game.

There are also parallels to the increasing use of computerization in software control devices, and my comments today are primarily dedicated to blood grouping equipment that is used in donor center settings and high volume labs,

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particularly because those are the areas that are regulated in particular by the CBER regulations.

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Characteristics of dedicated blood grouping and instrumentation are that they have a specific intended use and are designed to be used for that use. In many cases, that use may even be further specified as to a particular group or class of samples. For example, rather than blood grouping in general, it is blood grouping on donor samples.

Most of the equipment has been adapted from standard laboratory manual procedures, such as microplate technologies or research systems, such as the continuous flow Technicon analyzers. Early instrumentation was incorporated, many user defined test panels and operating conditions, and this is a transition that has occurred in that later instrumentation, and instrumentation that is coming out today is more defined and regulated from the standpoint of the options that are available to the user both from tests that can be run and how those tests are constructed.

Until recently, and with the exception of the STS-M that Gamma introduced in the mid-1980s, most laboratory blood grouping analyzers are fully open from the standpoint that they are not manufactured by reagent companies, and

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they are produced to be used over a wide range of reagents no specified by the manufacturer.

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The instrumentation manufacturer has also transitioned over the course of these years from the early seventies, when the Technicon AutoAnalyzers were in place until recently, and instrument manufacturers are compelled to comply with the 21 CFR Part 820, which is the device manufacturing GMPs, and is required, as well, to adhere to the CGMPs and the new QSR.

These regulations lay out the responsibilities of the manufacturer to define specification, to quality assure the elements of the design, and this is an area that the new QSR regulations has really taken under their wing and enhanced.

In addition, the responsibility of the manufacturer is to validate the system, not only from its production, but also its performance, to qualify the performance in trials within their facility and also by clinical trials, which I will get to in a minute.

The instrument manufacturer then submits a 510(k) or PMA for the instrumentation, and if there are reagents, dedicated reagents associated with it, also submits actually

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PLA requirements for those reagents or PLA amendments for those reagents.

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The clinical trials conducted by the manufacturers need to cover a variety of operating scenarios in order to assure that the systems will operate in a variety of different conditions. These conditions may have been or the range of operating conditions may have been identified through the performance qualifications internally, and then are validated through the clinical trials.

In general, diverse geographical locations are selected, as well as facilities of varying size and facilities that may have operator diversity, so that the full range of experience can be assessed.

The extent of testing is also something that has transitioned over the years. Early 510(k) applications for this type of instrumentation really encompassed fairly low numbers of samples, in the hundreds rather than the thousands, and yet, as well, over the course of time, there has been a shift in approach to testing, that sheer numbers of samples are not as critical as providing a range of critical samples to be tested, samples that perhaps would not be encountered in the normal population that was selected.

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So, a variety of types of samples need to be tested, samples which will also confirm the samples that are suitable to be used on the system. Depending on whether the system is an open system or has dedicated reagents, reagents also need to be looked at, and generally, if it is an open system, more than one manufacturer's reagents and more than one lot number. In general, the rule of three tends to apply, three lots, three sites as a minimum.

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User implementation and use of blood grouping analyzers prior to 1992, through the seventies and early eighties, primarily focused on the parallel serological testing. The number of samples varied widely, from a few hundred samples to a week's worth of testing.

In general, test plans and validation documentation was more limited because the word validation certainly in the seventies wasn't really part of our vocabulary in an extended setting. There was also limited software validation because the early analyzers did not incorporate a lot of software-controlled mechanisms. In fact, the early Technicon AutoAnalyzers certainly didn't have positive sample identification, software-controlled clumps of red cells spit out onto a filter paper,

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Validation in the early days, as well, through the mid-eighties, was also confined to validation of the software of the laboratory information or donor-management interface. Prior to 1992, donor centers who were making changes to their blood grouping instrumentation or methods of doing blood grouping instrumentation were required to submit their documentation to CBER for review prior to implementation.

I know that there was a wide variety of types of submission packages that were submitted from very limited submissions to very complex situations, and that really promoted the development of the July 1992 memorandum.

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This memorandum from CBER outlines changes to equipment and what was necessary to be done in order to facilitate a change in blood grouping instrumentation, and the justification for this memorandum was that obviously, CBER had done a lot of review of submissions both for reagents and the equipment, underwent independent reviews, and it was identified that based on this information that CBER no longer needed to review changes prior to implementation, and that these transition documents would be reviewed at the next inspection.

