U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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WORKSHOP ON STANDARDS FOR INACTIVATION AND CLEARANCE OF INFECTIOUS AGENTS IN THE MANUFACTURE OF PLASMA DERIVATIVES FROM NON-HUMAN SOURCE MATERIALS FOR HUMAN INJECTABLE USE

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MONDAY
OCTOBER 25, 1999

The workshop took place in the Masur Auditorium, National Institutes of Health, Bethesda, Maryland at 8:00 a.m., Mark D. Heintzelman, Ph.D., Chair, presiding.

Present:

MARK D. HEINTZELMAN, Ph.D., Chair
JESSE GOODMAN, M.D., Speaker
JOHN S. FINLAYSON, Ph.D., Speaker
DR. PETER NEUMANN, Speaker
DR. HANNELORE WILLKOMMEN
PHILIP SNOY, DVM
THOMAS J. LYNCH, J.D., Ph.D.
KEITH HOOTS, M.D.
MR. JASON BABLAK
BARBEE WHITAKER, Ph.D.

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(8:03 a.m.)

DR. HEINTZELMAN: Good morning. It's Monday morning. It's time to get going. I'd like to welcome everybody here. My name is Mark Heintzelman. I'm the chairperson for the workshop. I'll be introducing Dr. Jesse Goodman who is going to give the introduction and welcome.

We have just a very few administrative issues to discuss. I want to let you know that there is a cafeteria here. Getting there is not too hard. All you've got to do is follow the arrows and it's downstairs. Quite easy to do. I don't think they have an Dr. Atkins line, so for those of you who are pursuing such an endeavor you'll be on your own.

We got funded for this week and that's always a nice thing. President Clinton signed a continuing resolution which I think expires on Friday. So it's very happy our workshop is this week. He's making noise about not doing this again and trying to not put gas in the car, but having the car ready to go and don't start it is a real challenge and it would have destroyed our plans.

Our first speaker is Dr. Jesse Goodman. He is our Deputy Director for Medical Affairs at CBER. He's going to give you an introduction and

welcome and we'll begin our workshop on Standards for Inactivation and Clearance of Infectious Agents in the Manufacture of Plasma Derivatives From Non-human Source Materials for Human Injectable Use.

Dr. Goodman?

DR. GOODMAN: Well, good morning to you hardy souls. Since I've been saying to my children for the last two hours in various stages of trying to get them to school, one missed the bus, one was still asleep when I left home, so -- I think you have a small group here, but I think in many ways that should encourage you to speak up, have a real interchange here on this subject. But I guess I'd like to start out by welcoming you to this workshop on the Inactivation and Clearance of Infectious Agents from Plasma Derivatives From Non-human Sources for Use in Humans. My background is both as an infectious disease person and a hematologist, so I'm quite familiar at least some of these products and their importance.

These are, as you know, very unusual and special products which meet special needs and they range -- they're often lifesaving products that range from antivenoms to factors for people who have multiple antibodies and as such, although at the present time they tend to have small constituencies

and small amounts of use, they're critically important and lifesaving.

And as was pointed out to me, and Mark asked me to say hi here, unlike the situation with the human plasma industry and plasma derivatives, there really is no sort of safety net or set of universally adopted safety standards for this product. So that's what you're being asked to consider.

Now why in the world would one take this issue on now? And I think there are several points that I want to make about that. One is there's an expanding catalog of infectious agents of animal source which potentially contaminate products in humans. And of course, the parvovirus is an example that you're probably familiar with.

There's definitely an increasing awareness of the ability of pathogens to cross species and my area of research interest is in tick borne infections and we've worked on avian leukosis and babesiosis and both of these are obviously common infections of exactly some of the kinds of animals that the products you're interested in are made from and then used in humans. So there is an awareness of this transfer of pathogens.

I think perhaps even more important is

the realization that there are contaminants in not just animal, but human biologic materials which don't cause acute and obvious disease, so you -- we tend to think we have quite a good warning system because if something is wrong we will know about it. But as the situation with retroviruses indicates, there can be real problems in source materials that may have an outcome that is only apparent many years later and may not necessarily be easy to tie to the source material.

And then finally, I understand that this -- one of the oldest areas of sort of the plasma industry here of preparation of materials from animal plasmas also may have some room for expansion in the current biotechnological era in terms of things like development of transgenic plasmas, possibilities of making new immunoglobulins that will be used in human therapy.

So I think you'll hear an overview of these issues today and the question will be what can be done. I think first of all, the reason you're here is because we're all increasing our understanding of both the sources and the nature of these kinds of pathogens that may be in these materials and that has to increase. There's clearly a scientific need here. Again, there hasn't always

been -- xenotransplantation has helped stimulate interest in these animal pathogens which may be less obvious causes of disease and we need to begin to apply modern molecular methodologies to search for pathogens that might be important.

I think one of the things that you'll want to discuss is the parallels to human plasma and the potential for incorporating pathogen inactivation steps into the routine management of these materials. Can that be done without sacrificing biologic activity? Can that be done economically? Is that something that is necessarily uniform across different products or will it most likely differ for different products?

And this should be able not only to inactivate known pathogens because, as far as I'm aware there haven't been major crises in this area that you're here to consider today. It's not just the known pathogens you want to deal with. It's the unknown pathogens. It's affording some margins of error and again, this is where there is another parallel to xenotransplantation.

So just thanks to all of you for coming and considering this issue and I hope you'll discuss it carefully and the pros and cons of the various kinds of steps that you can take and begin to move

this field forward and I'd just like to thank Mark and the Office of Blood for inviting me to say hi and say that I would like to stay and listen, but I've got to run out and go talk about antibiotic resistance with the folks at CDER, so thanks very much and have a good day.

DR. FINLAYSON: Good morning again. As I look out here I'm afraid that the echo coming back may do away with what little hearing I have left. Nonetheless, I'm John Finlayson. I'm the Associate Director for Science of the Office of Blood Research and Review at CBER and I trust all of you are sufficiently familiar with us that we can use these three and four letter codes to represent our agencies.

Could I have the first overhead? Oh, I have the first overhead. All right. The first line there is an abstract of the title of this workshop which surely must deserve some sort of a prize for lengths of titles for workshops, but the point is I'm going to talk about plasma derivatives and try to tie this to our interest in plasma derivatives from non-human sources. I will attempt to give a historical overview, but as you will see from the next slide which I don't want just yet, the perspective that I'm going to take is not that of someone who has spent a

great deal of time with plasma derivatives from non-human sources. As a matter of fact, I suspect that my major qualification for speaking to you at the beginning of the program this morning is simply that I was the most historical person that Dr. Heintzelman found as he was wandering the halls of Building 29.

Nonetheless, I'm going to try and provide a historical overview and if I can have the next overhead. Could I have the next overhead, please? What I'm going to try and describe are as Dr. Goodman referred to, lessons learned from plasma derivatives from human source materials. Now throughout the day we're going to be talking about plasma derivatives because that's the term that we have become accustomed to, but I hope everyone is aware that the same considerations would apply if we were talking about material made from serum or whole blood or blood cells rather than plasma per se. So regard the term plasma as partially precise and partially shorthand.

However, in talking about plasma derivatives from human source materials in an attempt to give a historical overview, it is also entirely appropriate to consider the history with respect to animal plasma derivatives and there are several

reasons for this. The very earliest plasma derivatives that we had were from animal sources. If I could have the next overhead?

Already in 1890, Behring and Kitasato described antitoxins made from animal blood, animal plasma, animal serum, mostly, but not exclusively equine in origin. And these antitoxins have been with us ever since. Furthermore, not only the first plasma derivatives, but the very first biological reference standard in the world was in animal preparation. If I could have the next overhead?

Paul Ehrlich in 1897 was faced with the problem of standardizing the potency measurement of, I'll say this term in German, diphtheria Heilserums, literally healing sera. Or as we said a little later, therapeutic sera. We are fortunate to have a representative from the Paul Ehrlich Institut with us today and she'll be speaking a little later on the program.

Faced with the necessity for doing these potency measurements and for standardizing the measurement process, what Ehrlich decided to do was to choose one antitoxin as the reference preparation, determine its ability to neutralize toxin and then report the potency of the other antisera in terms of comparison with this reference standard.

Now closely allied to this procedure and closely allied to the two facts that I've said, namely that the first plasma derivatives were of animal origin and the first reference standard was of animal origin is the alliance to the legislative authority for the Center for Biologics Evaluation and Research. That is to say, CBER. These antitoxins or antisera were, when they came in use, prepared locality. In other words, if they were needed in the New York City area, they were prepared in New York. If they were needed in the Washington, D.C. area, they were prepared in the Washington, D.C. area. And sometimes they worked and sometimes they didn't work.

Now in 1901 there was a serious outbreak of diphtheria in St. Louis and so immediately a program was initiated for administering diphtheria antitoxin, again, locally prepared in St. Louis, this program was begun. Tragically, in this immunization and of course it was passive immunization, ten children died not of diphtheria, but rather of tetanus. Why did this happen? This happened because the horse from which the antiserum was collected had tetanus and in the rush to immunize, collect the antiserum, immunize the human recipients, it was considered that there was not sufficient time to do safety testing.

Well, as a result of this tragedy, if I could have the next overhead, Congress passed the Biologics Control Act in 1902. This act is variously referred to as the Virus Toxin Law and the Vaccine Virus Toxin Law and other shorthand terminologies. The point is that it was the predecessor of our current day Public Health Service Act.

Now if you publish an act for the control of something, you have to give some group the authority for enforcing it. And Congress gave the authority for enforcing the Biologics Control Act to a division of the Hygienic Laboratory. By that time, 1902 the Hygienic Laboratory had moved from New York to Washington, D.C. It's worth noting that the Hygienic Laboratory was the predecessor of the National Institutes of Health and the particular division that was given authority for enforcing the Biologics Control Act was the predecessor of CBER.

Now among the classes of products mentioned in the act, you see, was therapeutic serum and was antitoxin. These animal antitoxins and analogous products still exist and are still with us. If I could have the next overhead. They have been joined by a number of other products from animal sources and I have listed here animal species from which we have currently licensed biological products

and I might add that others are under development even as we speak.

If I could have the next overhead which is something if you think you've seen it before it's an indication that you are awake and oriented and paying attention, just to remind us that we're back on the track of seeing what lessons have been learned from plasma derivatives from human source materials.

Now to glean these lessons, we need to fast forward from the time of Behring and Kitasato and Paul Ehrlich and the Biologics Control Act enactment to the time of World War II. In the 60 years between the onset of World War II and the present, we truly have learned a great deal about viral clearance. If I could have the next overhead.

Much of the recently obtained information has come from such procedures as cell culture of the virus in question when Dr. Willkommen from the Paul Ehrlich Institut gives her talk, she will refer to these as relevant viruses, for example, HIV. In other words, the actual virus that we are concerned with that is inhabiting the plasma that is the source for our plasma derivatives.

Another powerful technique in recently obtained information is the use of cell culture of model viruses, for example, BVDV, bovine viral

diarrhea virus has proved to be an extremely useful model virus for the hepatitis C. And if neither of these is appropriate, we now have available to us a nucleic acid testing where we can test for the genome or parts of the genome of the virus in which we are interested.

Now if I could have the next overhead, we can see by methods such as these, we can determine the quantitative reduction in the viral load. That is to say we can quantitate the viral clearance. We can get an idea of the reproduceability of that clearance by a particular manufacturing step or series of manufacturing steps or an overall manufacturing process and depending on the particular procedure that's being used to eliminate viruses, we may even be able to get information by using these approaches about the kinetics of the clearance.

However, I'm not going to talk about these things because Dr. Lynch is going to be talking about them this afternoon. So for now, let us, as a certain program back in the days of radio, if there's anyone in the audience old enough to remember the days of radio, used to say let us return to those thrilling days of yesteryear, specifically to the time of World War II and look at the next overhead.

Here's some facts about the manufacture

of human plasma derivatives in the 1940s which is essentially when the whole industry began. There were no viral screening tests available to use on the source plasma. That is to say on the plasma donors. You could look at the donor's eyeballs to see if they were bright yellow. You could ask the donor if he had ever had jaundice, if you were really a forward looking blood collection center, you might even do one of the indescribably nonspecific liver function tests, but there were no specific tests available to screen for viruses that might be in the donor's blood and therefore the donor's plasma.

Moreover, the manufacturing process itself for preparing human plasma derivatives was still evolving. Next overhead, please.

So how could you tell that the product was, from a viral point of view? Safe, or conversely, that it was unsafe? And how could you tell that the manufacturing process was or was not clearing virus? Well, I think I should digress for just a moment at this point because sometimes we become very taken with our modern status and self-importance to say that even back in the 1940s and 1950s people were aware of the procedures that we have available to us today. That is to say, to culture a virus and to harvest that virus, spike it

into the plasma and see where it went during the purification process.

There were only two major problems in the 1940s and early 1950s with this approach. And that is one, since virtually nothing was known about the biology of the viruses that were in human plasma and could infect potentially recipients of plasma derivatives, there was no way of knowing whether these viruses that could be cultured and harvested were or were not good models for the viruses that you were interested in. So the best that they could do was to use a variety of these viruses with different physical and biological characteristics.

The second problem was that when such procedures were carried out in the early 1940s with a fractionation procedure which was a distant precursor of the way that most human plasma derivatives are made today, what we found was that the viruses that were used as tracers showed up in all fractions harvested. So even though there may have been some quantitative reduction in the viral load, it forced people to use other procedures for determining whether the material was virally safe and whether the process being used for manufacture had cleared virus.

So if we take a look at the next overhead we'll see some of these other approaches. Well, one

of the useful, I would say, intermediate approaches has been the use of animal models. But you see I put there parenthetically, eventually, because in the 1940s and the early 1950s these animal models did not exist. These models which are primarily primate models began to evolve at the very end of the 1960s and continued to develop through the middle of the 1980s.

However, one approach that was available from the earliest time was the use of epidemiological studies. Sometimes these epidemiological studies consisted of following the patient populations, that is, the recipients of a particular plasma derivative simply to see whether there was disease development. On some occasions there was investigation of adverse events and these too provided useful information.

The last thing that you see on the list there is studies with human volunteers. I would like to spend a little time on this for several reasons. First, because these studies were done it the late 1940s and the early 1950s, and there has been such a long lapse of time between then and now, these studies are not well known to many of today's investigators. And the other reason is that these are studies that obviously could never be done again, so it is worth seeing what information was taken away

from them.

If we look at the next overhead, here is an experiment which studied the effect of ten hour heating on hepatitis. Now the hepatitis that people were talking about in these studies, these studies were reported in 1948 and done a number of years earlier, was that this hepatitis was a so-called homologous serum hepatitis which today we know to be hepatitis B. If you will look down in the footnote down here and let me see if I can make this work, you'll see "icterogenic" pooled plasma. In those days, pooled plasma was a licensed product and you have probably all seen the posters showing the wounded serviceman lying on the beachhead and the medic there with the inverted rifle with the bayonet stuck in the sand and he's infusing this reconstituted plasma as part of the casualty resuscitation procedure. Well, it was known that pooled plasma carried the risk of transmitting so-called homologous serum hepatitis and in some occasions there would be pools, lots of this plasma which seemed to be particularly capable of transmitting hepatitis and these were designated "icterogenic" pools. So in this particular experiment, 10 milliliters of an "icterogenic" pooled plasma was mixed with 40 mls of 25 percent human

albumin which was the only way that albumin was formulated in those days and 10 milliliters of this mixture was after the treatments, which I'll come to in just a minute, was injected into human volunteers. You see that 10 milliliters of such a mixture would be equivalent to 2 milliliters of the plasma and therefore, presumably would transmit, have the potential for transmitting the infectivity in that plasma, those 2 milliliters of plasma plus any infectivity that might be present in the albumin itself.

The first treatment that this underwent, Group A, was nothing, simply to make the mixture and put it in the refrigerator. The second was to heat the mixture for 10 hours at 60 degrees Celsius. Now anyone who has ever tried to heat human plasma or serum at 60 degrees Celsius knows that you start to coagulate it or turn it into gelatin very quickly. So being able to do an experiment like this was dependent on finding stabilizers that would allow albumin to be heated for 10 hours at 60 degrees Celsius and in fact, to a certain extent, if diluted properly would allow whole plasma to be heated. So heating, you see, for 10 hours at 60 degrees Celsius in the presence of stabilizers or for 10 hours at 64 degrees Celsius in the presence of a somewhat

different mix of stabilizers eliminated the transmission of hepatitis and thus seemed to have been a very effective method for clearing virus. Now I'm not going to elaborate on this because let's take a look at the next overhead because the obvious question was well, suppose you took the "icterogenic" pool of plasma and simply fractionated it to prepare albumin. What would be the infectivity of the resulting product? And as you can see from Group A here, this albumin again, prepared as a 25 percent solution, just like the clinical preparation, but undergoing no heating, did not transmit hepatitis.

Now recall that two or probably even one milliliter of the "icterogenic" plasma when injected into human volunteers would infect at least half of them with hepatitis. Here we're injecting three milliliters and we're injecting a 25 percent solution which depending on how you want to do the calculations, amounts to at least 18 milliliters of the starting plasma and there is no evidence of hepatitis. When that albumin was heated and the same dose was given by the same route, again, no hepatitis.

When a much larger dose was given, something that is like a clinical dose or maybe twice a clinical dose that might be given by the route that

the clinical dose would be administered to one icteric and one non-icteric case of hepatitis was found. On the other hand, when this albumin was heated, no hepatitis.

Now let's go back and take a look at this line here. One hundred milliliters of 25 percent albumin, again, depending on how you want to do the calculations amounts to at least 625 milliliters of the starting plasma. This is plasma of which one or two milliliters would be expected to infect half of the recipients. And so the message here is that simply the purification process to obtain the albumin in a purer form and albumin in those days was prepared to a purity of at least 97 percent, simply the purification procedure in the absence of the heating was capable of the great reduction in the viral burden. And seeing that there could be virus still remaining, this was eliminated by the heating procedure which is consistent with the information that we saw on the previous overhead.

Now I mention to you that the procedure for purification, that is the manufacturing process itself was still evolving at this time. The method for manufacturing this albumin was a fractionation procedure which was called Cohn Method 6. That group that worked out these procedures under the leadership

of Professor Edwin Cohn at Harvard Medical School continued to develop methods and finally, eventually got up to Method 12. In Method 12, one prepared, among other fractions, what was called SPPS, Stable Plasma Protein Solution, which was made up as a five percent protein solution and as you can see when this was administered, hepatitis indeed was transmitted. This was a less pure preparation of albumin. It was rich in albumin, but only about 69 percent of the total protein was albumin. Nonetheless, despite this impurity and the fact that it could transmit hepatitis when it was heated for 10 hours at 60 degrees, again, the hepatitis transmission did not occur.