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This memorandum detailed requirements or implementation on the part of the user. Those requirements included calibration, validation, parallel testing, QC, maintenance, and an emergency plan.

Validation by the user needs to focus on those control operations that are either identified or spelled out by the manufacturer in a validation guidance document, and also identified by blood center in the review of their operations, so that specific control points are identified.

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That parallel testing that I mentioned also helped delineate what was required from a testing standpoint. The guidance document indicates that a minimum of 500 samples need to be tested over at least three days, and that they should be tested under representative conditions.

It may be required based on the local population to supplement with weak phenotypes, so that a true challenge to the system could be made.

Summary of experiences needed to be documented, as well as documentation and resolution of discrepancies. The resulting anti-D rate for this parallel testing has to be under 6 percent, and the reagents in use also needed to be documented.

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This guidance document indicated that these were the requirements if the facility followed the intended use of the instrument manufacturer, and also followed the guidance of the manufacturer or the requirements of the manufacturer for operation of the analyzer and also reagent validation or reagent qualification.

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Since 1992, there has been a further evolution. There is a broader use and regulation of software. There is a further transition of blood collection facilities to a manufacturing environment, which has created some different scenarios with respect to validation.

There has also been an increasing emphasis on a quality systems approach, and lastly, there is now a movement of automation from the blood center and high volume testing laboratory to the transfusion service.

All of these items I think lead us to a situation where better definition of user implementation requirements and validation requirements needs to be further defined.

Thank you.

[Applause.]

MR. WILSON: Now we will take it a step further with the historical perspective of site-assembled systems. Sheryl Kochman.

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Historical Perspective, Site-Assembled Systems

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MS. KOCHMAN: Much of what I have to say about site-assembled systems is very similar to what Debbie had to say about dedicated systems, but the focus is a little bit different. In component selection for a site-assembled blood grouping system, some of the things you have to keep in mind is that the individual instruments are usually considered general-purpose devices.

General-purpose devices are usually Class I devices. They are subject only to general controls and many of them are exempt from the 510(k) regulations, and they would all be regulated by CDRH.

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In comparison, dedicated equipment has a specific intended use before use in blood establishments. This intended use raises them to Class II devices, which means that they are subject to performance standards. They require a 510(k) or PMA submission with supporting data, and are regulated by CBER rather than CDRH.

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The gist of this is that when a facility chooses to assemble their own system, the responsibility shifts from that of the manufacturer to that of the user. It is the

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user's responsibility to evaluate and validate the instruments for the new intended use, that is, they are changing a general-purpose device or series of general-purpose devices to device intended for performing blood grouping in a blood establishment.

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One of the first things you would have to do is evaluate the instruments. There is a list of questions you would have to go through to determine which devices you are going to choose to use.

I am going to just skip over these because they are not really that critical.

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The most important thing that the user has to do is the instrument validation - can the site-assembled system consistently perform correct blood grouping.

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In this case, the testing that has to be performed by the user actually mirrors the testing that a manufacturer of a dedicated system would perform. We would expect testing in parallel with current methods. We would expect it to represent typical conditions of use. We would expect the typical sample mix to be covered.

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There should be testing over at least a five-day period and by at least two different operators. As Debbie alluded to, we now think that there probably should be about 5,000 unselected samples.

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And then a range of selected samples to cover various sample types, various sample ages, weak subgroups and variants.

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If you are interested in seeing what CBER has put in writing before, there is a Points to Consider document for the design of clinical trials for blood grouping reagents, and that docket is No. 91N0467, and that describes in detail the kinds of things we expect to be covered.

So, the testing that Debbie described in terms of 500 tests over a three-day period is what a user buying a dedicated system is expected to do. The user who is assembling their own system basically has to do the same thing that a manufacturer of a dedicated instrument does.

Overall, the desired outcome then is to obtain documented evidence which provides a high degree of assurance that the site-assembled system will consistently perform correct blood grouping tests, and, in reality, this is just process validation.

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MR. WILSON: Today's final presentation will be one user's perspective by Patti Rossman of the American Red Cross.

One User's Perspective

MS. ROSSMAN: Good afternoon.

On behalf of the staff at the American Red Cross, I want to thank you for the opportunity to participate in this dialogue today. I am a member of a group at the Red Cross whose function is to evaluate instruments, reagents, software, and processes that have potential for use the Red Cross testing labs, and to validate those systems that we choose to implement.

The American Red Cross performs validations in the National Testing Labs where our donor laboratory testing is performed, and in the blood regions where donor blood is collected, made into components, and distributed.

Generally, these validations can be planned and executed very efficiently. Sometimes, however, we encounter roadblocks that result in inefficiencies at best, and in long and resource-consuming validations at worst. I will describe these roadblocks as we have experienced them and provide some suggestions that will perhaps lead to resolution of some of these difficulties.

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One of the costliest roadblocks that we have run into in terms of consuming resources pertains to difficulties in obtaining documentation of vendor testing and installation. An important of our validation process is to develop the testing strategy for any new or changed system. The final Red Cross testing strategy, or validation strategy, includes the extent to which various functions of the new or changed system must be tested.

The first step in developing the testing strategy is to obtain a summary of the changes in the system from the vendor. From this information, we develop a Red Cross summary of changes. We request predetermined specifications and quality attributes of the product from the vendor.

Finally, we request a summary of testing from the vendor. We ask that this summary from the vendor include the results of modular or unit testing, integration testing, delta testing, systems testing, regression testing, and alpha testing.

We compare the vendor testing summary to the summary of changes and the specifications and quality statements. As a result of our analysis of all of this information, we develop our testing strategy. In the testing or validation strategy, we document our analysis and

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rationale for the extent of the testing that we will perform.

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We also ask for written documentation that the vendor has performed testing required by CFR 820 for medical devices if the process that we are validating includes a medical device that is new to our system.

For systems that contain a computer as a component, such as automated blood grouping analyzers, we ask for written assurance that the vendor has met the requirements listed in the Draft Guidelines for the Validation of Blood Establishment Computer Systems.

We believe that these guidelines should apply to medical devices, such as testing instruments, that contain a computer as part of the system. Clarification by the FDA that these guidelines do pertain to testing instruments and the instrument vendors would be very helpful to us.

Recently, we were preparing to validate a system that performs ABO/Rh, Syphilis, and CMV testing for use in the Red Cross. The instrument manufacturer did not give us a comprehensive summary of testing that they had performed on the instrument because the testing documentation was in Japanese and not available in English.

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Because we could not obtain and evaluate testing done by the vendor, we determined that it was our responsibility to perform testing that probably duplicated most, if not all, of the vendor's validation.

The result was a validation that included a 14-module beta test that has taken a year to write and execute. While this was a specific instance, we urge FDA to recognize that with a global economy, there may be a need to update the guidance to address language differences and other impacts that importation of instruments may have.

In other instances, vendors have stated that some design goals and testing information is proprietary. Thus, it seems that there may not be a clear understanding between the manufacturers and the end users as to what documentation is needed by the end user.

One of the actions that we have taken to try to remedy the situation of lack of vendor testing documentation is to specify in vendor contracts what they must supply for new or changed systems. However, it would be very helpful to us if the FDA could clarify whether or not the Draft Guidelines also apply to instrument systems that contain a computer as a component.

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Another unresolved validation issue has recently arisen. We have encountered a situation where a vendor specifies sample and reagent volume ranges for ABO and Rh in their SOP. We asked the vendor for written documentation that they had tested the limits of these ranges using the instrument for which the ranges were intended.

They sent us a letter stating that they tested the ranges on the predicate instrumental and had then received a 510(k) demonstrating substantial equivalence between the predicate instrument and the instrument that we were validating. However, they never tested the ranges on the new instrument.

We believe that such studies should be a part of the systems development process and should be performed by the manufacturer, who will have first-hand knowledge of the new instrument's capabilities and the manufacturer's expectations of it. We as end users do not have the appropriate environment in which to perform such developmental activities.

Another situation that we have encountered that creates a lot of discussion about the best way to proceed occurs when we are performing parallel testing to replace a current method with a new method, and there is no

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confirmatory or "referee" method by which to resolve discordant results between the two methods.