Well, you can ask, is this a real result? In other words, I just got through telling you that there were no specific viral tests available in those days so how did people decide whether or not there really was transmission of hepatitis? Well, first thing one would look for was jaundice and obviously if there was jaundice, the chances were very, very high that hepatitis had been transmitted.

If there were not jaundice, one did all of the liver function tests that one could get one's hands on, looking for serum bilirubin, bromsufalein test, the thymol turbidity test and other tests that

were in the armamentarium of the investigative physicians at that time. However, in I think testimony to the vision and face of the investigator who led the carrying out of these studies, namely, Dr. Roderick Murray, he bled these recipients of these products serially, obtained the serum, froze an array and kept the records on the faith that some day there would be specific serological tests for homologous serum hepatitis. And indeed, when 15 or 20 years later Murray and a different co-worker thawed out these samples, coded them, tested them under code, to make a long story short, the recipients who were said to have had hepatitis had hepatitis B and those who were said not to have had hepatitis, didn't have hepatitis B.

All right, let us move from albumin and ask what about other plasma derivatives? Consider the product that today is called immune globulin. Its major constituent is what we call today IgG. In the 1940s and 1950s, there were no effective stabilizing conditions to permit the heating of IgG or immune globulin and therefore it wasn't heated. Furthermore, there were no other known effective viral clearance techniques and so the use, obviously, were not employed either. Nonetheless, as I indicated, the methods for purification, that is, the

actual manufacture of the product was still under development and so we can take a look at the next overhead to see a comparison here.

Here we have the infectivity of immune globulin made from, that is fractionated from a pool of "icterogenic" plasma. Here we have it fractionated by Method 6 of Cohn and Method 9 of Oncley which, in fact, is the way that most of the immune globulin for intramuscular administration is still made today. And we can see here that a 2 milliliter dose of 16 percent protein solution and I might add parenthetically that this is very much like what is used today, 16.5 plus or minus 1.5 percent protein is the concentration of immunoglobulin that is manufactured and used clinically today. When 2 milliliters of this was administered to 10 recipients, no hepatitis was found and again, these recipients were bled serially and their sera tested again 20 years later and the results confirmed. Sixteen percent solution, 2 milliliter dose amounts to at least 32 milliliters of plasma and recall that the starting plasma, 1 or 2 milliliters would be expected to infect about half of the recipients.

Now, let's go to this elegant method, Method 12. When I say elegant, that is not irony. From a physico chemical point of view this was a

truly elegant method. The only problem was when that immunoglobulin was injected, 5 out of 5 of the recipients got hepatitis. Now you may say well, yes, but it wasn't a fair trial because you really were studying the route of administration here. That very well may be, but despite that this experiment spelled the death knell of Method 12 for anything other than a laboratory method for purification of plasma proteins. But it is legitimate to ask was this result, namely no hepatitis from the immune globulin prepared from "icterogenic" plasma by the method that, as I say, is still used today was this a real result or was one simply lucky or was one simply skimming off somehow the tip of an iceberg?

So on the next overhead, we see some of the follow up of recipients of immune globulin. Here we have a study that was carried out and reported during World War II. Eight hundred sixty-nine recipients of immune globulin evidenced no jaundice. Admittedly, a crude measure, but better than nothing.

In 1952, remember, we were still a little time away from the development of polio vaccine, so the only medicament that was available for prophylaxis for poliomyelitis was so-called poliomyelitis immune globulin, a preparation of IgG

from people who had recovered from polio and 2,800 recipients of this prophylaxis were followed and again, no jaundice was seen.

Also, in 1952, we were fighting a war in Korea and so immunoglobulin was being given as prophylaxis for what was then called infectious hepatitis or as we call it today hepatitis A and so 1,977 recipients of this prophylaxis were followed and these were followed both by looking for evidence of jaundice and by liver function tests and again, no product related hepatitis was seen.

Now I would say that the take home message at this point is it seems that immune globulin, despite the fact that it undergoes no deliberate viral inactivation steps, seems to be safe, but the reason for the safety is not clear. Now an incident that took place in the 1970s which in the interest of time I will not describe, this incident and the follow-up thereof suggested that the presence of some antibody, that is to say, antibody to the hepatitis B surface antigen or anti HBS, some antibody in the product itself was important for neutralizing any hepatitis B virus that might have escaped detection and might have found its way all the way through the fractionation process.

Furthermore, in the 1980s and the 1990s,

there were numerous occasions to perform very intensive follow up of immune globulin recipients both with respect to transmission of hepatitis and with respect to transmission of HIV which had reared its ugly head by that time. Some of these follow ups took place in the context of clinical trials. Some of them took place in the wake of reports of adverse events. Some of them took place in the wake of rumors. For example, in the 1980s, word got out that one recipient of RHOD immune globulin, RhoGAM and as you are aware RhoGAM is a trade name and I am using it advisedly here, that one recipient of RhoGAM had developed HIV infection. You can imagine that this lit up the switchboard both at the Ortho Corporation and at the FDA. And so an immediate intensive follow up took place involving both of those organizations and the CDC. It proved that eventually to have been strictly a rumor. The recipient had a number of other modes of becoming infected, but on this occasion there was very wide follow up recipients of not only this product, but other immune globulins.

Along in the early 1980s, we also had intravenous immune globulins developed and licensed and the recipients of these were followed in the context of clinical trials as well as post-marketing surveillance. There was no evidence ever of

transmission of HIV or hepatitis B virus. There were, however, some rare transmissions of hepatitis C virus including one set of episodes of transmission of hepatitis C virus by a U.S.-licensed immune globulin intravenous.

In view of this situation, FDA requested that all manufacturers of immune globulins, be they for intramuscular use or for intravenous use have validated viral clearance steps in their manufacturing process.

Now, to continue tracing the evolution of plasma derivatives we should ask what other major class of products evolved? And the answer is clotting factors. Now if we look at the early stages of plasma derivative development on the next overhead, we see that in their early stages of development albumin seemed to be safe from the viewpoint of transmission of viruses and we felt that we had a pretty good idea why this was so, that is, the purification process lowered the viral burden and the, by that time mandatory 10 hour, 60 degree Celsius heating was effective in inactivating viruses.

In the case of the immune globulins by contrast, they also seemed to be quite safe, but the reason was not clear. And again, I emphasize in the

early days there were no deliberate viral inactivation steps that were possible and therefore none was carried out.

When some decades later, clotting factors or sometimes they're called clotting factor concentrates became available it was known that these were risky products. In fact, they were called high risk products. Nonetheless, the benefit risk ratio was so high that it was deemed appropriate to use them. It was deemed appropriate by the FDA and the predecessor control organization. It was deemed appropriate by the manufacturers. It was deemed appropriate by the physicians and most importantly, it was deemed appropriate by the patients because these were truly life saving products.

Now I might say parenthetically at this point in the discussion, mainly because there's no other appropriate place to say it, some products that did not have such a high benefit risk ratio were simply taken off the market. For example, human thrombin was delicensed as a therapeutic product in the 1950s. It was shown that it transmitted hepatitis and there was an alternative product, namely bovine thrombin available. Human fibrinogen was taken off the market in the 1970s. It also was found to transmit hepatitis and as information

accumulated about its clinical use, it was found that its clinical benefit was very, very low.

Now, anti-hemophiliac factor was first licensed in 1966 and since then there have been numerous developments. Of course, the one that immediately leaps to mind is the tragic transmission of HIV to hemophiliacs who were receiving such preparations. But let us look at the next overhead and we'll see some of the progress in clotting factors since 1966.

First, there's been the introduction of specific screening tests for the plasma and for the donors. Now bear in mind that with the exception of the syphilis test, all tests for infectious diseases to which the plasma of plasma donors and blood of blood donors is subjected had been introduced since 1966 and 100 percent of the tests that we do for viral markers have been introduced since 1966, so all of this is within the time frame that is the history of clotting factors.

Second, there has been the introduction of deliberate viral inactivation steps. The first of these was introduced in 1983 and they became universal by 1985. You see below here, I have indicated discovery of methods for stabilization depending on the particular method that was used for

viral inactivation or viral clearance. Sometimes the introduction of a particular method was dependent on the discovery of a method for stabilizing the clotting factor so in fact it could be subjected to this procedure. Bear in mind that one of the major impediments to obtaining purified clotting factors in the first place was that compared with proteins such as albumin, they were much less stable, simply from a protein point of view.

And then finally we had over this time period since 1966 advanced purification procedures, procedures which were developed to obtain a purer protein, that is a higher specific activity, clotting factor, but which in fact, could be validated and very often shown to have a great deal of viral clearance capacity.

Now again, I am not going to discuss all of these items here because Dr. Lynch is going to talk about them this afternoon. I mean I certainly hope Dr. Lynch can live up to this advance billing that I'm giving him.

What I am going to do is to give a summary of some results of epidemiological follow up, much of which was, in fact, most of which was obtained in the setting of clinical trials of hemophiliacs who received antihemophilic factor and

if you look at the next overhead we can see that information that was gleaned over a number of years. Now, I should say that if we went back in time before that we would see that those earlier studies on human recipients were preceeded by studies with animal models and in fact, virtually of them were with the chimpanzee model. Nonetheless, because the denominators in those studies were considerably smaller than those that we have here, I think we can look directly at the results with human recipients.

Now also bear in mind that this all took place after 1985 and that means that the plasma, the donors of the plasma that was used to prepare those materials were being screened for markers of hepatitis B and HIV and furthermore all of these products were subjected to one or more deliberate viral clearance processes. After this point then screening for markers of hepatitis C came in as well. Products A, B, C and D are simply different U.S. licensed antihemophilic factor products. A prime is not a U.S. licensed product, but was made in manner similar to the method used to make product A and was licensed in a different country.

Suffice it to say without belaboring the denominators that you see that all of the numerators are zero. This is follow up of recipients of

antihemophilic factor, that is to say Factor VII concentrate. Factor IX safety data was mostly published later. These studies that I have selected here for reported in 1993 in a review by two employees of CBER, Drs. Bill Fricke and Dr. Mary Ann Lamb. But subsequently information on Factor IX concentrates became available as well with the same results so that we can say since 1987 there have been no, zero, transmissions of hepatitis B virus, hepatitis C virus or HIV by U.S. licensed clotting factors and there was only a brief episode in 1995 of the transmission of hepatitis A by clotting factor made by one firm.

So what do you say about these effective, I would even go so far as to say proven approaches to viral safety that have evolved in the decades since human plasma derivatives came into the picture?

Let's take a look at the last overhead and I think the message is that the combined use of screened plasma, that is to say screened plasma donors, validated purification steps and by that I mean not only validated from the manufacturing point of view, but purification steps to prepare a purer product also validated for their viral clearance capacity, validated deliberate viral clearance steps and certainly not to be forgotten adherence to

current good manufacturing practice. This combi approach has served us very well, so I think that the lessons are that not only has this combi approach served us well in the field of plasma derivatives, but to use a word that the computer people like very much, this approach seems to be exportable and in particular, it should be exportable in whole or in part to plasma derivatives made from non-human source materials.

Thank you.

(Applause.)

DR. HEINTZELMAN: My name is Mark Heintzelman. And I'll be speaking regarding the regulatory requirements for plasma derivatives. As soon as we can get the projector to come up. Our computers now are now very high tech and very safe and the one I have in particular has so many layers of passwords and security codes on it that if this takes more than three minutes this could take forever. So hopefully we'll be moving along quickly very soon.

I would like to thank Dr. Finlayson for that overview. I feel that he is eminently qualified to educate myself, in particular. He's been a mentor of mine since my career here at CBER and I always benefit greatly from listening to him.

His comment about the length of the title is very true and you have to remember that when you have a last name as long as Heintzelman, you tend to see length differently than many people and I happen to notice shortness and brevity much more readily.

Something to point out not generally noted is I've tried to avoid as much as possible the use of red and green in these slides for people who are red/green color blind. Projections like this can drive you crazy. I happen to know from personal experience. So they may lack luster, but I can read them for a change.

My name is Mark Heintzelman. I work with the Division of Blood Applications in the Office of Blood Research and Review, Center for Biologics. My talk is concerning the regulatory requirements for plasma derivatives in the United States.

Page down, please. The title is Standards for Inactivation and Clearance of Infectious Agents in the Manufacture of Plasma Derivatives from Non-Human Source Materials for Human Injectable Use. Long, but for a reason because there are a number of animal derived products that get manufactured into a variety of final applications and we wanted to try to make this so that when you read the title you would at least recognize that we're not

talking about in vitro diagnostics or a variety of other products.

Next slide. I will discuss the regulatory requirements for plasma derivatives that pertain to pathogen reduction and try and review them at all stages from pre-IND through post marketing because while there are a number of products that are licensed that are made from plasma derivatives, there are -- and we have many manufacturers who know the regulations, many manufacturers and consultants here, who know the regulations incredibly well. We are hoping to address some of these issues to people that were newcomers to the field also, so there may be a minor amount of review for those of you with a considerable amount of experience.

Which products? Well, specifically we're talking about plasma derivatives, regulated by the Center for Biologics Evaluation and Research within the Office of Blood Research and Review, not those regulated by the Office of Therapeutics and not those regulated by the Office of Vaccines. Though we may share the same concerns, we may in the long run end up in the same place for those products, but we're here to talk about blood and blood products.

Of course the issues that are pertinent are zoonosis and safety. When considering this

product line, it is important to compare the two steps in the manufacture of human plasma derivatives. Setting standards for pathogen reduction in animal derived products should be no less rigorous. I think Dr. Finlayson has done a wonderful job of showing how our base of information has come from human success stories in restricting and reducing viral and pathogen contamination.

Examples of infection that can be quickly recognized when sourced from human plasma or serum do to their rapid rate of infection are well known to many of us. For products manufactured from animal plasma or serum, the infection rate can be much more gradual as is suspected say in the course of BSE or for an opportunistic pathogen of animal origin in aggressive infection with high morbidity and mortality is also possible. So we see the gamut on both sides of its ability to demonstrate itself epidemiologically.

We're going to discuss now, and as I said I would review the regulations. I realize that reading the regulations can be the greatest cure for insomnia known to mankind and I will try to keep it from falling within that purview, but I will review the regulatory pathway to eventual licensure for these products, trying to point out at appropriate

intervals where these pathogen reduction and removal or inactivation schemes can be gleaned from the guidance and the documentation that we have.

First opportunity to discuss this issue is at a pre-IND meeting. Certainly a formal meeting, typically conducted with a sponsor prior to submission of the IND. Prior to filing an IND we encourage that you meet and discuss source materials and pathogen reduction concerns with CBER when you have a product that may have within it this liability. This is a great opportunity to lay the groundwork.

At this point in time a really good recommendation to a manufacturer is to ask them what is your intended use statement to be? If your intended use is clearly defined at the pre-IND stage, you will certainly find that is a much more direct path to the final testing of your hypothesis in accomplishing the Phase III pivotal trial, rather than deciding what your intended use statement will be after completion of the Phase III pivotal trial. So it's really a good first question to ask.

Of course we're now faced with changing technologies and changing technologies bring new species into production and new concerns and the discussion that we have today will be certainly based

upon the five or six species that Dr. Finlayson pointed out as being a manufacturing species for these products. I'll mention Dr. Snoy's talk in a while. He will cover these animal issues and requirements in detail.

As everyone knows the pathway to licensure should begin with pre-clinical data, Phase I, Phase II and Phase III testing within the IND. These regulations are found in Title 21 Code of Federal Regulations, Section 312.

Another good opportunity that presents itself as the IND progresses is at the pre-Phase III meeting. Typically, will have met with the sponsor prior to the filing of the IND. Generally, there are a number of conferences and calls, sometimes even meetings required during Phase I and II, but before you get into Phase III it's highly recommended that you meet and discuss with CBER in detail the plans to make sure that you have consensus as to where you're going. So at this opportunity is also a very good opportunity for discussion, to discuss and agree on the pivotal trial and the validation requirements for the product. These would include pathogen reduction and pathogen inactivation standards.

After having completed your Phase III, you'll be considering submitting your license

application and a pre-licensing meeting is essential. Here, we find final agreement for pathogen reduction can be identified, now that you're going to be scaling up and begin talking about providing final large volume of your product. Scale of manufacturing and appropriate validation requirements are identified. If you will be going from pilot to scale we have a number of guidance documents that concern themselves with those requirements, but there are instances where scale up does dramatically affect the production modality. And can require a new look at viral or pathogen reduction inactivation standards.

The licensing requirements, of course, are found in the Code of Regulations, Title 21, Section 314.

We'll find as we go through this talk and as John began to point out very concisely when he reviewed the Cohn and Oncley fractionation steps and methods that many manufacturing steps will have pathogen reduction capability. The value of those steps should be identified and quantified and not just looked at as serendipitous.

Additional specific steps may be required to be incorporated into the manufacturing process as you proceed to consider pathogen reduction and inactivation.

These typically are seen as steps such as solvent detergent treatment and heat inactivation. Dr. Lynch, who did make it will be here and discuss these steps in detail.

Now I'd like to begin with a very brief quick overview of some opportunities to discuss pathogen inactivation at the IND stage up through pre-license. Now the manufacturer has met and discussed in detail with CBER these requirements and we have some documentation that's available to you to help get through the filling out of the form 356H and to eventually obtain licensure.

A document that is very pertinent to this issue is our CMC guidance. This is the chemistry and manufacturing and controls and establishment description information for human plasma derived biological products, animal plasma or serum derived products which was issued and finalized in February of 1999. This document, we always have to say this, this document represents FDA's current thinking on the content and format of the chemistry and manufacturing controls and establishment description information for human plasma derived biological products, animal plasma or serum derived products. Current thinking is current thinking, subject to change and modification as technology and time

advances.

I'm going to review a number of areas within the document where are steps taken or steps are identified that can serve to address the issues of pathogen reduction and inactivation. First of all, we find in the general information section two definitions a statement about virus clearance. The number of principles may be used to demonstrated expected removal or inactivation of infectious virus. That's a very nice way of saying that CBER is open to technological advances. It recognizes that there are standards that are out there, such as solvent detergent and heat treatment, but new, novel creative methods that render a product safer without adulterating its activity are always being sought after and would readily be considered during manufacturing.

The manufacturing scheme may include steps which are intended to specifically address removal and steps which specifically address inactivation. This was the first time that I was able to encounter specific notification that we consider these issues to be separate and distinct even though they may result in the same end product where we are looking at removal and inactivation. Removal serendipitously may be through the

fractionation process and intentional steps added in for inactivation.