We have encountered this in the case of antibody screening and CMV testing. If the new method is potentially better than the current method, we are reluctant to say that the new method fails the parallel test if it does not match the current method results because this would result in maintaining the sensitivity and specificity of the older, potentially inferior, system.

Such was the case when we standardized antibody screening in our labs by converting all labs to microplate technology. The old method was different in each location and the new method was frequently more sensitive than the old method.

In the case of CMV testing, where no confirmatory test exists -- and the reason I mention CMV is because we do perform it on the same instrument that we do our blood grouping -- one manufacturer's package insert recommends the resolution of discordant results using a 3-out-of-4 algorithm.

Unfortunately, one of the 4 tests is a very subjective manual latex card test. The manufacturer had to have two techs read the card test and agree on the results to generate the data in a package insert.

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We could not perform the latex card testing with two techs to resolve discordant results because this is not in accordance with the procedures that are used in our operating environment.

Another area of concern that we have relates to performance claims in reagent package inserts. We talked about this a little bit earlier. We have tried in the past to use the performance claims in the package inserts of reagents that we use as an indication of the performance that we could expect in our operations.

We believe that the performance of reagents in a validation should match package insert claims, but often they do not because in many instances, package inserts were generated in environments and with systems very different from the ones that we are now using.

Without applicable performance data, it is difficult to prove in a user validation that the performance is as intended by the manufacturer. We believe that sensitivity and specificity claims should be generated by the vendor for the system that is being marketed and implemented.

These claims should be based on an adequate number of samples and the claim data should include standard deviations. Without standard deviation information, the

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claims become a minimum instead of one point in a range and the expected result for our validation is difficult to determine.

A final comment about validation issues listed on this slide is that it is imperative that the manufacturers tell us about changes in the manufacturing process of reagents before we receive the reagents for use.

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From our experience as a multi-site blood banking and testing organization, the American Red Cross has recognized the need for a validation approach designed specifically for standardized multi-site organizations.

Because our 8 National Testing Labs have standardized instruments, software, testing SOPs, and training, we employ a test bed approach to many validations. In the test bed approach, the functionality of the new or changed system is validated in one of our National Testing Labs, rather than in all 8 labs.

The validation is executed, discrepancies are resolved, and any required changes are requested. If the changes are long term, a work-around is developed and is incorporated into our SOPs.

In the next phase of validation, at least for major changes, a system test is written and executed. Once

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again, the system test is performed in only one of our laboratories. The system test validates the complete process, including the assay, the instrument or instruments, the software, the firmware, the Red Cross SOPs and training, and the vendor SOPs and training, in the Red Cross operating environment.

Most of the parallel testing takes place in the system test. The final phase of validation is the User Acceptance testing. User Acceptance testing is performed at each of the National Testing Labs.

Because of the extensive testing done to validate the process in the test bed site, the User Acceptance only needs to validate those functions that may be particular to each installation or each site.

A limited number of samples are tested as part of the User Acceptance. This number is determined after analysis of results of the first validation and the system test. We want the FDA to be aware of the test bed strategy for a multi-site standardized system because this approach allows us to implement new technology sooner than we could if we had to perform the same amount of validation at every site.

As my time is up, I want to thank you again for this opportunity.

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[Applause.]

Summary

MR. WILSON: I will try to make my summary brief.

We heard about dedicated instrumentation and what are the attributes of what it takes to develop and to validate such equipment and how it has changed over the years.

Secondly, we heard about home-grown systems where individual components are wedded together at the test site and the elements of concern relative to validating performance.

I guess in closing we had a user approach where an array of concerns relative to large-scale testing and validation has created I guess some unique problems although from what I can see, they may not be unique to large systems. Some of these problems apply to small systems also.

With that, I guess one of the questions that I could try to answer from the last -- let me make a comment first about the dedicated systems. It is important to bear in mind that many of these instruments that are wedded together may never have been intended to be wedded together, and it surely would be the site's responsibility to ensure that whatever answer you are trying to get out of these

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wedded-together, general-purpose instruments, that all the parameters of validation are addressed in the validation plan, and it is all put together and executed before you put such a product on line.