Under Part 1 of the CMC section within the introduction, going from the general information to the introduction, we find the starting materials for human plasma derived products are known to be capable of transmitting infectious disease and many of the infectious agents of primary concern have been identified. There's nothing surprising here.

It goes unsaid, but it's not included within the document that for animal plasma derived products a different set of agents is of concern, but no less concern than for human plasma.

Part 2 within the biological substance product component of the document, C, methods of manufacturing and packaging within the manufacturing methods. It says (1) starting materials. Materials used in the processing and collection of the biological substance should be fully described. Such a description could include any endogenous pathogens within the species that are being used for production.

1(a). For purchased raw materials, representative certificates of analysis from the supplier or the manufacturer's own acceptance testing results should be submitted. It's typically

interpreted in to mean that that would include identification of any potential pathogens.

(b). The tests and specifications for materials of animal source that may potentially be contaminated with adventitious agents, for example, bovine spongiform encephalopathy for fetal bovine serum and viruses and products of human and animal origin should be fully described. Here we find a direct notification that we would like to have information regarding any potentially contaminating viruses identified at this point. And it should not be just construed to be limited only to viruses. Any pathogens would be appropriate to identify.

Information or certification supporting the freedom of reagents from adventitious agents should be included in the submission. That goes unsaid. In-depth discussion regarding the quality of the animals used in production will be discussed by Snoy shortly. I will not pursue information at this time regarding the species and the pathogens of concern, but continue on with the regulatory pathway for these products and their relationship to the reduction standards that we will discuss, hopefully, when we get to the discussion panel, leaving the information for the specifics regarding animal serums and production with Dr. Snoy.

Under process controls within the CMC guidance document there's validation data should be provided for a number of processes.

A description of the validation studies which identify and establish acceptable limits for critical parameters to be used and in process controls, to assure the success of routine production. Reference can be made to flow charts and diagrams. Certainly critical areas to determine appropriate levels for would be in pathogen levels during the processing.

Validation studies for the purification process or a description of the validation of the purification process to demonstrate adequate removal of extraneous substances such as chemicals used in purification, column contaminants, endotoxin, antibiotics, residual plasma proteins, nonviable particulates and viruses should be provided. Yet another notification that we are looking for this information for these license applications.

Within microbiology is an unusual twist to this, but a description of the validation studies for any processes used for an activation of waste for release into the environment should be provided. If you're going to be releasing waste into the environment as a result of your manufacturing process

and that waste is contaminated with animal pathogens, that too should be identified and corrected. So it's a little bit out of the manufacturing stream within the final product, but still within the concept, overall, of pathogen reduction and removal.

Within specific analytical methods 1(b) is the statement lot release protocols including specification, ranges of representative lots of the product should be provided. Specifications may include, but are not limited to biochemical purity which may, for example, include PCR testing of the final product to look for pathogen DNA or RNA, safety, which I'll discuss later, but safety is clearly one of the regulations we have that directly addresses the issues associated with pathogen reduction; appearance, pH, residual moisture, excipients may or may not be, endotoxins and sterility.

Under (f), specifications, analytical methods, excipients; (b) refined for noncompendial excipients, tests and specifications should be described. For novel excipients, the preparation, characterization and controls should be described. As technology continues to move forward, novel, the statement here for novel excipients leaves wide open manufacturing techniques that will undoubtedly

include derivatives from animal, serum and plasma and the need again to consequently identify those pathogens that may be removed or inactivated throughout the process.

For inactive ingredients of human or animal origin, you need to provide certification or results of testing or other procedures demonstrating their freedom from adventitious agents. So direct correlate to these excipients and their possible contamination with adventitious agents.

An impurities profile needs to be provided. A discussion of the impurities profile with supporting analytical data should be provided. But certainly within an impurities profile for anyone whose product may contain zoonotic organisms we would want to see it addressed fully at this time. As you can see, we begin to build a huge foundation upon which these issues are addressed and found throughout the regulations.

It's an understatement to say, please be sure to consult the CBER listing of guidelines, policy statements and points to consider as you go through your license submission. Within the document, the CMC document for plasma derivatives, at the back is a complete listing of the guidelines, points to consider and policy statements that are

referenced throughout it. And there are a number of opportunities and many of these separate documents to find again specific references to pathogen reduction requirements found throughout each one of the individual steps. I didn't list them all because there's a huge number and they're constantly being updated. These are all available on the web.

Also, and within the CMC document, you'll find the international conference on harmonization guidelines mentioned for specific issues and those are the rules that we are following also.

Now we've, in a very cursory overview considered IND, the opportunities during the IND to discuss pathogen removal or inactivation, talked about important documentation that is requested throughout the licensure process. Let's look at licensure and post-marketing and those regulations to see where once again we find specific mentions of steps that would help to render these products safer.

Under 600.3 in the definition section, (p) the word safety means the relative freedom from harmful effect to the persons affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time. It's not a direct mention here of

pathogen reduction, but certainly coming down with hepatitis, HIV, West Nile Fever or virus infection or any of these other pathogens that are out there would be a direct step back to our regulations where we have very strong statutory authorization.

Again, in the definitions section, purity means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipients or deleterious to the product. Impurity here can be taken to mean that whether the animal pathogens that may be found in the products made from animal sera or plasma are infecting human beings and showing disease is not important. The fact that we can find them means that he product is not pure and the regulatory authorization is quite clear on that matter. So again, we find good statutory authorization for requiring removal of these products or products that don't contain them here in the CFR.

Under 610.13, purity, products shall be free of extraneous material, except that with is unavoidable in the manufacturing process described in the approved license. How you interpret unavoidable becomes a very big issue.

Now what I've done is I've gone through and I picked some of the additional standards for products that are licensed. You may have noticed

that our CFR is kind of on the Atkins Diet itself and has lost considerable weight in the last five to ten years and there are a number of products that are not found there any longer, but some of the regulations are still there and I looked through the CFR to try to find specific instances where even though this is for a human, where pathogen reduction and/or inactivation is mentioned so that it's clear that the stance that CBER takes is very much so directed towards that goal. And here for human albumin, albumin human, excuse me, under 630.80, under source material, the source material of albumin human shall be blood, plasma, serum or placentas from human donors determined at the time of donation to have been free from disease causing causative agents that are destroyed or removed by the processing method. So we can start with the material that may have some contamination with pathogen in it, but the regulation identifies that those need to be destroyed or removed during manufacturing.

Under 640.81, processing for albumin human, heat treatment is noted. As Dr. Finlayson pointed out with the original identification that when the value of heat treatment was first come upon, in the regs we find heat treatment, heating of the final containers of albumin human shall be in within

24 hours after completion of filling.

Heat treatment shall be conducted so that the solution is heated for not less than 10 or more than 11 hours at an attained temperature of 60 degrees centigrade. Heat treatment obviously as was seen in those earlier experiments is an effective method for reducing hepatitis within the recipients.

Under 640.90, plasma protein fraction human, we see similar information provided. Not too surprising. Source material. The source material of plasma protein fraction human shall be blood, plasma or serum from human donors determined at the time of donation to have been free from disease causative agents that are not destroyed or removed by the processing method as determined by a medical history of the donor and from such physical examination and clinical tests as may appear necessary for each donor at the time the blood was obtained. So specific mention again that your starting source material has to be well identified.

Again within the plasma protein fraction, (e), we find heat treatment. Heating of the final containers of plasma protein fraction human shall begin within 24 hours after completion of filling. Heat treatment shall be conducted so that the solution is heated for not less than 10 or more than

11 hours and at attained temperature of 60 degrees C.

The next product line that is included in this is 640.100, immunoglobulin human. Source material. The source of immunoglobulin human shall be blood, plasma or serum from human donors determined at the time of donation to have been free of causative agents of diseases that are not destroyed or removed by the processing methods as determined by the donor's history and from such physical examination and clinical tests as appear necessary for each donor at the time the blood was obtained. So this is an early recognition that the donor as the source for these products will always be of question and the manufacturing process needs to be stepped up to assure that the products come through safely.

Within manufacture, 640.102, manufacture of immune globulin human, sterilization and heating. The final product shall be sterilized promptly after solution. The statement, clearly such sterilization would be a good inactivation of any final contaminants that might be found.

So many manufacturing steps designed to provide a high level of protection to these products will help forestall a disaster. The threat of emerging infectious diseases requires a constant

watch for new risks which will pose new threats to products made from animal sources. We should not just assume that because we have such a tremendous safety level with the products in that there's been no real outbreaks of problems from animal-derived products as I mentioned here, that that's how the present and the future will continue to take us. Having a level of assurance that these products are treated effectively so that pathogen inactivation and pathogen reduction are identified and prevent any future catastrophes that may occur as a result of emerging infectious disease is critical for us to consider. It's the purpose of the workshop today.

I have a case study that I want to discuss in a moment that is just an overview of where we missed it with human and the threat of after having missed it with human and preventing that from occurring with animal is incredibly important. I believe that a proactive position is a far better one than a retrospective explanation. And in these days there is a lot of explaining that goes on at all levels. As a matter of fact, on several hills I can think of and we would like to very much consider that we can be more proactive in our requirements for safety for these products.

I have a very brief case study that I

wanted to point out regarding hepatitis C virus and contamination that occurred not too long ago in products of human source and final use. What I've done here is I've simply looked at CBER's position as events continue to unfold and discussed steps that CBER took in a regulatory fashion and left out many of the specifics regarding manufacturers and product lines because my talk is to consider the regulatory requirements for these products and I believe that this shows in a fairly straight forward example how we have gone forward and addressed issues when things have gone wrong and this is what we're trying to prevent.

On January 8, 1992, CBER wrote a letter, wrote to all U.S. licensed manufacturers of plasma derivatives in an effort to facilitate the implementation of new procedures for inactivation of infectious agents in plasma derivatives. These were, of course, from human source or whole blood and recovered plasma.

Subsequently, in January and February of 1992, CBER wrote to all manufacturers that were not licensed, but had pending license applications for plasma derivatives and those that had IND applications in as well with similar, within the same text.

On May 23, 1994, a letter was sent to all U.S. licensed manufacturers and all manufacturers with pending license applications for human immunoglobulin preparations. The letter acknowledged that various manufacturers of immunoglobulin for intravenous use -- oh boy, excuse me. The letter acknowledged that various manufacturers of immunoglobulins for intravenous use were at various stages of progress, i.e., some had introduced virus inactivation removal steps. Others had violated virus inactivation and removal steps.

Part of the manufacturing process in some of the clinical trials with products made by incorporating viral inactivation steps. CBER was not aware of the status of progress with regard to comparable work involving intramuscular immunoglobulin and specific immunoglobulins for intramuscular use. CBER requested that recipients of the letter reply with plans for progress in this area. Okay, that was an example of a proactive step taken by the Center.

On December 27, 1994, OBRR wrote to the appropriate license manufacturers informing them of OBRR's intent to begin HCV RNA testing in all human immunoglobulin products that had not undergone one or more validated viral inactivation/removal steps.

So you can see that there have been times where CBER has moved forward directly setting the level of safety at a technologically achievable levels through PCR testing to increase the safety profile of products. A well validated pathogen reduction scheme could have prevented the transmission of hepatitis C in these products and many other pathogens from plasma derivatives.

That's the extent of my discussion. Thank you.

(Applause.)

DR. NEUMANN: Good morning. I'm from the Bureau of Biologics and Radiopharmaceuticals for Health Canada, I guess we're considered the CBER equivalent. And if the first slide goes up, now this is in contravention to all the rules and regulations regarding what makes a good slide, but I'm not responsible for the title. I can blame that on Mark.

Furthermore, it's good to be speaking fairly early on because anything that I don't cover I can say will be covered by Tom Lynch later on in the afternoon or Dr. Willkommen and after my talk it's nice to have some backup.

I would like to say that I think you'll find actually a handout of my slides in your package. To keep people awake I think you'll find that was

the penultimate version and there's a few spelling mistakes and other changes that might have to be made that will be on the slides here.

What I've done is taken the -- I like the word current thinking of the Bureau of Biologics with respect to plasma-derived products and essentially drawn parallels to it for what our thinking would be on animal derived products.

Now on the draft paper, next slide, if you can read that, guidance in the the manufacture of plasma derived products, human plasma derived products and this is what essentially the bureau uses and as an internal guide to reviewers in order to insure consistency of applications in front of us from manufacturers of plasma derived products. In that guide, you can see on the next three slides covers the table of contents. Some of these will be covered in my subsequent slides and I think if you'll look at the next slide as well, these cover essentially, some of these, I must say were cribbed, not entirely but derived from some of the ICH guidance documents on federation of biotech products derived from cell lines. Some of them were CPMP guidelines. Some of them were EMA. Some of them were also the FDA guidance or industry documents so in typical Canadian fashion these tend to be a hybrid

of earlier regulatory guidance documents.

Now the next slide essentially describes what we're looking at today and this is -- you have a manufacturer here and this is an animal derived product, the sacrificial dog in this case and the manufacturer is, I think you can even see here he seems to have a smile on his face, but he's probably in the business for profit. I mean that somewhat cynically actually. And this is essentially the discussion of our product today. We have an animal derived product being used in human and physician oversight of the undoubtedly, in this case, adverse reactions that's likely to occur.

Next, please. Now one way of evaluating the risks of animal derived products would be looking at in decreasing risk order would be those animal diseases for which there's evidence of transmission and human disease. There's all sorts of known zoonotic diseases, pox viruses of bovine and other origins, rabies, menangle virus, swine flu, equine infectious

-- equine encephalitis, hendra virus and of course, more recently BSE and vCJD. This list could go on forever. I think we are discovering anybody that subscribes to ProMed has seen that almost every day new viruses are emerging which may have some animal

and human pathogen and I think we're looking at things like West Nile Virus and so on.

So these would be the things of first consideration. Secondly, those for which there is animal disease but no evidence of transmission or disease in humans. We're looking at things like porcine parvovirus for which there's no evidence of either transmission or infection as evidenced by seroconversion. Equine infectious anemia, there's -- it doesn't appear to be infectious to humans. Louping ill, foot and mouth disease virus, pseudorabies, there are a host and a huge range of animal viruses for which there are no human infections associated.

Next. Third level of risk would be those for which there is animal disease and the theoretical transmission of risk to humans and this might be things like other prion diseases, scrapies, ruminant TSEs. The only ruminant TSE we're aware of at the moment, obviously, is BSE and variant CJD and the other ruminants that have been identified as having TSEs, they're not likely to be used as a source for human plasma and last, but not least, there's no animal disease and questionable evidence of transmission, but there's no human disease shown yet. PERVs, there have been possible seroconversion, but

even this is a little bit questionable and as Dr. Weiss two and a half years ago pointed out that under certain conditions PERVs could be transmitted to human cells in vitro.

Now what this doesn't take into account, of course, and this is almost on a case by case basis, what the benefit risk of any of these particular animal derived products are. Despite the theoretical impossible risk of animal virus transmission to humans, one still has to look at whether or not these are critical life saving drugs and that's another factor to be looked at.

Next slide, please. Now what I've done here is on the left hand side taken note of our guidance documents, those things which we consider important for reducing risks of human diseases from human derived plasma. One of the things we look at, of course, is the prevalence of relevant infectious disease compared to Canadian and U.S. sources. If we were receiving plasma from non-North American sources we would want to see that the relevant infectious diseases, if there happens to be endemic diseases in some other area, those would be taken into consideration and a parallel with animals is that for bovine sources, we're looking for BSE countries of origin and whether or not there is any consideration

or not, but free of menangle virus, for instance, if that happens to be a consideration; ruminant TSEs if there is to be another ruminant used other than bovines.

For donor selection, well, we look for equivalency of the donor history and risk assessment criteria compared to Canadian and U.S. practices. In animals, one might very well look for a specific pathogen free herds or flocks. Donor animals could be retested prior to successive leads. These are for animals who are not sacrificed or evidence of relevant vaccination, if one has concern about rabies transmission then animals would be expected to be vaccinated against rabies or they happen to be a rabies-free country. This is something that may be considered, are there surveillance programs for slaughterhouse operations in which the local agricultural regulatory agencies may require oversight or perhaps an on-going program looking for viral diseases in the herds from which these plasma products are derived.

Next please. Another thing we're looking at is test kit comparability. We're looking at the sensitivity taking into account, strain variation of viruses and the regulatory oversight of the manufacturer of the kits. For animal source

material, one could identify commercial test use if such exists and a regulatory oversight for their manufacturer or if there are no commercial kits available, then the reference procedure is used. An awful lot of these screening tests are in-house methods and they would have to be very well validated or reference to other referenced literature sources.

Another thing we would look at for plasma derived, human plasma derived are procedures associated with reactive test results such as donor referrals, re-entry algorithms, trace back, look back procedures and quarantine procedures. Some of these things may not be and cannot be applicable to animal source material.

Now another thing we look at, doing a history assessment, written and oral questionnaires. Now what we might be looking at for animal source material is animal health history which is on-going veterinary assessment of a flock or herd and if you have a Dr. Doolittle available, then they could be asking animal risk questions. This is the original Dr. Doolittle. I think it was Rex Harrison, not some other actor.

Donor testing, since these tests have been known to transmit diseases, all these screen

tests have come into account and for animal source material you'd look for disease free status and test as appropriate for species, for instance, nucleic acid testing for porcine parvovirus.

Next. For human source material, we're looking at post donation information and this is information exchanged between collection sites and manufacturing, if it's found that the donor didn't meet health criteria, develops disease or risks, have been identified, and subsequently found positive for viral markers for which they were originally found negative. And the assessment of PDIs and you would defer the donors and retrieve plasma units.

Considerations for animal source material may be that the herd be monitored for known diseases, seroconversion. If the disease had been identified in a herd, one could retrieve plasma of other animals in the herd. If donor animal is subject to rebleeds, then that animal would be restricted or eliminated from further donation and plasma which hasn't already been pooled could be retrieved.

I won't be the first and probably not the last person to say that size matters. Limiting pool size would reduce the window period collection or risks including the risk of including units contaminated with an agent for which screening can't

be done. Similar considerations could be made of animal source material, a lot of it depending on the number of -- the type and material being produced. If this is a material that's a large volume material, that's likely to be used only once or twice during a patient's lifetime, that would have a different profile than those products for which there's on-going therapy is required such as hemophiliacs require weekly or biweekly infusions. For each of these human derived sorts, upper limits should be established of each product taking into account the number of lots and number of units in the pools for specific product to which the users are exposed, the infectious disease risks associated with the products and if they're added as stabilizers they should be ideally derived from the same pool as the product. Here we're looking at albumins almost exclusively.

Nucleic acid testing of pools. There should be validated methods of suitable sensitivity for different genotypes and the specificity must be supported by documentation to reduce risk of hepatitis C. Each assay line used must include controls expressed with reference to international standards. For animal source testing, not testing of pools for appropriate viruses depending on the species, for viruses for which screening tests are

not sufficiently sensitive. For instance, PPV could be tested for pigs. Or not testing when the validated inactivation removal processes have not been demonstrated. Again, if there has been some risk associated with animal derived plasma, then indeed one could develop a NAT test to reduce the raw plasma as a source of contaminating material.

Next, please. The quarantine of plasma units. Now this is being widely used in the ABRA industries in North America. This is a period of time to allow for the retrieval of units prior to pooling, based on subsequent positive results of donor testing or post donation information. This is possible for animals subsequently bled for plasma and it could be possible for diseases identified in the herds. You could retrieve units from other animals. Now this is a "could" not a "should" but this is something for consideration, that if there was a quarantine period allowed, one would be able to retrieve plasma units from those animals which are being held in quarantine, plasma units in quarantine if subsequent disease is identified in the source herd.

Next. A lot of these are going to be covered by Tom Lynch. Following activation of removal procedures, this specific step must be

introduced if the removal of a virus is a major factor in the safety of the product or if the manufacturing process itself doesn't remove infectivity. And similar considerations can be given to animal source material. Heat treatment which has been described quite well, for albumin, if it's used as a stabilizer can also protect the virus from inactivation. Therefore, worse case scenario consideration should be given in which case high titered spiking experiments should be used in which albumin itself is a very good stabilizer of virus and I think this same consideration would have to be taken into account for animals. Animal albumins and other stable products through which they're being used as a stabilizer, the same considerations can be taken into account.

Now animal albumins aren't typically used as stabilizers in animal products so maybe this is not a consideration here.

Next. Solvent detergents. This has frequently been described for human derived plasma as a cassette. I think the New York Blood Center has described it as such and an in-process solution should be free of aggregates particularly when you're considering this, that might harbor virus. Therefore, maybe filtration before treatment can

remove some of these aggregates. Inside these aggregates could be viruses that you're well-protected from the effects of solvent detergent. And for animal sources, again, we know the toxicity and effective range of solvents and detergents to be used for human derived plasma. For animals, known animal viruses, such as PERVs, solvent detergent would very likely inactivate these kind of viruses and a whole host of unknown envelope viruses waiting to be discovered. I think in some cases maybe the unknown, if one isn't looking for them, you're not going to find them and to some extent the use of solvent detergent will be a way of proactively looking at -- treating animal source plasma so that you don't have to wait to find when the next zoonosis will be found in humans.

Next slide. Viral filters are being widely used now and they're now even being used in recombinant products and recombinant products just to remove risks of, in the most case, murine viruses which for the most part haven't been shown to cause any disease, but these manufacturers are using viral filters, along with solvent detergent treatment and coagulation factors. However, if you're using viral filters sometimes the filters themselves can affect yields. Perhaps there might be an activation of

coagulation factors and obviously it's essential that filter integrity tests be done in process control and scale down comparisons with production scale.

For animal source material, its broad usage with human derived processes and it's possibly, a lot of these filters are already validated for a host of animal diseases and in some cases it would be a relatively innocuous and easy step to introduce. For human immunoglobulins, low pH, usually a pH of less than 4 inactives certain viruses, depending on time, temperature and the composition of solution. And this may also be applied to certain animal immunoglobulins.

Next. Now I'm appropriating the use of the words "relevant viruses" and "model viruses" here from some of the CPMP documents and they do seem appropriate, so I didn't invent a word of my own. The relevant viruses are either identified viruses that pose risk and for which spiking studies can be done. Model viruses are those for which infectious spiking studies cannot be done. For instance, if a virus cannot be grown in vitro such as hepatitis B or hepatitis C. And for animal sources, we'd be looking at spiking studies would be done according to the potential risks to humans. That doesn't tell you very much, but again, on a case by case basis, one

would have to look into these.

And on the next slide there's a table showing you relevant and model viruses for human plasma derived products: HIV, it is a relevant virus for both HIV 1 and 2; hepatitis B. Manufacturers frequently use pseudorabies viruses, other envelope DNA viruses and perhaps along with pseudorabies manufacturers have used a host of herpes viruses and there really is no practical system for hepatitis B validation using in vivo models. I have yet to see people using duck hepatitis virus. Actually, I've seen one submission that's used that. You do go through a lot of ducks. Hepatitis C virus, BVDV, sindbis has been used. BVDV is particularly a more relevant model and BVDV strain should be used that has a high physical chemical resistance. For B-19, an appropriate model would be porcine parvovirus. It seems to be the most closely related model to B-19. Hepatitis A is a relevant virus for coagulation factor studies. You can grow hepatitis A and consideration should be paid to possible interfering antibodies, if you're looking at immunoglobulin preparations and the immunoglobulin preparation itself should be free of anti-hepatitis A antibodies. And prions, not much can be said about them and the models that people have been using, scrapie models

and so on, may or may not be appropriate for the prion disease of consideration.

Next, please. Now these may be relevant in model viruses for animal plasma derived products. And all of down here is a list, an array of viruses or virus families with a representative species of virus which have an array of genomes, envelope, non-enveloped and resistance to pH and chemicals and different shapes. And again, prion diseases, there may be various hosts that could harbor these and has high resistance to pH. The thing that could be said about prion diseases is there may be some evidence of partitioning of prions, at least it has been shown with the plasma derived albumins, for instance, which have been shown to decrease prion load, at least if one is using a scrapie model by about four logs.

Next, please. Now the conduct of viral spiking experiments, I think a lot of the work has been done for us. The ICH technical requirements for registration, etcetera, and these are for biotech products. And some of the considerations for the spiking experiments have already been dealt with in that document. Essentially reduction is the sum of the individual factors. Less than one log is not considered significant. Steps with four log reduction are generally considered significant for

package insert claims. This is above and beyond those serendipitous fractionation steps which must be used in the manufacture, but coincidentally do remove viruses. And considerations could be given for animal source material and the conduct of spiking experiments. As I said, the work has been done for you.

Next. In the conduct of viral spiking experiments, there are specific precautions that are outlined in that ICH document. Things like avoiding aggregation with high titered preparations. The dilution effect on the spike of stabilizers. A few years ago we received submissions in which in the same submission they demonstrated that a difference of 10 percent on the stabilizer used would make a remarkable difference on the degree of viral inactivation and yet, the dilution of the spike and their spiking experiments haven't taken that into account. When you have a 10 percent spike, you obviously have a 10 percent reduction in the stabilizers that are being used in the product and that has to be accounted for.

And again, steady scale versus production scale, all of the parameters that one measures, all the end process controls and things that ones looks at at a production scale must be mimicked perfectly

in the study scale.

Next. Further limitations, the tissue culture virus that's in a production step may be different than the native virus. People may very well be using laboratory strains of virus in their spiking experiments and sometimes these get passage to some degree and they may no longer reflect what wild type viruses exist and this is another consideration to take into account, that the viruses used in these spiking experiments must from time to time be

re-passaged from wild type viruses that one might expect to contaminate a product. And the reduction values of identical procedures should not be included unless they're justified. If you have a column fractionation step and it requires a specific type of column, two subsequent steps cannot be pooled together and considered two separate reduction steps.

Next. Specific points to consider, for instance, for immunoglobulins, unknown and envelope viruses. Before steps were introduced, there was instances of hepatitis C transmission. You're looking at these particular products. You're looking at a very large volume, but low frequency and I think these kind of considerations have to be taken into account of what your product is, how it's used and

what the lifetime risk to the recipient may be. For coagulation factors, we know that hepatitis A and B-19 risks have been associated and both of which are highly resistant to inactivation. Again, we are looking at -- I shouldn't say we, manufacturers are looking at ways of reducing hepatitis A and B-19 risks by introducing PCR technology to reduce the burden of the raw material. I think we've all learned that anticipating that there will be sufficient neutralizing antibodies in these materials, particularly for immunoglobulins, that both hepatitis A and B-19 have been shown to have such high titers that there is not sufficient neutralizing antibodies in any of the pools. There has been cases of B-19 in which it was assumed that there would be sufficient neutralizing antibody, but B-19 is one of those bugs when a donor happens to be viremic, they have titers of about 10 to the fourteenth and with that kind of viral load, practically no degree of neutralizing pooled sera could possibly neutralize that much virus.

And again albumin, it has an excellent safety record and there's been some evidence of prion partitioning. We have seen some studies from manufacturers where there appears to be at least a four log reduction due to partitioning of prions in

the albumin fraction.

Next, please. Now this tends to be my thinking. If it can be done, do it. I think we shouldn't be waiting for something to happen, particularly when there are cassettes, if you will, of known procedures for viral inactivation and they can be introduced into animal derived products without further reduction or loss of yield from these products and that manufacturers should be looking at ways of reducing either known or unknown risks with respect to animal derived proteins.

Thank you.

(Applause.)

CHAIRMAN HEINTZELMAN: Well, we're scheduled for a break now. We're a little ahead of schedule. That's good. Maybe we'll leave a little early. Why don't we take a 15 or 20 minute break, does 20 minutes sound okay? Twenty minutes gets us back at 10:30 and we'll reconvene with the European Union perspective. Thank you.

(Whereupon, the proceedings went off the record at 10:10 a.m. and went back on the record at 10:36 a.m.)

CHAIRMAN HEINTZELMAN: We'll reconvene, please, and get ready for our next speaker.

(Pause.)

DR. WILLKOMMEN: Ladies and gentlemen, it's a pleasure for me to continue now with the European perspectives and I have heard already this morning the position of the Food and Drug Administration, from the Canadian people and I must say we have not so many differences. I can stop here already. Okay?

(Laughter.)

DR. WILLKOMMEN: But I want to speak, of course, and I have thought that it would be fine or it would be interesting or maybe interesting for you to compare or to demonstrate to you the European requirements of life safety testing of many titered products derived from human or animal sources.

I'm sorry, I forgot to introduce myself. My name is Hannelore Willkommen. I am from the Paul Ehrlich Institute in Germany. It is a national authority for sera and vaccines and this institute is very much responsible for the development of national guidelines in our field and is very much also into development of European guidelines.

So I want to speak about this and I hope I can give you some interesting information. At the beginning I want to summarize, I want to give you an overview about the guidelines which are in place. You know, the European Union consists of 15 countries

at the moment and we have a high need of guidelines in order to summarize our position, to find a common position in many aspects.

This is the background or this is the reason why we have a lot of guidelines in place. So these are the guidelines and I want to go through only very quickly. I want to mention these guidelines which cover these products derived from human or animal material.

First, these are the guidelines for plasma derivatives. This was revised in September 1996 and it is now a new version of this guideline is in place. And here, you see the source of the guidelines, if you go on home page of the European Agency, you can find all these guidelines and can read them.

So this guideline said how to test the source material, how to -- this guideline says also what's the capacity of the manufacturing process for the removal and inactivation of viruses. What does the figure have to be for the result.

The second guideline here, note for guidance on virus validation studies, this guideline says how to perform virus validation studies. And I think it's -- I'm quite glad about this guideline and I will come back later on a little bit on it.

So this is a guideline which you also know about. It is an ICH guideline, saying something about the quality and biosafety, especially about biotechnology products. And I have it here on the list because this guideline is applicable also for monoclonal antibodies which are derived from mouse ascites and so it is also animal and is a material used for the manufacturing derived from animal materials.

So next is a guideline for guidance on minimizing the risk of transmitting animal spongiform encephalopathies agents via immunosera products. This guideline was finalized in this year and there's also a newer version of an older guideline, but I don't want to come back on this one. I think it is -- you understand, it is another issue.

So we also have a guideline which was developed already. It started to develop in 1996 and -- sorry, in 1993, and it was finished in 1995. It is a guideline about the use of transgenic animals in the manufacture of biologic immunosera products for human use and we think that this guideline is already a little bit old and should be revised in some parts.

And then we have a new draft guideline and I must say it is at the moment the draft or the suggestion from our Institute. We discussed it

already in the biotech working party, but it is not finished from the discussion in the biotech working party. It is not finished and so it is a draft and maybe it more or less demonstrates opinion of our institute.

And it is a guideline about the production quality control of animal immunoglobulins and immune sera for human use. We think that especially for these kind of products we need some regulation and need also some regulations for Europe. At the moment, these kinds of products are on the market on the basis of a nationalized sense. There are no products in place already which has a European license.

So as a general approach, biosafety means the absence of infectious viruses and we are speaking or I am speaking only about viruses at the moment. I don't speak about the prions.

This means that the source material should be tested or it should be controlled. The manufacturing process should have a high capacity for removal inactivation of viruses and in some cases it may be useful also to test intermediate products or to test the final product.

This is a general approach and we think that this approach is also applicable for this kind

of product derived from animal material.

Let me go now through the different guidelines and show you the differences in the regulation or the state of regulation. I want to mention also what should be changed or what is under discussion at the moment.

These are the guidelines, ICH guideline here. It's a number of European -- and it is a guideline which covers the most of the monoclonal antibodies and the most ICH source material. You see, it is required to have close colonies and these colonies have to be tested for many, many viruses and it is very accepted that these testing is necessary and tests have been developed which are relatively easy to perform and you have no discussion about the need to test such a lot of different viruses. It is good, I think, I mention it because it is a starting point for our discussions.

With regards to the requirements on the capacity of the manufacturing process, we have an expression in the guideline that the manufacturing process should be substantially higher than the lab contamination in the source material. Very often we have contamination with retroviral particles and so in this case it should be substantially higher. It is not clearly defined. Here, it is to be considered

on a case by case basis.

Testing of the final product is only in some cases required, only if the source material contains the viral contaminants and then it is limited on some lots only.

So what is expressed in the draft that I want to remind you? It is, at the moment, our draft, draft for animal immunosera and immunoglobulins. We know that it is a little bit difficult and it is not realized in each case that animals are held in closed herds, but we think that it should be at least well monitored herds. If you are thinking about larger animals that is nearly impossible for the manufacturing. They say they can't hold the animals in closed herds.

At the moment we have products on the market in Germany which came from rabbit, goat, sheep and horses. So we think that these herds have to be tested on the freedom of infectious agents and at the moment there are no requirements, no advice from industry what they have to test and we think that it should -- virus lists should be developed and should be given to the consideration of the Ministry and also of the control authorities. I will come back on this point later.

So there are no specific requirements at

the moment for the capacity of the manufacturing process to remove inactive viruses for performing virus validation studies. This guideline is applicable and it is a guideline which is also applicable for the blood products.

We have to consider in the case of these products, we have to consider not only species specific viruses, very often the products need to be absorbed in human material, it is so at least in the state of anti-T cell sera. And if it is the case, we have all to consider the presence or we have to control the absence of human viruses and for all the steps of this manufacturing validation process. We have to consider human viruses too.

The final product is over here only required in specified cases, if it is not possible to arrive at the contamination of the source material.

So what is with human products? The idea today, you know, we have the development of the donors. We have a very -- we have a lot of regulations for the selection of donors and the testing for the absence of viruses. You see normally it is tested for HIV, HBV, HCV, and in Europe the HCV-RNA testing for plasma pools is introduced since July of this year. All manufacturers have to perform these testing and the pools have to be free of HCV-

RNA.

The capacity of the manufacturing process should be very high. We have a special guideline for it. So testing of this capacity has to be performed according to these validation guidelines.

If I summarize the requirements in some words, then I can say it is required a high affectivity for the manufacturing process, in most cases, two effective steps which compliment each other in the amount of action required.

The testing of the final product as in each case is not sufficient in order to demonstrate the safety of the product and it is so because of the statistical reasons or because of the statistical limitations, but the safety -- we think the safety has to be demonstrated by other measures.

In some cases, can it be useful? As an example, if you look at the contamination with parvovirus B-19, it is very informative to test the final product. So but it is not the general framework or it is not normally required.

So products derived from transgenic animals, I mentioned already that we have in all the guideline here and the guidelines is sufficient we think with regard to the source materials, with recommendation to the source materials. It is

required, of course, that animals shall be held in closed colonies. It is required that animals have to -- or the colony has to be tested or it has to be controlled in the absence of specified viruses. But the guideline gave only some examples of viruses which should be considered. There are no specific requirements for the capacity of the manufacturing process, but it is, of course, expressed that the process should be effective in the removal or inactivation of viruses and it is mentioned too that mycoplasma should be considered because if not as a source material of these products, mycoplasma can go to high titers in this material.

And again, there are no specific requirements for the testing of the final product. So now I want to make some remarks to the source material testing. If you compare the animal material with the human material we can say okay, the human material is a high risk material. It doesn't work. Yes, it's a high risk material. You know the contamination is chemical. It's pathogenic for humans.

In the case of animals, you don't know exactly what the risk level is. We know that animals can also have virus infections which are -- can have viruses which are pathogenic for humans, but they

have also, of course, viruses which are non-pathogenic for humans. We have to select, the system of selection of donors in place, testing of donations and here we ask for what we think we should have close herds if ever possible. We should have monitored herds. We should perform the testing of plasma pools. As an example, if it is not possible to avoid the contamination of the herd. As an example in the case of rabbits, you cannot or it is very difficult to avoid the contamination of rotavirus and it should be also with reovirus. And it should be then a measure of testing of the plasma pool that the manufacturer can demonstrate that the pool contains antibodies. That means that this virus is present in the flock, but he can demonstrate that as a means that he has no infectious virus in this plasma pool. We think that it is also an important point and we will come back on this later.

So we will go to sheep, horse, pig, also used for this and for animal sera and so on and we have also some products under development which use egg as source material. And the mouse for the ascites fluid. So there are known general recommendations about viruses which should be tested for.

Let me come now a little bit more

specific of immune sera in immunoglobulins because it is the topic here of this conference. And for lymphocyte T-cell immunoglobulins or sera and we have to comment that these products are used in immunocompromised patients. Antitoxins are the old products. They are already a long time on the market. It is also seen bacterial in viral agents. We have anti venoms against venomous snakes, scorpions and spiders. These are a group of the preparates which are on the market in Germany.

If you are looking on the development of products from transgenic animals then I was a little bit surprised and impressed from the data which I saw on the conference in April of this year in Boston. And Mr. Velander demonstrated here with high concentrations of these kind of products can be received in the animal material. I think this is an upgrowing field and we will more and more be confronted with such kind of products.