I want to also say that it is FDA's current thinking that any software package which is sold to wed independent instruments together constitutes a new instrument system, and that the manufacturer of such software really is the manufacturer of the entire system. They just simply employ components as being assembled basically by the software. I just wanted to articulate that fine difference

Relative to some of the remarks that were made in the last presentation, one thing that might help is that FDA, via CDRH, has recently issued a guideline for the validation of instruments with what they call "embedded software."

That is a draft guideline that was issued I guess about three months ago for comment, and the objective of that would be to evaluate nonspecific -- I don't want to say nonspecific, that sounds bad -- but it is a general guideline for any type of instrumentation that has embedded software not specific to blood grouping or viral marker testing, et cetera.

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So, we would view that that is where we would be heading relative to the instrumentation. You want to also bear in mind, just as we have issued our guidance out of CBER for blood establishment computer software systems, that while the CDRH guidance is general in a sense, not specific to any given instrument, what makes the regulation of software and software-driven products through CBER different is that we have two sets of regulations to deal with, not one like CDRH has.

CDRH does not have specific regulations related to their products. They are all general with the exception, I think, of contact lenses or some such. CBER's regulations are entirely different. We have requirements for blood processing, and our position is that if you have an instrument that is taking the place of a manual process, then, that system needs to be validated, not only to ensure that it meets what the FDA, through CDRH, has defined as what is expected for a validation of a computer system, but also because of the regulations required by blood need to be validated to ensure that it is not missing those elements required in the blood regs.

I want to also say that CBER does not expect that manufacturers build Cadillacs all the time, it is okay to build a Volkswagen, the point being that you don't have to

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acquire a system that does everything that is required by the blood regs.

What we are saying is that the elements that are naturally expected to be performed based on the blood regs, you would have to put in your labeling that, in fact, this doesn't do this, and you would have to provide a work-around.

But the point is that CBER does not require absolute full-blown systems. What we are saying is that those areas, which would normally be expected. For example, if you are talking about a system where -- I am talking about an extreme example, which will probably never occur -- you are going to use a system to do ABO blood typing, and it doesn't do B or it doesn't do AB, it does A and B, but it doesn't do AB, so you have got put in the label this doesn't do AB, but we would let you use it. We don't know why you would want to use something like that, but the point is that that would be part of the blood regulation integration.

I wanted to say those points upfront, a little bit of caveats, because we don't want to come across us dictating design of these products.

With that kind of a preface, I would be happy to open it up to the panel or the floor for any questions. This is a very, very difficult area to deal with because of

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instrumentation, which is not manufactured oftentimes by the reagent manufacturers. This question is about validation and how deep does one go if the instrument is being changed, or the software is being changed from when the manufacturer supplied the instrument, you know, what are the issues involved with that.

Maybe, Patti, could you maybe raise some of your questions again? We will try to deal with them, at least get it started?

Open Discussion and Proposals

MS. ROSSMAN: Let me look at my slides and see what my issues were. I think a lot of it does have to do with us being able, as the end user, to receive documentation from the vendor.

We are required by the regulations to have in our possession documentation that such and such has been performed, the delta testing, alpha testing, regression testing, boundary testing. If we can't get that information from the manufacturer, then, it has been our view, and I think Len just validated this, that we have to do it ourselves.

So, I guess one of my issues is, is there a way that we can facilitate getting this information in the future, or making it not required?

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MR. WILSON: I am going to try to keep this simple. Is this a system that has not been changed from when it was purchased from the supplier?

MS. ROSSMAN: That is correct.

MR. WILSON: Well, has this product been 510(k)'d?

MS. ROSSMAN: Correct.

MR. WILSON: Under the 510(k) clearance system -- this would be CDRH or is this from CBER?

MS. ROSSMAN: CBER.

MR. WILSON: Then -- I am choking here. The reason why I am choking is because there isn't any element of validation or software with instrumentation that is easy. It is not easy for FDA either, so there I have said it, but I don't want a misinterpretation.

Part of the problem is that when we get certain discrete -- well, how about this, is this okay, and you are seeing an FDA person choke on a microphone right in front of you now, because what we have difficulty with is, well, is that all there is, is this really what you are talking about? If you notice, I said were there any changes to the system, because sometimes changes to the system can have a distinct different impact.

One of the places where have been coming from is that -- and I think you will find it in one of the guidance

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documents -- maybe, Molly, you could help me out with the title of it -- that was recently issued by CDRH, where there is a recognition that users of instruments which are purchased, that have embedded software, are a distinct disadvantage. They are not going to be able to validate it to the level at which the manufacturer validated it.

How do you know how to read the code or, you know, all the different parameter testing? I think where we are coming from is that the first place to start is what is the intended use and how do you validate the intended use.

The idea is that if it is ABO blood typing, then, you are talking about validating it from the point of view of the process validation guideline, IQOQPQ.

Now, if there are problems with -- like I said, if there is changes, if the site decides to make changes in the instrument or the software, all bets are off, because then the validation, which has been performed to obtain a 510 clearance is no longer valid, and there is no such thing as a partial validation. It is either validated or it is not.

So, maybe, Molly, can you answer that? Is there something that I have strayed or missed on?

MS. RAY: Maybe just a couple additional points that might help to clarify it a little. There are several documents that are out that might be useful. Number one,

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there is a draft document out for comment. It is called "Off-the-Shelf Software."

If you incorporate any off-the-shelf software into your own computer system, it will help you to identify what testing you would need to do, and it would be based on the intended use and how you incorporate that software into your computer system.

There is another document out. It is another draft document on the general principles of software validation. That would apply to any validation of any software. The information as far as what you are asking for, source code, detailed design specifications.

A lot of companies view that as proprietary information, and will not share that with you, but as part of your contractual agreement, or as part of qualifying your supplier, you can identify in there what your needs are insofar as that if that vendor switches operating systems, switches hardware requirements, et cetera, that they notify you of how it may impact the functioning of your system, so that you can then validate those changes.

A lot of those documents are available. If you go to the CDRH home page. CGMPhome is the address, and a lot of the documents are there, as well as new items on CDRH, so hopefully, those will help answer some of those questions.

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MS. ROSSMAN: As I understand it, and what we generally go by is that if we can receive written documentation of the required testing from the vendor, then, that's fine, but if we can't, doesn't the responsibility then fall on us to generate that ourselves?

MR. WILSON: Which validation portion are you talking about, are you talking about validation of the software itself or are you talking about validation -- again, what I tried to say was that from the user perspective, it's IQOQPQ.

The instrument manufacturer may have a validation program for performance that they may elect to share with you, you know, you could maybe take a look at this kind of thing, but installation qualification, operational qualification, and performance qualification are the responsibility of the site that is using the instrument - did you install it correctly, do all the lights and buzzers work, does it give me my right answer. I mean that is a very, very narrow view, you know, limited view of it, but that is where I think we are headed.

MS. RAY: Additionally, the software manufacturer, they are responsible for doing their unit and integration level testing, the verification, so if they are the ones

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that possess the source code, they have to do the code walkthroughs, the code reviews.

They have to do the unit level testing structurally for their branch and all their logic testing. If you do not have access to that, and you have purchased that system, what you are responsible to do as the user is site validation and that system level testing for your intended use, and there is a document that was put out by the Office of Compliance that addresses that.

MR. WILSON: Just be careful about definitions. What some people call system level testing is different from what others -- you know, so just watch it in terms of the definitions. That can be very, very confusing to even seasoned individuals in the systems.

We had a really tough time with that. All the manufacturers would send software packages in to us, and they would have their own definitions, so we had to work that one through first. They, oh, oh, you really do have it, you just didn't call it that, what we thought it was, or what was in the FDA glossary, which is what we thought was going to be the common denominator, but, you know, we don't dictate how you develop your products, we just evaluate how to clear them.

That's it?

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MS. ROSSMAN: Can we talk about the parallel testing when there is no confirmatory testing or no gold standard? We have had really a lot of problems with that one.

MR. WILSON: If I understand correctly, well, first of all, there should be a validation plan in any good experiment to resolve discordancies before you start your process. The notion that you are going to get perfectly concordant answers in any type of an experiment is not realistic, and you really need to evaluate how you are going to resolve these things, and it depends on the marker that you are testing for.

If you have ABO discordancies, that would likely be handled largely differently from syphilis discordancies because of the nature of the test, the type of marker, the clinical significance, et cetera, et cetera.

As was stated at one of the last BPACs, in IVD type of testing like this, there are no real gold standards, there is only gold-plated standards, and I can tell you from the point of view of the blood-borne pathogen testing, what we typically tell manufacturers to do is that when you get a discordancy, you throw the book out, you do everything you can to resolve that, go all the way through to PCR, but I don't what specific concerns you had.