Safety of the source material. Now I want to go a little bit more in detail to this. We should have closed herds, but we don't have it in each case. We mean that the animal should be zoological tested of animals elected animals before entering the colony and at regular intervals thereafter. This would be done and we have to give

the companies some guidance which agents they should consider.

We think that epidemiologically consideration should be taken into account. That means if a virus is absent in the country of origin, then it is not necessary of course to test against this virus. But in order to demonstrate or confirm this an official certificate should be provided by the industry and as a background of this, a compulsory notification of clinical suspected cases should be in place and also clinical laboratory notification of them.

So these various factors of testing directives animals and on the side of epidemiological considerations should give us information and knowledge about the absence of viruses in the source material in the animals used as donors.

And I told already the testing of plasma pools should be required appropriate in vitro and in vivo tests should be used and if human material is used for absorption as an example, then also human viruses have to be considered.

Which viruses should be tested for? This is a very sensitive question. I mean and we think that viruses that are pathogenic for animals and humans, the so-called zoonotic or the transzoonotic

viruses as used now, I mean in this sense, but also animal specific virus, there's a possible potential to infect humans should be considered. So the route of application, heatlh of recipients should be considered to. This means that in that risk benefit analysis has to consider all these points. In the specific analysis associated with degenerative oncogenic immune supressive or diseases like meningitis and encephalitis and hemorrhagic fevers, all of these viruses should be taken into consideration.

So now I will show you some lists and I will start with a well known virus. I don't want to discuss them. I will only show you these in order to demonstrate what we think at the moment in Europe and I want to repeat that these at the moment, the position of the Paul Erhlich Institute where you have to discuss at this point again and the next meeting of the biotech working party in November and I think the biotech working party agreed with this suggestion, then it will be sent to the CPMP and will be finalized by the CPMP and if CPMP agrees, of course, to the -- it will be finalized and released for consultation. So it will be public then.

I started with the murine viruses because we have no discussion about it and you know that it

is a very long list of viruses, but the industry has found a good match demand in order to handle it.

These viruses are grouped again into groups, the first group is human pathogenic viruses. The second group are viruses which should be taken into consideration because they can cause disease, of course, especially in animals.

So these are the lists of viruses which we think should be considered if rabbits are the animals of production and we think that -- I want to repeat it, on the one side, the animal should be tested again for agents or other considerations should be taken into consideration epidemiological. There's an epidemiological situation of the country of origin should be considered from the industry and of course, the industry has also to take note from new emerging diseases which occur in the country of origin.

So these are viruses we have some problems with with regard to the products which we have on the market with reovirus and with the rabbit. Rotavirus is really not a problem which cannot be solved.

These are the second group of viruses. As you see that some of the viruses are mouse specific viruses and they can be tested also in the

MAP test and the antibody -- mouse antibody production test and the company uses this test for this reason here too.

So if we look on goats and sheeps then we have also a long list of viruses which should be taken into consideration and if you are familiar with them you will see that some of them are restricted in specific areas. Some of them only -- we had only a very small outbreak of them and these are not all viruses which are distributed widely or broadly distributed or occur in many countries. But we think the industry should go through the list and should consider all of them and should say what the situation -- what they think about these types of viruses.

So I could continue. These are equine viruses and is the same system which we have used here. So I won't stop with this list and so the guideline which we drafted will only contain these virus lists because at the moment it's only these species are involved in the manufacturing of immunoglobulins or immune sera.

So I want to come now to the second point, namely, the testing of the manufacturing process or the capacity of the manufacturing process for removal and inactivation of viruses. And here I

mention again the guidelines which has to be used for it or which basis has to be taken. It is here the ICH guideline which as you know is applicable for cell derived products and also for products from our monoclonal antibodies and this is the guideline which is applicable for human and for plasma derivatives which are made from human plasma or also from animal plasma or products from other body fluids and tissues. And it is also applicable for products derived from transgenic animals.

The guideline that's here, this guideline is a little bit stronger than this one, especially with respect of the demonstration of the robustness of the manufacturing processes. Here the requirements are very strict. And this is expressed here in this part, you see, production parameters which influence effectiveness of the process to inactivate and remove viruses should be explored and the results used in setting a proper and precise limits. It is a very hard requirement for the industry, I mean, and it is not realized in each case and we think that the manufacturer which performs or which produces products from animal materials should consider this and should perform studies which demonstrate, which can demonstrate to us reliably the effectiveness of the stages for removal and

inactivation of viruses. And I think we have an agreement or a common position where you saw -- I mean, a common position in all agencies which spoke today that we think that the methods which are used for human products should also be used for products which are derived from animal material.

And the guideline -- maybe that you know it, also gives some recommendation for performing the studies and we think these are parameters which are very important in order to reflect on the one side accurately the manufacturing process and on the other side to receive data which really are -- which really are convincing and demonstrate the robustness affectivity -- affectivity and robustness of these processes. And you'll see here all the generic studies are currently not sufficient and this is a guideline which is used for the plasma derivatives too and it is very important, we know that if you have a partitioning process that means that the virus is partitioned into other fractions are removed during manufacturing. Then you have a higher variability in the process and you have to demonstrate very carefully what the inference of the parameters of this procedure is. The inactivation is easier to validate and you have to investigate, you have to study here is a kinetic inactivation

procedures and in general are better to evaluate or the data can better reflect the effectiveness of this procedure.

Choice of viruses, of course, this is also as a general recommendation, viruses which may contaminate the product, viruses which could present a wide range of physical and chemical properties as possible. Any virus used in the validation study is a model virus. We think that it is important to consider and of course, reliable and efficient preventative of infectivity should be available.

The NAT testing or this detection of the genome virus can be of help if you validate and manufacture or if you have the task to validate the process.

We think that our intention is to receive data which reflects real process conditions. With regard to the choice of the viruses you'll see it is very well defined in the European guideline which was these have to be used and in the case of coagulation factors, it is required to test also with hepatitis A virus and parvovirus.

In the case of the animal seras, it's not so good to find and it can't be tested. It has to be considered on a case by case basis and in general retrovirus should be involved. Herpes viruses are

normally included and enveloped viruses, of course, because they are very often more difficult to remove or inactivate.

So if we are asking for robustness of those studies, then I think that it's not so easy to perform this and we think that it's a basis, we should always as a correct downscale process which would be evaluated on affectivity of removal or inactivation and then variations should be made and some parameters which seems to be important should be controlled so that's manufacturing process or the manufacturer can consider the inference of different parameters and can establish really a safe process which is reliable in its inactivation parameter. As a result of the studies, critical process parameters should be defined and so the definition of worse case conditions which you often see in various validation studies should not be applied so much because sometimes the defined worse case conditions are not really the worse case.

So the requirements for the process capacity, you can read the text. It is attached here. It says that the manufacturing process should incorporate a fact of validated steps and in most cases it is still able to have two distinct effective steps which complement each other and at least one of

the steps should be effective against non-enveloped viruses.

This should also be the case for animal sera and murine sera, we think, but it is really not the case at the moment. In most cases, these preparates are already long-time on the market and the distinct inactivation stages such as heat treatment is not involved in this procedure. We should consider this, but we should also consider the value of these products. We need them on the market and we have to give, we think, the manufacturers guidance so that they can improve and to receive the time for it to improve the manufacturing process, to improve the safety of this product.

So I'm at the end of my talk. If I summarize, you know, the safety is -- viral safety is the absence of infectious viruses and we think that it's the control of the source material is really important and the principles which we have with regard to human derived products should be applicated also on this kind of products and manufacturing needs a high capacity for removal inactivation and additionally in specified cases experimental testing of intermediates of final product should be performed.

And I think that it would be valuable

if you would in the future requirements which are internationally accepted and so that we have an agreement in the ICH process in the requirements which would be set for these products.

Before I end I want to mention we know that the animal viruses are different from human pathogenic viruses, but we often don't know what they really do and I think we don't have so much information what has happened after application of such kind of product. And the knowledge which we have about illness, about infectivity of viruses based normally on the normal, on the natural route of transmissions and we don't have it in the case of such kind of products. Of course, in the risk of benefits analysis which we have to do, case by case, we have to consider all these points which are important for this product and so I mean we should go step by step forward that we also have no safe products with regard to these kind of products which are derived from animal material.

Thank you.

(Applause.)

CHAIRMAN HEINTZELMAN: Pretty much that concludes our morning session. I see we are scheduled for lunch from 11:30 to 1. We're about 15 to 20 minutes ahead of time. What I would suggest we

do is that we break for lunch now, if that's okay with everyone. And reconvene a little earlier, say 12:30, so we pick it up on that side. I see a little nodding. That's the puffin signal for we got it right here. So let's break now and reconvene at 12:30. We'll start with Dr. Snoy's talk concerning animal health standards and go forward.

For those of you who drove here today, if you're not familiar with parking at NIH, if you give your parking place up, it's forever. So take that into consideration. I'll see you at 12:30. Thank you.

(Whereupon, at 11:10 a.m., the workshop was recessed, to reconvene at 12:30 p.m., Tuesday, October 25, 1999.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(12:33 p.m.)

DR. SNOY: Mark has taken a lot of grief about the length of this title which is the Inactivation and Clearance of Infectious Diseases. I guess I would have argued that it should have been longer and I would have inserted for the prevention of viral contamination and failing that, the clearance and inactivation of infectious diseases in the, I use the word animal plasma rather than non-human.

My talk is about animal health standards and curiously enough that's reflected here in the title. I also use as a kind of jumbo business card and I've included my phone number, fax number and probably more useful my e-mail address, because if one of the purposes of the today's workshop is to kind of establish a dialogue and begin talking about what kind of things we can do to assure that the freedom of infectious diseases of this animal plasma, then if you can't communicate with me, then I guess I won't go any further than that. So I would suggest the e-mail and go with that.

Now in the interest of providing safe biological products made from animal plasma and also in the interest of providing guidance for industry in

the animal health standards that the Agency feels are relevant to preventing viruses, I'm going to present the animal health standards that we believe would help assure the safety of the plasma products.

Now it goes without saying, although I'm obviously going to say it anyway, that the knowledge about and the ability to diagnose animal diseases has increased greatly since these products made from animal plasma were first licensed. And the same can be said for the standards of housing and care and feeding.

In addition, there have been new diseases that have been discovered since these products were first licensed, or old issues like scrapie and TSEs that have become new issues in the sourcing of biologicals from animals. So as I said, most of my talk will describe the animal standards and the animal care issues which we would expect to be included in a BLA in order to assure the safety of animal plasma products. And another way to look at that is how that I, as a reviewer, would be looking for in reviewing a BLA.

So as I said, I do say actually that the overriding principle here in my mind anyway is that rather than just depending on downstream processing to clear potential viruses, that I think we all agree

it would be preferable to keep the viruses out of the bulk product and that would be a preferable way to go than just depending on clearance steps.

So the next slide, please. I thought I'd, in an attempt to build consensus towards that, I thought I would begin by discussing a few instances in which the actual biological product and the material from which it was made was not clear of viruses.

Now unless you've been out of the universe for the last five years, you're aware of the SV-40 story in polio vaccine. And it was -- in 1960 it was discovered that SV-40, which is a polyoma virus, was a potential contaminant of IPV vaccine and had been since about 1955 when it was first put into use. At the source of this virus was the macaque kidney cells from which the vaccine was made, and it was known that this virus could cause tumors in laboratory rodents and the discovery that this was in the vaccine, obviously, caused quite a flurry of activity and interaction with the manufacturers, public health officials and the precursor of what is now the Center for Biologics.

So much effort went into dealing with this issue, after the horse was out of the barn, to use an analogy which as a veterinarian I'm prone to

use.

So following this, the vaccine -- the cells that were used to go in the vaccine were required to be shown to be free of SV-40, and a number of epidemiologic studies ensued which showed that there was no public health effects of this virus in the material. And that's the way things were until the early 1990s when the issue returned and DNA sequences homologous to SV-40 were shown to be in a number of human tissues, mesotheliomas, ependyomas, and osteosarcomas, to name a few.

And so once again an inordinate amount of energy, time and research went into determining what the effects of this contamination were and I might say that the issue is still not completely settled. Now while issues like SV-40 may provide research direction for some, I think it's safe to say that the Agency would just as soon prefer to not have had this in the biological to start with, and that's the direction that we're going to try to go into today.

There were some interesting things about SV-40 which are relevant to our discussion today and one that -- one is that in spite of the fact that this was the polio vaccine was grown in the cell culture system when they were using the macaque kidney cells, there was no evidence of viral

infection. There was no cytopathic effect, no effect on the cells that were grown. And therefore, it was not picked up that there was a viral contaminant and it wasn't until there was a change in species in the monkey that was used to grow the cells that the virus was detected.

And the other interesting fact is that the SV-40 proved to be relatively resistant to formalin inactivation. So I guess the moral of the story is you can't always depend on infectivity -- demonstrating infectivity just by the use of cell culture systems, looking for CPE. Obviously, it has to be a cell that's susceptible to the virus and also that inactivation steps don't always remove the virus.

A number of other incidents of biocontamination of biological products. About the same time, yellow fever vaccine was shown to be contaminated with avian leukosis virus and more recently measles and mumps vaccines were shown to have an RT activity that indicated that retrovirus gene expression in those vaccines which originate from chicken cell substrates was possible and caused much concern about the possibility of transmitting that virus in the measles and mumps vaccines.

Well, again much energy was spent in

assuring that the retrovirus associated with this RT activity did not replicate in human cell lines nor in peripheral blood mononuclear cells. So again, a bullet was dodged, but not without concerted effort.

Murine monoclonal antibodies were first licensed in 1987 amid concern of the presence of a type C endogenous retrovirus in the -- both in the mice in which ascites fluid was harvested from monoclonal antibodies and also in murine cell lines. It was shown that this endogenous retrovirus was universal in all the murine products, so the bottom line is that while these criteria were established in which the titer of the virus present in harvested material was quantitative, inactivation procedures then had to demonstrate that that titer virus could be removed from the material and then their infectivity assays for final release of the product.

And then undoubtedly, you're familiar with the endogenous retrovirus in pig tissues used for xenotransplantation. This is also a type C retrovirus which cannot be removed by closed breeding systems or by rederivation techniques. So this problem was discovered after several INDs had begun which used porcine tissue, and the discovery that the porcine endogenous retrovirus could infect human cells and cell lines resulted in all these INDs

placed on hold. What followed again was much time and research energy and expense in demonstrating that both the human serum and peripheral blood line nuclear cells showed no evidence of infectivity and once done, then some of these INDs have been taken off hold.

And then finally, probably more germane to our discussion today is the episode of Factor VIII in porcine parvovirus. The Factor VIII was thought to be free of porcine parvovirus until a change in laboratories that examined the presence of the virus by PCR, showed that there was parvovirus in the product, and this is particularly relevant because parvoviral infections in pigs is subclinical. Parvovirus itself is fairly instable to environmental inactivation in many inactivation steps that are used in processing biological products, and also has been shown to move, to jump from species to species, as evidenced by the early outbreak in the late 1970s of canine parvovirus which was shown to originate with feline parvovirus known as feline distemper.

Well, the story had a good ending. It was shown that there was no antibody development in humans and again after much interaction between the agency and the manufacturer and research effort, the issue was addressed.

So again I use those as examples as why we should strive to assure ourselves that a few reasonable and practical means that the bulk material that we start with is as free from viral contamination as we can make it. So we won't have to address these after the fact.

Next slide, please. Now this morning Mark referred to the CMC guidance which is here which was published this year and deals with in a kind of outline fashion the animal health standards and issues that we were most concerned about when reviewing BLA for these products that are made in animal plasma. But don't feel alone. There's a number of other guidelines which also address the animal health issues and the requirement for health screening of animals used for human biologicals, and one is the points to consider document for products made from transgenic animals. This was issued in 1995.

Next slide. As I mentioned before about the monoclonal antibodies, there's a section in there about animal health screening and animal health issues and one, the Cadillac of animal health screening and infectious disease issues is the -- what's currently the draft Public Health Service guideline for xenotransplantation. So if you want to

feel better about this you can read those, and since that tissue cannot be processed before transplanting to humans, then there's a higher standard of requirement for freedom from infectious diseases.

And now I wanted to allude briefly to CFR 600.11. There's a fairly brief description in there of the number of issues which are relevant to using animals for production of biologicals, and it addresses such issues as the number of caretakers, requirements for sanitation, the requirement for daily observations, removal of animals that are ill from production, competent veterinary care and quarantine. And also in there is a requirement to make sure that animals that are used for production are immunized for tetanus. So I would just emphasize that if I failed to mention that further in the talk that there is a provision that production in animals be demonstrated to be immune from tetanus. So you might want to keep that in mind when you're developing your health programs for animals.

Next. So the remainder of my talk is pretty much filling in the details that we would be looking for in BLA as outlined in the CMC dealing with products made in plasma for human use in making animal plasma. It breaks down the animal issues into these five areas. And the object here, I think we

should say up front is to basically have specific pathogen free herds. And a lot of the specific steps that I'm going to speak about in a minute address steps that will help establish these SPF herds.

Next slide. And it begins with qualifying animals for production. I think it's safe to say that in the BLAs we're not looking for SOPs, but a summary of what should be established, written procedures which will deal with the sections that I just outlined in this case for qualification of animals for production, and the first thing that needs to be addressed is the quarantine requirements of animals. Either the quarantine at the start up of putting a herd together or the addition of animals to an existing herd.

The CFR which I alluded to, 600.11, states that there should be a minimum of seven days of quarantine, and I would argue that that should be more like 14 to 21 There's a number of animal diseases which require longer than a seven day quarantine period, so I would look at the CFR requirements for quarantine as being minimal and would recommend a longer period.

Now during the quarantine there must be daily observation and recording of those observations by a qualified person. This wouldn't necessarily

have to be a veterinarian, but it would be a trained caretaker who could contact the veterinarian in case of problems. This should be an all in, all out situation. In other words, a cohort should go through together if it's a 14-day quarantine period, then the animals come in, remain in a cohort for 14 days and then be discharged. No animals should be added during that time without extending the quarantine period.

And there should be procedural and also physical barriers to the quarantined animals versus the actual production animals, if that's the case. In other words, these animals should be held a physical distance, and even would be in a separate building from the production animals, and they should have separate staff that takes care of the quarantined animals.

The source, if this is a start up herd, then the source animals should come from a herd with known health status. In other words, they should be specific pathogen free animals, and also obviously if you're dealing with a species that has spongiform encephalopathies, then the animals should be sourced from a country that is free of spongiform encephalopathies.