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MS. ROSSMAN: In this case, we were talking specifically about CMV, although we have had this issue with other testing, but I guess my question to you is the Red Cross is one of those organizations that takes a very literal approach now to package inserts.

We have been cited many times across the country for not doing so, and especially since we are under a consent decree, we try to go by the letter of the law, and we are very careful about that, but we also want to be reasonable.

In this case, the vendor or the manufacturer of the reagent described how they validated the CMV reagent, and we felt obligated to follow their method of validation, their algorithm, and obtain their data.

Is that reasonable or that overkill?

MR. WILSON: In any IVD, the performance claims in the package insert, analytical sensitivity, analytical specificity, clinical sensitivity, clinical specificity, or reproducibility are expected to be able to be met by the users. That is the basis for the approval of a test, and that is just simply truth in labeling, so within reason, you ought to be able to achieve those types of results.

Now in terms of a CMV, one of the concerns that you would want to have is that since CMV detection rates

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vary drastically throughout subpopulations in the United States, you could have various prevalence, but you ought to have the same, what we call, "relative" sensitivity, and in those particular instances, the clearance of those 510(k)'s were based on consensus results. That is 2 out of 3 or 3 out of 4, and I thought that the test kit package inserts articulated where the discordancies were, and there was a sensitivity or specificity calculation that was based on that, and my view would be that if a site were able to run a statistically valid sample, and achieve statistical equivalence, then, that would constitute adequate validation for that performance characteristic.

Do you want me to say that again? Okay. I was trying to generalize. We don't think that user have to rebuild test kit performance criteria. What we want to know is that the users are able to use the test kits and achieve the same performance characteristics as the manufacturer claims, notwithstanding differences with populations. I mean you pick one that happens to be quite variable.

But if you had a number of discordants in there, and it was not different basically from the number of discordants that is in the test kit package insert -- by the way, there is also a document called, "A Summary of Safety and Effectiveness," which is also available for 510(k)'s, or

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you could request a 510(k) redacted through FOI, and you can look at some of the information that is in there, that would be considered public knowledge. That might help a little bit.

MS. ZERGER: Can I just make a comment here? I think especially with CMV, I think some of the terms that you have used up there, statistical equivalence, does that mean if you state a performance claim of 95.6 sensitivity, and somebody gets 95.5, that they have failed, because that is the way customers are interpreting those, and I think that that is a little unfair because the manufacturers, 510(k) at this point could be nine years old.

The assay that you compare to may not even be on the market any longer or there is new assays that you are being compared to, that were not available at the time of your 510(k) submission.

I have a little bit of trouble saying, okay, you know, we say in one comparison study or whatever that the sensitivity was, you know, XYZ, and they are a tenth of a point below that, they have failed.

MR. WILSON: That is why I said statistically, and a manufacturer's sensitivity in a package insert has to be met, has to be reasonably capable of being met, or the test

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kit gets recalled because it is not meeting its labeling claims. That is not at issue here.

The question is whether or not they can assure themselves that when they run this test kit, they are getting more or less what the manufacturer intended the test kit to be able -- that level at which it is able to be performed.

Now, we are not in a position right now to discuss statistical power, et cetera, but the bottom line is that if the sensitivity of the assay is 99.5 percent, running three specimens that are positive won't necessarily tell you that you are able to get the same level of performance, you know, all things considered, you are running it correctly, the equipment is set correctly, I mean you are talking about just isolating it to the test kit, not the other several items that could possibly go wrong.

Now, do manufacturers need to reproduce the clinical studies for validation? No, I don't think so. I don't think it is that high, but I think it is fairly obvious that most people would say running three specimens is probably absurd.

When you have a marker like CMV, and half or 75 or 95 percent of your specimens are positive, then, you could

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run at least several hundred up to 1,000 specimens, and you also want to validate for your intended use.

If you have got a big system that you are running large numbers of samples in, you really probably want to upgrade your validation proportional to that level of samples, because you are also looking at many things, can you run this instrument through several shifts with turning over -- I mean the whole picture of validation, just getting that performance characteristic met with a reasonable statistical power, you know, maybe 80 percent power or something like that, that might be sufficient.