The quarantine should conclude with a

thorough physical exam by a veterinarian, and part of the quarantine period should include serologic screening and if you -- and obviously you're going to establish a closed herd, and for a closed herd the serologic screening should meet or exceed that screening that you're doing, that you're performing in the herd, and I would argue for exceeding the -- whatever your list is, and we'll talk about that in a minute -- but your list of agencies to assure that you don't unintentionally introduce a viral contaminant to the herd that you may not necessarily be testing for on a regular basis.

Next slide. Husbandry issues which should be addressed in the submission, the type of housing is critical. Do the animals go out in pasture? Are they raised behind barriers? What's the limited access to these animals? This is part of the raw product and access to these animals should be limited. There should also be some sort of security for the animals. What's the fencing situation? A lot of sponsors have double fences to try to keep unwanted animals out.

The frequency and method of sanitation can be summarized. Again, these would be written SOPs that are in place, but a summary of these would be adequate in the BLA. There should be one, if not

two, methods of identification, ear tags, tattoos, implantable devices, so that you can trace, if you have an outbreak of disease in the herd or maybe pick up one animal, you should be able to trace the plasma from that animal forward in the processing.

Records should be kept lifetime, and that should include all illnesses and antibiotic use, vaccinations, wormings, and those should be -- those records should be present with the herd, not in some distant location.

And finally, feed components should be known. The obvious issue here is freedom from mammalian source to rendered protein, but there's also other issues which are chemical and microbial contaminants, and there should be a periodic analysis of feed that again goes into the beginning of the product for human use.

Next slide, please. It should also be summarized in the BLA description of the procedures for immunization techniques. You'd want to include adjuvant use, the route of inoculation, number of boosts and how the antigen is prepared and what type of analysis it undergoes to assure that it's not contaminated with either bacteria or a viral contaminant which would then go downstream, obviously.

Bleeding protocols should be summarized. In other words, how is the bleeding done. Is it a plasma pheresis unit? The frequency that the animals are bled and where this is performed. It is generally accepted that the procedures should be performed in an area separate from where animals are housed and the sanitation of these areas where procedures are done would be of a higher level than the actual, than the animal housing area.

And one final comment about this, obviously, all procedures which are done to the animals, whether it's immunization or bleeding, would be approved in this country, would be approved by an animal care and use committee which reviews all animal procedures. And this would be, I again, I would say this is required by the USDA, but this would be good backup to have if say an FDA inspector comes in and sees some technical part of the immunization that they're not comfortable with, the fact that this has been reviewed by the sponsors animal care and use committee may go a long way to addressing concerns that they might have.

Next slide. Animal health is obviously the cornerstone of the animal health program and in the application, there should be a description of the veterinary support. This can be either the contract

person or it could be someone on the staff. If it's the local dog and cat guy who comes in every six months and just looks around, that's probably going to be a point of discussion when you make an application to the agency. And this is also a good place to get input on what infectious diseases are of concern and which infectious diseases should be screened serologically in the herd. Day observations, again can be made by animal care staff, but there should be a written and established way that the staff can communicate problems to the veterinary support people.

And there should be periodic serologic screening. I will, at the end of the talk, I'll present some lists for the species that we're dealing with today that will serve as kind of a beginning for discussion of what agents are concerned in these particular species. But this should be done on a regular basis, again, in order to establish that your herd that you're using to produce human biologicals is, in fact, an SPF herd and this would be expected to be done on a regular basis.

The quarantine we've talked about. Again that serologic screening should at a minimum match what's being done in the herd, and I would suggest that it would even have additional agents that could

be of concern in that particular species and not to forget bacterial and parasitic diseases. There should be periodic screening, for example, TB in ruminants and a number of other species would be an expected part of a preventive medicine program and a health program for the animals, and there is also periodic evaluation for internal parasites or periodic worming of the animals.

And finally, any unexpected deaths would be necropsied as part of the health surveillance program, and I also argue that a certain percentage, 5 to 10 percent, say, of the animals that are discharged say for poor production or just discharged from the herd should be necropsied completely and serve as the sentinel animals in the herd.

Next slide. Then finally, just as you would include a description of the area in which material is processed, you would expect that there be a description of the facility, the animal facility, and this would include the animal holding areas and that would include the areas to any pathologic agent introduction, again security, and what steps are taken to limit the access to this herd.

Again, the animal procedure areas should be separate and would be expected to have a higher level of cleanliness than the animal holding areas

and there should be -- should address the equipment used to bleed the animals and the cleaning of that equipment, including validation of the cleaning procedure and also the removal of the agents that are used for sanitization.

And then finally, if multiple products are made in a facility, there should be a way of segregating the animals that make the various products so that there's no potential for mix up. That could be keeping animals for different products in separate pens and -- or including different colored ear tags or some system for easily and visually identifying which animals are with which product.

Well then finally I'm going to present a list of agents for which I would recommend that the herd be screened for serologically, and I've chosen these based on a number of reasons. One, these are all, first all you mentioned they're all common to the United States. If the herd is in a foreign country then there would be additional agents that would expected to be screened for. Most of these agents have a significant viremia phase and many of them are also shown to infect -- some are shown to infect human cells or at least there's no data that exists that shows that they don't infect human cells.

And several from viruses, from families of concern -- for example, like herpes viruses and retroviruses which are known to possibly transform cells.

Next slide, please. And I'll -- since there's no handout, I'll go slowly through these. You have to consider that these are works in progress. The reason I included my e-mail address on the first slide is that some of you may feel that there need to be additional viruses added to that and I'd certainly be happy to entertain those comments.

Next slide is sheep serology. And another caveat is if a lot of these -- not many of these sheep diseases have vaccines which will prevent them, but if, for example, the slide on the equine diseases, there were six diseases there for those. There are approved vaccines and if the herd is on an approved vaccine schedule following manufacturer recommendations, then it's my opinion it would be confusing to try to do serologic screening on those animals. So you would not have to do the screening for viral diseases that are being screened, that are being vaccinated for.

Next slide. There's been a lot of activity and interest in porcine viral diseases as a result of xenotransplanations and some of these agents are fairly recent discoveries, the porcine

circovirus and the swine hepatitis E virus.

And then if you're really adverse to doing serologic screening, my rabbit serology list is a short one. Unfortunately, it is -- go ahead with the next one. There's the complicating factor that you don't get a whole lot of plasma out of rabbits, so you're going to have a pretty large colony. But the good thing about rabbits is generally you can have some fairly significant barriers to the introduction of disease, and there are other serologies that can be done to demonstrate SPF status which would -- kind of the standard serologic screening for rabbits includes nonviral diseases like bordotella bronchoseptica, and Tyzzer's disease and also CAR bacillus.

So with that I would close my comments, again saying that I think it's a kind of primary principle that we should do everything we can to avoid and to minimize the presence of viruses in the starting material, the raw bulk and the steps that I've outlined here will go a long way to providing that extra measure of safety.

In addition, of course, this is in addition to the downstream processing of viral clearance and validation.

(Applause.)

MR. LYNCH: Good afternoon, everyone. I'm Tom Lynch. I'm with the Division of Hematology in the Office of Blood, and I've been asked to provide a description of current and under development clearance methods for viruses as they're used in plasma derivatives. I'd also like to talk briefly about validating those methods and some practical considerations in their implementation in a manufacturing process.

Next slide. As you've just heard, Phil and several other earlier speakers talked about safety measures that can be taken to assure the safety of the source material. There are limitations, however, such as those which we encountered in controlling the safety or quality of human plasma. Test methods always have thresholds associated with them and one can only test for one what one knows about and therefore unknown viruses or emerging viruses will escape these sorts of precautionary measures.

Therefore, a second level of safety is built into the manufacture of products such as plasma derivatives which could include clearance steps during the manufacturing of the products which is what I'll focus on today. But there's also a possibility of additional testing during

manufacturing of intermediates or the final product.

A third layer of precaution exists with respect to the use of the product in the field, i.e., the clinical experience. And while not a topic for today's discussion, this forms an essential part of the safety net. One should be aware of the consequences of the use of one's product in order to assure that adverse events are detected early enough that precautions can be taken.

Next slide. As we've all been using the word, clearance includes both methods that inactivate viruses and methods that separate those viruses from the manufacturing product. Individual manufacturing steps can contribute to either, and those steps could include those that are specifically designed and incorporated into a manufacturing stream in order to remove or reduce a viral risk, and they could also be steps that are principally intended to purify a product, but which serendipitously clear viruses as well.

In the ordinary course, each clearance step is validated as to its effectiveness and reliability, independently of the others, although in principle, there's no reason why several steps could not be validated in concert. And finally, that last

statement implies that multiple independent steps within a single manufacturing process could contribute to an overall safety profile for a product in most cases.

Next slide. My list of current viral clearance methods is drawn from my experience with the plasma derivatives and the recombinant analogs. There are other methods that have been used in production of viral vaccines and so forth, but this list is useful enough for our purposes.

Those methods that work by inactivation can be broadly separated into those relying on heating of a product or of an intermediate and those that work by chemically inactivating viruses.

The first successful inactivation method applied to a human plasma derivative was heating the final container of albumin at 60 degrees Centigrade for 10 to 11 hours. John Finlayson mentioned this. This process has since been applied to other plasma derivatives which can be heated, usually in bulk and as a process intermediate at the same or similar temperatures and time.

A variant of this is to take a dried lyophilized product, usually in the final container and heating that material for anywhere from 60 to 100 degrees Celsius for anywhere from 1 to 150 hours,

depending on the temperature. The most common combination, I guess, is somewhere around 80 degrees Celsius for 72 hours, several products in Europe and the rest are so treated, but there are other variants.

Where this is done in the final container, as in the case of albumin, one has the advantage, distinct advantage of precluding the reintroduction of viruses once the inactivation has been performed.

Vapor heating is a method that was developed fairly recently in which a bulk intermediate is lyophilized and then rehydrated to a very tightly controlled residual moisture content and then is heated under controlled pressure for 60 to 80 degrees for the specified time. This again is done in bulk and finally there's an older method. I don't think this is used, I'm sure it's not used in the United States. I'm not sure whether it's used elsewhere, where lyophilized intermediate could be suspended in a solvent and heated under those conditions.

Chemical inactivation methods that are most frequently used is the so-called solvent detergent method. In its most frequent application it involves the use of an organic solvent called

tri-n-butyl phosphate and one of the number of nonionic detergents. This has been very successful in reducing the risk associated with envelope viruses, but because it works by dissolving lipid envelopes it's ineffective toward non-envelope viruses.

It was recognized early on that for very fragile viruses, fractionation by alcohol during the basic production of some of these products can also inactivate to a limited extent some of these viruses and another production method, the use of low pH or the low pH in the presence of pepsin, is included in the manufacture of some intravenous immunoglobulins, and this procedure has a certain capacity for inactivating some viruses.

The removal steps include partitioning, which is an example of steps that are designed primarily to purify the product, or nanofiltration, which is a relatively recent advance in the filtration field which uses membranes with a small enough pore size so that viruses can be excluded from a product small enough to pass through them. Some of these membranes also may work by partially adsorbing viruses, but that's an ancillary mechanism.

The purification steps can be -- well, for human plasma derivatives ethanol fractionation is

still the foundational method for purifying these proteins, and some limited partitioning of viruses has been shown during the crude fractionation of the paste from which these products are made. But other precipitation steps analogous to the cone fractionation may exist in other product categories depending on the method of production.

A more sophisticated, perhaps, method revolves, involves chromatography and because this tends to be a higher resolution technique, in general, some more robust clearance of viruses can be demonstrated in some cases.

Next slide. There are, as I mentioned, other viral inactivation methods. This is an incomplete list of some of them. They fall generally into the categories of irradiation techniques, other chemical inactivants and photochemical techniques that might be thought of to be a hybrid of the two.

Some of these methods such as the use of beta propiolactone are old, but still may be useful. Others like ultraviolet or ionizing radiation have been tried in the past unsuccessfully, but there's renewed interest in these methods and they may yet be adapted successfully to inactivating viruses and biologics.

There's a lot of interest these days in

photochemical methods, particularly because of the possibility that they may be useful for inactivating viruses present in cellular components from blood.

Next slide. And it's important for these methods to bear in mind that there are two basic mechanisms by which they can operate. One is the so-called direct reaction or Type 1 reaction where the photosensitizer such as a psoralen is activated and then directly reacts with its macromolecular target, in this case nucleic acid which psoralens, of course, are capable of cross linking either between or within the strand.

A second very different reaction occurs with these photosensitizers. They work by being activated by whatever light is being shown on them and then giving up the photon to produce a reactive oxygen species. That singlet oxygen then reacts with the molecule that's the target for the inactivation. I'll get back to the significance of these two mechanisms a little bit later.

Next slide. Okay, in any of these methods when one is thinking about implementing them in a manufacturing process, there are three fundamental concerns, I think, that one must take account of. First, of course, is the compatibility with the product. It does no good to inactivate all

the viruses in the world if you kill your product in the process. Secondly, one must consider how effective the method is, and that's done by validation studies on the small scale showing how much of viral clearance capacity a particular method has.

Finally, the reliability of the method in a production environment also has to be demonstrated and that's done by process validation and the application of GNPs. Now you've heard something about this, these two before. I'm going to repeat some of that, but since I already had the slides made up it's my bad luck. Starting though with the compatibility of the product, next slide, it's good to bear in mind that methods that inactivate viruses do so by inactivating what is basically a super molecular biochemical complex, so many of these methods will inactivate a protein product just as easily as a virus. And this can happen, these bad things can happen by a number of mechanisms.

First is, of course, simple thermal denaturation that you may encounter in heat inactivation methods, but also in methods that rely on irradiation.

A second common adverse effect would be chemically modifying the product and this is, in

fact, most possible with most methods although they're usually associated with chemical methods of inactivation.

Free radical oxidation I've suggested may be a problem, especially with radication and photochemical methods. When you are generating reactive oxygen species, that species is terribly indiscriminate about what it oxidizes and it could be your product.

And finally chemical contamination is an issue that has to be addressed when one is introducing potentially toxic or mutagenic chemicals into a manufacturing stream, and there must be some assurance that those chemicals are removed or converted to non-toxic forms by the subsequent manufacturing process.

Next slide. To demonstrate that a viral inactivation technique is compatible with the product one must first consider whether one is dealing with a new product in which the viral inactivation step is part of the manufacturing process from the get go. There, the preclinical and clinical studies that one is doing already for licensure should be designed to show that the product is safe and effective, and so the question of the impact of a viral clearance step is incorporated into those -- into that undertaking.

However, there's a different problem that emerges when one takes an existing product and method by which it's made and tries to change that manufacturing process to include a new step to remove or inactivate viruses, and there, the challenge is to demonstrate the comparability of the product made by the new manufacturing method to that of the licensed precursor.

One can do that on any of three levels, depending on the perceived level of risk. If one is capable of doing a detailed chemical or molecular characterization of the product, one can compare it in great detail before and after the change was made. And if comparability can be established by that method, one is home free.

In many cases, though this degree of characterization is not possible, either because the molecule itself is terribly complex or the product is a rather complex mixture of biochemicals. One might then have to proceed to in vivo studies using a relevant animal model, for example, to show that the behavior of the product is not altered.

However, it is not always possible to identify an animal model that is sufficiently predictive of the behavior of the product in humans, and in that case some sort of human clinical trial

may be required to establish comparability after a major manufacturing change.

Next slide. Okay, moving on to demonstration of the effectiveness of a viral clearance step. I usually think of this as breaking down into four basic operations. One is the necessity of establishing a scaled down laboratory model of the production process. This is because it is usually undesirable to introduce large quantities of virus into a manufacturing facility, so one usually does this in the laboratory.

Most of these viral clearance validation studies are done by spiking very high titers of virus into the product and then measuring the reduction of that virus by the subsequent manufacturing step. One quantifies this reduction and then compares the reduction of viral challenge to the anticipated risk associated with the product. So I want to touch briefly on each of these four.

Next slide. First of all, the clearance method has to be scalable for this paradigm to work. And again, one faces different challenges depending on whether the product is a new product, in other words, if one is developing a manufacturing scheme from scratch, or one is trying to introduce a new manufacturing step into an existing production

process.

The design of the laboratory model in any case should include all of the critical procedures used at full scale and it should adopt the production methods as far as you can. This is not always possible. Production methods are not necessarily directly scalable, but where methods have to be modified, the impact of those modifications ought to be addressed.

One needs to identify all of the critical parameters by which the process is either controlled or evaluated and those need to be controlled in the laboratory scale down study. And among those would be relative values such as volumes or geometries that of necessity change when one scales down the process, or

absolute values, such as time and temperature, which should be carefully controlled as absolutes.

Next slide. The sine qua non of validating the scaled down model is its performance. This is usually established by making multiple runs of the scaled down laboratory model and statistically comparing its outcomes with the manufacturing history, if one exists. The purpose of that is to show that the two, the laboratory and the full scale method, are substantially equivalent. There are very

often differences, and these need to be carefully evaluated to assure that they don't affect the predictability of the laboratory scale result to the effectiveness of the production method.

Next slide. Moving on to the spiking itself, one first has to select a virus to use in such studies. As I said most are done by spiking experiments, which is made possible by two technical requirements First is the availability of high titer stocks to add to your product, and such stocks do not always exist for each and every virus of concern. And secondly, there must be viable methods for quantifying those viruses and that usually means the ability to grow the virus in a susceptible cell culture model system.

We've already heard about the distinction between relevant and model viruses, and the point Hennelore made about all viruses that are available in the laboratory being, in fact, model viruses is well taken. But in any event, the viruses should be selected when model viruses are used, should be selected by either similarity to a known risk that one is trying to evaluate or for a rather broad spectrum of characteristics such that the viral safety in the blood of a product in a broader sense might be established.

Next slide. The quantification of the viral reduction when a spike sample is subjected to the manufacturing step is most often done by infectivity assays. I almost said "always done by infectivity assays", but there is, in fact, a lot of interest in adopting biochemical assays such as PCR or reverse transcriptase to quantifying virus during these steps.

If, however, one is to use a biochemical surrogate, if you will, one should consider carefully establishing the relationship between the biochemical surrogate and infectivity itself. Because the intact infectious virus is what is relevant in these studies.

Depending on the nature and characteristics of the viruses, plaque assays which are quantitative or limiting dilution or end point assays which are quantile in nature can be used and have been used in the past. And within these general categories of assays, a number of general characteristics should be considered, things like number of replicates that are included in the assay and the size of the dilution steps. Both speak to generating sufficient data for sound statistical analyses.

The experiments should include positive

controls to guarantee the recovery of the initial spike and to eliminate the possibility that the test article itself interferes with the assay. And the clearance study should also include appropriate negative controls to assure that the assay has the requisite specificity and the test article isn't, in fact, toxic. But within these constraints there have been a wide variety of assay designs that have been successfully used in the past.