I am being hesitant about coming down with a concrete number because there is no clean way to come out with a concrete number here. We have wrestled with this very, very meaningfully, and it is very, very difficult within FDA to come up with, well, here just do this, and, you know, everything is fine.

Well, it changes with every site. We talked about a test bed being representative for the whole system. That assumes that everybody is going to do everything exactly the same way in every one of the satellite sites, I mean that is a pretty big assumption, and is the computer system going to be the same in every one of those sites. Oh, we were running Windows 3.1 here and Windows 95 in the other,

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because we didn't -- well, it is not exactly the same. I am using these as basic examples.

You also need to bear in mind that any of these issues -- because I can assure you they are complex -- you know, give us a call, send us a fax, we will do our best to try to articulate where we think the best cut is.

We probably won't be able to resolve all of the issues here, but at least we are trying.

MS. ZERGER: Mr. Wilson, I have a question for you. How would you suggest that we advise a customer, let's say, that wants to validate our CMV assay on our analyzer.

Let's say that someone is running their current test of record on CMV is a method to which we have not compared ourselves to for the purposes of our 510(k) submission. When we did our 510(k) submission, we obviously did not do clinical trials in parallel to everything out there. Okay?

So, they are using a test method or a manufacturer to which we have not compared ourselves. How would you suggest that we advise them on this issue of sensitivity and specificity, then, because there will not be data in our package insert that shows relative sensitivity and specificity to that particular manufacturer or method?

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MR. WILSON: I will not be able to answer in any measure of detail here because it will vary from test kit to test kit. What I can tell you is that if you look at the transcript of the June BPAC, I believe it was, where I had to talk about CMV serological testing relative to leukoreduction filters, I believe I said -- but please check the transcript -- that none of the test kits has relative sensitivity less than, I think it was 98 percent or whatever the number was. I think that is a place to start.

Why does one conduct site validation for a CMV kit or any other test kit? You can run it correctly and, presumably the manufacturer's test kits -- the test has not deteriorated so it performs to its labeling claims. So if you can somehow get it to performance to its labeling claims with some measure of confidence, then you are mostly there.

The controls need to be performing appropriately, or calibrators. You need to make sure that your people are trained properly. It is more than just simply looking at the sensitivity and specificity number, but those numbers need to be addressed.

A reasonable statistical approach is probably going to be acceptable. We are not looking for, in most instances -- I have to wiggle a little bit -- in most instances at statistical equivalence which means that you

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would have to determine what statistical difference you are willing to accept and then do a sample number which is capable of giving you 80 percent power with a 0.05 alpha and things like that.

I don't think we are looking at that. What we are looking at is a reasonable assurance that the sites know what they are doing and they have done a good job of integrating the test.

MS. WEILAND: I just wanted to comment that one of the things that I think centers have fallen into is when they look at the '92 guidelines and they see a minimum of 500 samples tested across three days, they forget that part of that stricture is under representative conditions for their operating systems.

One of the problems in startup that I have observed is that while they test those samples, they test them in their off hours or they work them into the workflow. Yet their workflow is 1,000 to 1,500 samples a day. They run into problems when they then begin to implement because they have not validated, really, under representative conditions as to how the instrument is going to be running.

MR. WILSON: The process validation guideline even articulates that it should be sensitive to personnel, abilities, fluctuations, et cetera. You really want to try

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to evaluate it in a worst-case scenario, your operating worst-case scenario.

Any further questions? We have run a little bit over but I think we could take one more if someone has one.

I would like to thank all of you on behalf of FDA, Sheryl, Helen, myself, Molly Ray. Joe Wilczek has done a great job putting together these facilities for us. Again, we would like to thank all of our speakers and we hope that this was productive.

I think FDA learned a lot. We know where many of the concerns are. We know where many of the challenges are. There are a lot of elements that we have listened to today that are not going to be easy to resolve.

As I have said on a number of occasions, we may not be able to resolve all of these in one fell swoop, but I think a concrete step forward with industry can be considered to be a productive effort. Any guidance documents that we will developing you will have an opportunity to comment on based on our good guidance document rule. We would welcome comments.

Thank you very much.

[Whereupon, at 5:24 p.m., the proceedings were adjourned.]

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