Next slide. Well, once one has accumulated all this data, one can calculate a clearance factor, i.e. the reduction in viral titer that the manufacturing step achieved, and then compare that with an anticipated risk if that is known. For human plasma derivatives, for the major viruses, this is known. In some other cases, I can imagine that it may not be entirely defined what the risk is.

A safety margin is calculated by this comparison which is simply the excess capacity of the manufacturing process over the level of the anticipated risk.

Next slide. Now more than one clearance process can be, clearance step, can be included in the manufacturing process. If those two steps are very similar, they can't be relied on to add

additional safety over and above each other. However, if the clearance steps are based on some independent operating principle, they can be combined to yield what is usually referred to as accumulative log reduction factor. Examples of this are, is the combination of results from removal or inactivation steps such as heat and nanofiltration, or two or more steps of the same type, such a solvent detergent and heat provided that they work on different operating principles.

Next slide. This is an example from one of the U.S. Factor VIIIs. This series of studies actually performed eventually with six viruses of various ilk and three steps were validated, a chromatography step, solvent detergent treatment and dry heating of the final container. Each of these steps is sufficiently different from the others that the contribution of each of them can be considered in calculating a cumulative log reduction factor for the product. So this is an example of this principle in operation.

Next slide. Okay, the reliability of the method, as I said, depends on the first instance on full scale process validation. The whole purpose of process validation is not to reestablish the effectiveness of the method, but simply to

demonstrate that the production process is adequately controlled. That means that the operating parameters that you've identified is important in laboratory can, in fact, be controlled to within the specified tolerance in the manufacturing facility, and that when those parameters are so controlled, the product that has the required quality attributes can be consistently made.

Next slide. In order to carry out a full-scale process validation study, one needs to know what one is trying to accomplish. That means defining the requirements and goals of the process, identifying and specifying the critical parameters that are used to control and to evaluate the process. One then takes this information and the procedure itself and develops a steady protocol to evaluate the process, executes the study and analyzes and evaluates the outcome. A fairly straightforward undertaking, although complex in application.

What one needs to know is everything that one can about the process and the product, and one needs to define what controls are needed, what parameters need to be controlled and used to evaluate the process in ordinary manufacturing.

Next slide. In a setting other than viral validation, process validation can often be

used to define or refine these operating and process parameters and establish or refine the valid operating ranges. However, for a viral clearance step one is constrained by the process that is defined in the laboratory clearance study, and one needs to control these parameters to within this predetermined range in order for the laboratory viral clearance validation study to be relevant to the manufacturing process.

Manufacturing of a product can extend for many years in essentially the same form, but there may be instances where the need to revalidate a manufacturing process arises. There are some reasons listed on this slide. When major changes are made to the equipment procedures, materials or the product itself, one has to consider whether revalidation is necessary, whether equipment malfunctions or process failures, unexpected nonconformities of the product, that may signal a need to revalidate processes. Variability in outcomes, stability test values or AERs, or complaints associated with a product may also be danger signals that would trigger a need to revalidate.

Hand in hand with process validation is a more general collection of precautions known as good manufacturing practices. This is a whole other talk,

so I won't say anything more than what's on this slide. The goal of good manufacturing processes is to assure the consistency of manufacture of a product with its required poly attributes. Consistency is the key here.

So the facility and equipment that one uses has to be appropriately designed and qualified. Adequate written procedures have to be in place and followed. The processes have to be controlled by in-process measures and specifications that define successful outcome, and where the unexpected happens those deviations and failures should be completely investigated and resolved.

Next slide. This is sort of a transition slide. Everything that I've said has developed from practical experience in dealing with risks of human viruses, particularly in the manufacture of plasma derivatives. One would think that these principles hold true as well for other agents such as spongiform encephalopathies, but there's a great deal of uncertainty as to the truth of that proposition. We are restricted in some measure by lack of knowledge and lack of technology. We have no useful convenient and accurate screening method for these agents. Current infectivity assays, using laboratory animals are time consuming and expensive and generally aren't

used in the field. There are no known methods for inactivating TSEs that are compatible with manufacturing biological processes, although clearance, during purification, i.e., by removal has been demonstrated for some products.

Probably the best precaution that one can take during these days is to exclude VSE or scrapie endemic areas from sourcing animal materials. Similar precautions were taken in the human arena by restricting the UK donors, people who have resided in the UK for six or more months, from donating plasma. But the application of TSE clearance methods is still somewhat in the future.

Next slide. But this is not so for other viruses that one may encounter in animal source material. So in the last couple of minutes I want to touch on how one would implement some of the considerations that one ought to keep in mind when one is considering implementing any of these techniques.

Heat is, as I said, one of the first and most broadly applied viral clearance methods. Here, it's critical that the temperature that is known to be effective in inactivating viruses is maintained uniformly throughout the product or the process intermediate over the specified time needed to fully

inactivate the viruses that may be present.

The heat inactivation can be carried out on final containers or process intermediates. Again, pasteurization of albumin was one of the first, but one can terminally dry heat final containers of lyophilized products as well. When one is heat inactivating an in process intermediate, one is usually working with a far larger volume of material and the control of temperature uniformity becomes a major challenge. If one, for example, is using a large tank and a liquid intermediate, the temperature profile of that tank has to be mapped carefully and controlled consistently during use.

Dry heat and vapor heating very often require longer times and higher temperatures to achieve equivalent inactivation levels, and it has become apparent that the amount of residual moisture in a lyophilized intermediate that is to be virally inactivated by heat is an important, if not critical, variable.

Finally, most -- many biologics are inherently instable under heat, and stabilizers have to be used to preserve biological activity. Of course, stabilizers can stabilize viruses as well as product, so a careful balance has to be struck between preserving the activity of the product and

inactivating the viruses that one is afraid may contaminant the product.

Next slide. Chemical methods of inactivation rely on exposure of the virus to the chemical. So it's critical that the chemical be mixed into the process intermediate uniformly. If one cannot maintain the minimum effective concentration throughout the solution for the entire inactivation period, one cannot rely on the effectiveness of the inactivation technique itself.

Many of the chemicals that are used or considered for use are toxic or mutagenic or they may give rise to toxic and mutagenic by-products during the reaction. These need to be carefully considered in order to establish reasonable extents to which they must be removed before the final product is used.

Also, one -- in establishing standards for residuals of these contaminants, one should also consider the extent to which patients who use a product will be exposed to that product, so for example, a product that is used regularly for a lifetime, such as coagulation factor, could pose a greater risk of cumulative exposure than a product that may be used only once or twice in a patient.

And finally, there is always the problem

of derivitizing the product itself by the chemical reactant, and one should carefully examine the activity, bioavailability and immunogenicity of the product.

Next slide. Radiation technique which is really still in development in most cases, again, uniformity in terms of exposure of the product to the source of illumination is important, perhaps less so for gamma irradiation than it is for UV and visible techniques. In these cases, methods have been devised for illuminating very thin streams or films of the product in order to achieve the necessary uniformity.

Heating effects are secondary and not the basis for effectiveness of these techniques, but have a large potential for inactivating or damaging the product. Many of these effects can be controlled by controlling the rate of irradiation or the environment in which radiation is carried out.

I mentioned singlet oxygen production before in the context of the photo inactivation techniques. In the type II reactions where singlet oxygen is, in fact, the basis for the technique, about the only thing one can do to constrain the risk of oxidizing the product is to localize the reactant itself, the photosensitizing agent. But in other

cases it may be possible simply to perform the photo inactivation in reduced oxygen or water environments which are the source of the singlet oxygen. And of course, the photo chemicals, if one is doing photo chemical inactivation, or the derivatives, raise many of the same chemically related issues as I showed you in the previous slide.

Next slide. Chromatography now is a more benign technique. This is a separation technique, but it tends to be rather complex in execution. A lot of parameters have to be considered, relative volumes, flow rates, solution, volumes, back pressures, things like that. Quality of the resin with which one packs a column is important, so it's a rather complex series of parameters that should be considered in a chromatography step either as a purification tool alone or as a purification tool that's been validated to clear viruses.

Many of these chromatography resins are expensive and there's a tendency, understandable tendency to reuse them, but if one is to do that, one faces a dual challenge of validating the continued effectiveness of the column as a purification tool and as a viral clearance tool and this has posed difficulties in the past for some.

And finally, if one is going to re-use a

column resin, many times in some cases, one needs to have effective cleaning and regeneration procedures in place to prevent the build up of infectious material and other contaminants on the resin as it's used.

Next slide. And the last example is nanofiltration. This technique has the virtue of being very well understand, having a very well understood mechanism and also being rather benign to the product and relatively straight forward process controls in terms of operating a nanofiltration step. And for these reasons it may be the easiest of the viral clearance methods to incorporate into an existing process.

However, one has to recognize that the effectiveness of nanofiltration is somewhat limited for the smallest of viruses and if one is making a protein of very large size, very high molecular weight, one's choice of an effective nanofilter membrane is constrained by the fact that your product may not be able to go through.

And I think that's all I have to say. So we have a break next?

(Applause.)

CHAIRMAN HEINTZELMAN: We continue on a little ahead of schedule. That's great. We'll have

a break now, 15 minutes. I show 1:50. So what do you say 2:10, make it a 20 minute break. At 2:10 we'll reconvene.

I'd like to remind you that in the packets that you receive when you picked up your little name cards, there's an appraisal form and I would very much so appreciate it if you would fill it out when we're done with the day. We benefit greatly from hearing what you have to say and it's an effort to constantly try to make these workshops more useful to you through your own feedback.

I was asked if you have to include your license number or your IND number on those. The answer is no. Your anonymity is just wonderful and your truthful statements are greatly appreciated.

So let's take a break and we'll get back at it at 2:10. Thank you.

(Off the record.)

CHAIRMAN HEINTZELMAN: Okay, well, if we could get set to go. I wanted to introduce our next speaker, representing the National Hemophilia Foundation. We have Dr. Keith Hoots. Keith is a Professor of Pediatrics at the University of Texas, at M.D. Anderson Cancer Center. He's a Professor of Pediatrics and Internal Medicine at the University of Texas, Houston Medical School. He's a Medical

Director for the Gulf States' Hemophilia and Thrombophilia Facility and he's the Vice Chair for the Medical and Scientific Advisory Committee at the National Hemophilia Foundation. Keith also serves as a member on the Advisory Committee on Blood Safety and Availability which I pointed out to him was recently on TV on C-SPAN so that for those of us that weren't able to attend the hearing, you could catch it on the tube.

So here's Dr. Keith Hoots.

DR. HOOTS: Thank you very much, Dr. Heintzelman. It's a pleasure to be here and I appreciate the invitation. I'm here under the aegis at least of National Hemophilia, but what I'm going to say pretty much reflects my perspectives rather than any institutional perspectives and what I thought I would do, I actually wanted to hear some of the presentations before I finalized what I was going to say and I'm hopeful that that will be beneficial to you. It certainly has been beneficial to me because it reiterated in my mind part of what I thought was the situation with non-human derived products and it also left a few avenues of at least for me to raise, I think, that have been partially alluded to and perhaps might be at least a little provocative for some discussion.

So I thought I would entitle the remarks, "Safety Vigilance and Total Quality Improvement, Lessons Learned from Human Derived Plasma Products." It sounds a bit presumptive, I think for me to talk about all the lessons we've learned from human plasma derived products, but I have at least lived through many of the errors that John Finlayson talked about this morning in terms of the impact, particularly as it relates to people with bleeding diatheses, but also not exclusively so. I mean I've been involved with HIV care of osteosarcoma patients who were transfused with single pack red cell units in 1983, so the impact is certainly something I'm very conscious of and I think very close to what I've done over most of my career.

And I think it's probably maybe a little trite, but apropos but in this particular conference that we start by using the avian metaphor that the hemophilia population ascribes to itself which is the canary in the mine shaft for blood safety. This has taken on everything from I guess sympathetic terms to sometimes almost pejorative terms, but it is important because obviously if you think -- it was Dr. Lynch who alluded to this in a previous talk. If you think about the number of individuals that the average person with hemophilia is exposed to if they

use plasma derived clotting replacement products over a lifetime, it's inordinate. An average lot of Factor VIII contains 60,000 donors per pool and so you extrapolate the fact that perhaps an average person may use anywhere from 3 to 10 lots per year for a lifetime. It's huge. Obviously, that's been modified more recently for some individuals who have come to rely on recombinant factor and I'll talk about some of those issues as we go along.

The evolution has also kind of taken on its own rubric, as it were that purity is better. There's been actually debate about that. I mean it's intuitive, I think, that the more pure things are the better off they are, but safety doesn't always necessarily comigrate with purity, but certainly as we got from Dr. Lynch's previous talk, in many cases it does because if you remove extraneous risk factors then simultaneously -- and purifying your final end protein or your final end product, then it makes sense that you'd get there a little bit better.

So what I thought I'd do is mention four safety or four basic principles that we discuss in one way or the other on the Committee for Blood Safety and Availability almost every time out. And you've heard most of them already talked about in far greater detail than I'm going to discuss them this

afternoon. The first is screening, testing, quarantining and pool size, all of which have to do with surveillance for blood safety. The second you heard just discussed in great detail, clearance, attenuation, spiking experiments. The third we don't really think about in terms of animal derived products, but I want to bring it up, again, trying to see if there's any lessons we can learn from the human plasma derived situation which is retrospective identification. And I'll go into that one in just a moment. And finally, one that we don't usually think about very much in detail either, related to animal derived products and that's availability. But I hope I can give you an example from my own experience in the hemophilia community to let you know that the evolution of safety still does run smack dab into availability issues on occasion. And it's important as we implement new strategies to enhance safety that we keep that in mind.

So with regards to screening, I mean you have heard from experts in the field, the types of viruses that need to be screened for depending on the animal source of a product, so I'm not going to reiterate that. Testing and quarantine, we've also heard discussed. I think taking my hat as the prescriber of a product, I absolutely implore that

every bit of testing be done that can be done and every bit of screening can be done with the caveat that I'm well aware that that adds to the cost. But if there's anything that's even remotely considered at risk or if it's a surrogate for something that might be at risk, strong consideration I think has to be given to doing it, again, without completely throwing out the baby with the bathwater by making the cost so prohibitive that you end up with no product at all.

Pool size is an interesting issue. Generally that's a human plasma derived issue, but I think I can tell you, point out some times where at least on the human side it may have some analogies to animal derived proteins. On the Advisory Committee, we spend a lot of time with constituent groups discussing the optimal pool size for plasma derived products. If you're a mother of a child with severe combined immune deficiency and you need intravenous gamma globulin or A gamma globulin and you need IgG, you want a pool size that's relatively large because you want to have a lot of phenotypes of antibodies so that your child is covered to the broadest array of diseases in the environment. By contrast, if you're the mother of a child with hemophilia, you want the smallest pool size that you can get because for you

it's a pure and simple safety issue. The fewer exposures you have per lot, theoretically, the safer things are for you if you're using a plasma derived product.

And clearly, then you arrive at issues of competing risk. And one of the ways that we address that on the committee was to make a very strong recommendation to Dr. Shalala that she consider making recombinant factor products available for all people with hemophilia and all constituencies for which there are available presently which would thereby remove their competing risk out of the situation and allow them the optimal decisions to be made on behalf of the constituencies for which a recombinant product is not feasible like intravenous gamma globulin, because there, with 10 to the 15th potential phenotypes that you need to have, you couldn't possibly have, at least theoretically, I don't think, possibly have a recombinant product that would work.

Well, does that have any relevance to the situation with animals? Well, I think it may, but it probably doesn't have to do anything with competing risk. It probably has to do with what I'm going to get to in a minute which is retrospective identification. And trying to figure out, perhaps,

hopefully never, but if it's necessary after the fact what may have happened from a product. The larger the pool size and the larger the heterogeneity of the source, the more difficult it is to track.

Let me to come to that for just a minute because I just want to lead right in before I do that to talk, just mention about clearance attenuation and spiking. Here I'm putting purely an advocacy cap on. And saying that the technologies that we have that are proven should be utilized regardless of the source material. That's my opinion. Anything that has proven scientific efficacy for reducing X number of logs of Agent X that has any remote resemblance to a human virus or an animal virus that could potentially become xenogeneic should be implemented.

So that said let's skip ahead then to talk retrospective identification. This is where the question of pool size may come in. Ordinarily in retrospective identification in human tissues of blood safety we talk about look back. We are really trying to target who is at risk from some donor. Well, obviously, we're not worried about the source animal. That animal is long since gone, sacrificed to get the product in many cases, if not, at least, would be sacrificed in lieu of if there was any question about risk. But the product for that animal

could be very important to identify some, as yet, identified new disease. If there was any remote idea that it could have originated therefrom. One of the principles that have been applied in human technology is the concept of mini pool matrices where manufacturers can pool the plasma from several individual humans, put it into a pool that is then from which aliquots are collected and saved, and from which screening and testing of all the targeted viruses and other pathogens are done and then it's mixed into a pool and then the larger pool is then TSE retested and if you get anywhere along the upscale of the mini pool testing that there's a positive for any serologic event, you have the advantage because you know exactly when the small component was added in and you can go back and quickly arrive at a potential source for it. That's probably not going to happen to the same degree with animal derived stuff, but one of the things that we learned very, I guess, poignantly from HIV in the hemophilia population was the benefit of having stored sera and in the case of animal products I would say -- I would make at least a plea for consideration that some sort of stored source material that's been itemized and frozen away from at least a mini pool if not from individual animals be

done, so that in some of these identified even remotely suspicious to have originated with a product that's derived from an animal source, you can work backwards and you might have then material for which you can exclude using that testing, a lot of known pathogens, but also then if there's a little material left, you might actually look for DNA sequences that you don't have any idea about or in the cases of TSEs you might not be able to do anything, but you might be able to inoculate it into some host animal that might see if they get diseased. I don't know. I just raise that as a potential because without the sources that, for instance, in HIV in humans that Elaine Istra had in working with Jim Geddert here at the NCI, the information that it took to figure out when hemophilia was first inoculated with HIV wouldn't have been forthcoming for years and it wouldn't have been anywhere near the circumscribed level. In addition, clearly those samples were absolutely key for Montagnier and for Gallo when they were identifying HTLV III and then also for even samples that we collected in Houston served a very important source material for the development of HTLV ELISA by Abbott Labs.

So all those things, kind of a lesson I think that we could learn here. It's not so costly

as it might first seem, particularly if you used a mini pool matrix theory to do that and again, I raise it to be provocative, not because I've had enough real time to go through all the logistics and say that it's completely feasible, but keep that in mind anyway.

Availability, and this is where issues related to total quality improvement come in, I think. The example I want to use is porcine Factor VIII produced by Speywood Labs in the UK. It was first developed in kind of an impure form in the late 1970s, but it caused anaphylactoid reactions in humans. And then a poly electrolyte technology was developed. I allowed most of the porcine antigens to be removed and Factor VIII from those porcine derived sources could then be given to humans. Well, why use porcine if you've got human, if you've got a human disease? Well, because about 30 percent of people with Factor VIII deficiencies who have congenital deficiencies and about one per million per year of the general population who get acquired hemophilia from natural antibodies against Factor VIII need alternative therapies, because you give them human Factor VIII, they'll neutralize it instantaneously and they get no hemostatic effect. It turns out that porcine epitopes are clearly different in many cases

in human, but they still get the same ability to activate thrombin generation and get a clot formation. And we can actually measure prospectively which of our patients that has high response, that is an anamnestic antibody to Factor VIII would predictably respond because the epitopes are different between human and porcine.

I should add that the antibodies that you get in both those situations, either the allo situation like in hemophilia or the autoimmune situation like you get with post-partum or with cancer in some cases, I mean just ideopathically, that is people just show up with Factor VIII inhibitors and suddenly they have hemophilia where there's no family history whatsoever and it's all because of the antibody. But in each of those situations, the antibodies that are produced are polyclonal. They're not monoclonal, so they attach to several semipredictable places on the Factor VIII molecule. But because of that that's why porcine has a very important place in armamentarium.

So why am I telling you all this? Well, the reason I'm telling you that is because this particular situation was borne out by the fact that in the course of really getting this implemented into our therapeutic armamentarium, and becoming somewhat

dependent on it kind of was, took about ten years. And about the tenth year that porcine parvovirus was detected in the source pig plasma and suddenly we had no porcine Factor VIII which was appropriate, because we didn't want to give our patients pig parvovirus. We didn't know if it was endogeneric or not. And the FDA didn't do it. And that was an appropriate rule. It served to point out the fact that surveillance is important. That vigilance and clearance is important and quarantine is important because all those were applied to the situation and finally about a year ago we started getting released lots because the company had implemented a quarantine and a testing procedure using essentially equivalent of MAP for pig parvovirus and excluding all the source plasma from the pigs that were not positive out of the pool. So what that tells you is, or what it says to me is that it is a component of TTI, but at least until the next stage, without going into detail because some of it is

semi-proprietary, but what that did was spur the company on to enhancing the technologies that are in the processes, in the pipelines now for going the next step for purity and even the next step perhaps even to ultimate purity and sequencing. All those things came out of a process that was implemented by

CPMP and FDA that said vigilance is absolutely critical and if you don't have absolute purity, then you have to be ever more vigilant and you have to do what's right and then once you've satisfied at least that the short term risk is resolved, in this case, by showing that all the pigs who had parvovirus are out of the pool don't be satisfied with it, but go on to the next stage and enhance the technology based on the availability of new techniques to try to get an ever more pure, in this case, porcine Factor VIII. But the same would apply, I think, for any analogous situation.

One lesson, I think to be learned from the porcine situation and it goes back to what I was talking about in terms of the quote look back for animals, there's another look back that occurred for that which was because you could do NAT testing for porcine parvovirus sequences the CDC had us in hemophilia treatment centers call in our patients that we knew had had multiple exposures to porcine Factor VIII uninhibited patients, draw blood on them, send it to CDC for NAT testing and see if they had any

anti-pig parvovirus in their serum. Virtually, they didn't. But that's again, once again it points out that the circle does come full when it comes to

surveillance and that the more you have available, if we had already had blood sera we wouldn't even have had to call people back in and we would have the answer in days instead of in months and I think the same analogies might apply.

So those are the issues that I wanted to say in terms of safety/availability and then I want to talk about to kind of -- for the last part of the talk to talk a little bit about animal sources for hemostatic and thrombotic agents that I see why I feel like I have a vested interest as a treater in the issues that have been discussed today.

Well, we've heard about transgenic animals and you saw from the slides that were presented that Factor IX is one of the big targeted proteins to be made from transgenic animals, but since we're -- many of us are now not only hemophilia treaters, we're thrombophilia treaters and because we now know that the genetics of thrombosis plays an incredibly important role in the diseases that were all considered environmental in the old days like heart disease and stroke, but particularly so in young people because young people usually don't get those diseases and they don't get clots, but if they have an inherited defect in protein C, antithrombin, Factor V or combinations thereof, they do. And it

tells you right then that the models that we use for inherited hemostatic disorders probably apply and perhaps the therapeutic models as well, certainly replacement seems to make all kinds of sense and it's already being implemented in terms of antithrombin for AT3 deficiency and now investigationally protein C, inactivated protein C for protein C deficiency. All those proteins could potentially be or actually are being made from transgenic animals. So the safety of those transgenic animals is absolutely paramount because these are in many cases individuals who have never been exposed to any blood product in their life, young children who have a DVT, unexpectedly, and you diagnose them and you need to replace them, at least until they're back in their steady state, until their vessel injury is over. So we want to be sure that those transgenic animals follow to the letter everything that Dr. Lynch talked about and every sort of screening that we could possibly do because they're going to be grown in 30 years and they don't want to wake up one day and have to worry about rib back if we can avoid it. And so screening is important, but if that were, forbid, ever to happen from a transgenic animal which I don't think is likely because their premise of transgenic mammary produced proteins and purification is pretty

reassuring because not only do you have -- start with a pretty pure animal, but then you purify the final product. But let's just say it happened and you at least want to be sure that we've done everything up front to protect it.

Those animals have, I think, yet to be exploited potential as well to reduce risk overall because there are several, I shouldn't say a lot, but there are several pro-coagulant defects that -- we're dependent on human derived products for -- at the present time because there's such a small cadre of individuals that it's not economically feasible to go through all the clinical trials. Even with the drug status that it takes to treat a Factor V deficiency, for instance, so we have to use source material like fresh frozen or solid treated fresh frozen which is one step up, which is good. But an even better step up would be if because of the pharmaco-economics, if transgenics turned out to be easily induced and if the same implementation and investigation to market IND could be streamlined with appropriate safety margins, then it might be cost effective to make a product, a recombinant Factor VIII, a recombinant Factor X for those particular populations which would then take them completely out of the risk factor of any plasma derived products. So I would put that in

as well.

One of the other things that's clearly important to us are excipients that are either animal or human derived. We want neither, ideally, and I should say that we're leaning in that direction with recombinant Factor A. What you may not know is that recombinant Factor A requires in its native molecule requires stabilization. By and large, that's been done with human serum albumin and so even though as you heard this morning HSA has been a remarkably safe product with particularly the onset of the CJD etcetera, the idea of getting any human source out is considered optimal, if not ideal. So two of the newest products that are -- you have finished IND and are really the PLA level include a B domainless Factor VIII. There's a truncated form of the molecule that doesn't require such stabilization and one in which there's an alternative stabilization made in sucrose instead of albumin.

Now each one of those results in an ever purified product. I would say that we continue to explore whether it's animal or human derived plasma sources, any sort of stability things that can be implemented that avoid extra risk should be undertaken. Again, cost being an issue and if it means the person can't get a product they need to

stay alive, then you take the risk. But if it means you can work out a pharmacoeconomic model that's okay, then obviously you eradicate the risk if you can.

That also applies actually too, in terms of the vectors that are being made for gene transplant -- as you probably know there are three trials ongoing for hemophilia A and B respectively in the U.S. with the first gene transplantation. One used adeno associated viral vector and one used an ex vivo adeno viral, excuse me, a nonviral construct for transduction and the other one uses an adeno viral, anyway, and their work also on antiviral vectors as well. Obviously, all those vectors are prepared in cultural systems and it's very important, obviously, that the excipients that go into those culture systems be as pristine as at all possible and if the growth factors can be derived from non-human, non-animal sources they should, but if they are to be derived, if they're absolutely essential that along the way they need either/or that those excipients go through very, very rigorous quality control to make sure, even though again purification hopefully would remove some of risk.

We heard discussion previously about TSEs and certainly for the blood safety committee that's

been a hot issue and certainly for the -- and for the committee on safety and availability as well. We have -- the recommendation as you heard has been to exclude donors from the UK, both in the United States and in Canada with certain arbitrary limitations on how long they've been there, trying to balance off the risk of safety with the inevitable impact on availability.

Certainly TSEs give the greatest pause, I guess, at least in 1999, to me, about animal source material. It's disconcerting to know that a TSE can go from scrapie to bovine to human in some sort of what seems to be a fairly rapid succession of events at least over decades, if not over years. And I guess if it can happen once, it can always happen again. And those are the ones particularly that are in -- I think we have to be very insightful about how, what we've learned already and certainly what we need to know which is being able to screen for variant, but also to implement strategies both that involve all the issues you've heard about today, to reduce the risk that that could happen if another TSE were to jump species, but also so that we can quickly take what we've learned with variant, all learning about how to screen for a sequence that we don't know what the sequence is yet and for the next time

around, hopefully implore that quickly in the screening processes for not only variant, but perhaps it would be a surrogate for other TSEs as well. Who knows? But that's kind of out there.

So those are the issues that I wanted to raise. I think it's been an incredibly interesting conference from a clinician's s point of view because I obviously spend lots of time worrying about plasma derivatives and recombinant products and traditionally, except for porcine Factor VIII, less time on animal derived products because of what I do, but the reminder of how important porcine Factor VIII has been for selected patients and the fact that we would, many of those patients can bleed to death without a product like that is a true reminder that you're doing is important and why the safety associated therewith is also exceedingly important.

Thank you.

(Applause.)

MR. BABLAK: Good afternoon. I'm Jason Bablak. As they're getting my slides ready here, I am Director of Regulatory Affairs for the International Plasma Products Industry Association. And having technical difficulties at the moment. There we go.

We've been asked to give kind of an

industry overview as to what our experience has been with viral inactivation and perhaps what lessons can be learned from the plasma industry to be taken to the industry that uses non-human source materials.

Next slide. Just as an overview I'm going to go through a little bit about who IPPIA is, what our members are, what we do. Then I'll talk about our industry experience and give some information on a particular case and then I'll give a little information on Factor VIII and how that's happened over the years of introduction of different viral clearance and inactivation technologies and the effect that's had. And then we'll summarize and I guess we're going to save questions for the discussion at the end.

Our members, we have four members: Alpha Therapeutics, Baxter Health Care, Bayer Corporation and Centeon. Together, these four members produce approximately 80 percent of the U.S. market and about 60 percent world-wide. So even though there's only four members it's a large chunk of the entire world market.

As I stated earlier, we use human source material. Virtually all the plasma is actually source plasma which is collected through a process called plasmapheresis where the whole blood is taken

out, separated in a machine and then the red blood cells are put back in and the plasma is collected. Usually between 600 and 800 milliliters per collection. These are commercial donors, so they are -- and they're able to donate more frequently than whole blood donors, so they come back approximately one or two times a week as opposed to I think the whole blood is 58 days.

Really what we do is we separate the therapeutic proteins from the rest of the plasma and we end up with products such as albumin, coagulation products, Factor VIII, Factor IX and some of the other specialty products, immunoglobulins, IVIg, some specialty immunoglobulins and then there are other specialty products as well, such as the alpha 1 proteinase inhibitor.

Something that's interesting about this is this is a relatively old industry when you compare it to other biotech companies and so a lot of these facilities and processes are existing and so the new technology is placed over top of the existing technology and that can -- while it's beneficial in certain ways, it also has some problems of getting the equipment licensed, getting everything up and running and the effect that it has on the market both in costs and in availability.

Next slide. As an industry representative I always have to put this slide in to preach the good things that we do. Some of the things that we've done above and beyond what the FDA has required, the qualified plasma program which actually Barbee Whitaker who is going to talk about me is going to go into a lot more detail, but that's really a way of managing the source material. Some of the new things that we're working on besides that, nucleic acid technology testing for the three main viruses, HIV, HBV and HCV. The industry has a voluntary commitment to implement testing for all of those by the end of the year 2000. Hepatitis C has already been implemented. HIV should be implemented by the end of this year and then HBV by the end of next year.

We've implemented a voluntary pool size limitation as Dr. Hoots was saying earlier. There was a maximum limit of 60,000 donors per finished product and that was something that was again done voluntarily by the industry to respond to consumer concern about donor exposure and also to get a better handle on the manufacturing process itself. Patient notification. We've also implemented a voluntary system that allows us to keep a registry of patients who voluntarily want to be notified if there are

withdrawals or recalls and we can immediately get information to them if there is a need to do that.

This one slide actually could be my entire presentation because this is the industry experience with viral inactivation and I'll just read part of that. There's been no transmission of HIV, HBV or HCV since the introduction of screening tests and inactivation procedures in the United States when these procedures have been done properly. I think that is a very important statement. This was an FDA person, Dr. Ed Tabor at one of the BPAC meetings and this really goes to show how powerful this kind of process can be and how important it can be.

Next. Another interesting comment. The GAO, General Accounting Office did a report for Congress and they came up with this statement, viral clearance techniques have made the risks of receiving an infected plasma product extremely low when manufacturers follow the procedures in place to insure safety. So these procedures have had a very large impact on the patients who use these products and they've made plasma products virtually risk free.

Unfortunately, it's not free in terms of supply or dollars and a lot of times when you introduce a technology such as this you can have a loss of efficiency, so in manufacturing you end up

with a lower yield from your starting material. To the end user, this results in usually a higher cost in the product and it also has sometimes the impact to limit supply or end supply for a certain time while either process is changed over or other things are addressed to make sure that the product is safe.

Next slide. I want to give you some examples now of how this was implemented with Factor VIII because I had this information and it's a dramatic impact so it makes sense. No treatment for Factor VIII. There's approximately 250 international units per liter without any kind of viral inactivation. As different technologies were introduced, it reduced and sometimes dramatically reduced the yield from the same starting material. So, for instance, with dry heat, you went from 250 international units per liter down to 175 and with pasteurization it goes all the way down to 100, so when you're starting out with the same amount of starting material, it dramatically limits the end product that you are able to sell to the consumers and one of the things that has to be understood is the throughput for a lot of these Factor VIII plants is not changed dramatically overnight and so if you have a facility that can throughput a million liters a year, and you go from 250 international units per

liter down to 100, there's a dramatic impact on supply or there can be.

Next slide. Now one of the things that our industry has figured out is as you get used to the technology and start tinkering with it you can increase the yield and for the inactivation procedures that are in use currently, many of them, the yield has gone back up to the original yields without inactivation or close thereto. So once you get some experience with something, you can usually figure out you can get some efficiencies back by tinkering with it.

It also has a dramatic impact on costs. As you can see in 1983 before there was really any viral inactivation, the price per international unit was about 10 cents. By the time 1988, when all the manufacturers had converted over to very robust viral inactivation procedures, the price had gone up to 34.7 cents per international unit which is a dramatic increase. And then it continued to rise. And it has stabled out since then, but I guess the point is when you introduce new technology it does have an impact on the end user.

In this case, for Factor VIII, it also had an impact on the market. In 1988, when all of the manufacturers had switched over, not only was

there a reduction in efficiency, but much of the earlier product that was not viral inactivated was removed from the market and so you had in 1988 a shortage of Factor VIII. And so this again had a dramatic impact on the consumers, now and in certain instances it may have been a worthwhile decision to do that given the fact that the products may not have been very safe and that's a decision that has to be made based on the new technology that's being implemented and the risk from the previously released material. But this is a dramatic example that in 1988 the market went -- the availability went way down and there was shortages, widespread throughout the country.

The plasma industry that I work for, basically we have a way of dealing with risk to the products through viruses and we break it down into two different ways. First is you work with the donor and you try to limit the incoming viral bioburden. Beyond the requirements that the FDA has set up as I talked about earlier, we have QPP, the quality plasma program which is something, like I said, Barbee's going to talk about that more, but really what that is is a way to enhance the collection of plasma at the collection site. We've also introduced NAT testing which is much more sensitive than testing for

antibodies and so that gives us, it closes the window period and allows us to be even that much more sure about the starting material.

And then on the manufacturing side, you either want to exclude or eliminate whatever residual bioburden might be left and when you do this you have to prioritize based on the risk to the final product, so for Class I risk, these are known, clinically significant pathogens, with demonstrated potential for transmission by plasma derivatives. Class II, known pathogens. They're either clinically non-significant or certainly not as significant as the Class I pathogen and it may resist the effects that have already been put in place to deal with the Class I pathogens. And then there are others, they're either known or unknown pathogens that may theoretically be transmitted through plasma, but there's not enough science or evidence yet to justify putting them in as a Class I.

For Class I HIV, HBV, HCV, for plasma based products, the existing donor screening testing and viral removal practices are effective and result in extremely low risk from these pathogens as has been evidenced by no viral transmission since these efforts have been put in place.

Future activities are intended to refine

current practices and improve cost effectiveness and with that you might also include improve yield, because that's just as important as the actual cost of doing the procedure.

Next slide. For Class II pathogens, these would primarily for us be non-lipid enveloped viruses such as hepatitis A and parvovirus B-19 and with these viruses what we need to do is evaluate the potential for addressing these viruses based on the risk to potential users and the feasibility of success. Obviously, it's not worth spending -- increasing the price of this dramatically if the success you're going to have is only going to be marginal. So one has to justify the increased expense that might be incurred with an outcome of a safer product.

Currently, we're focusing on reducing the risk of parvovirus B-19 transmission by screening and removing high titer donors. The industry has put together a voluntary commitment to put together some kind of standard on parvovirus B-19 and that will be implemented by the end of the Year 2000 as well. And also increased or additional work on inactivation technologies for these more resistant viruses.

Then the other category, a typical example of this would be CJD or the variant CJD.

Here, there's a lot of research going on right now to determine the potential for transmission through plasma derivatives and also the potential for removal. One of the benefits for some of the earlier viral inactivation techniques is that you can get incremental use from that by removing either emerging or unknown viruses through the same procedures that you're already doing for the known viruses. And there's been some studies that show that the CJD causative agent is partitioned through some of the fractionation and also through some of the viral clearance techniques. So that's sort of getting more bang for your buck.

And there's also a need for surveillance programs for users to determine if there are new agents being transmitted or if they're not. That's also beneficial to know.

One of the things the industry has done is gotten together and done some collaborative research and formed the consortium for plasma science which is a for profit company with a goal of enhancing the safety of blood plasma and derivatives. Basically, it's focusing on sterilization techniques for source plasma and this is basically a program that funds research particularly aimed at sterilization and other types of inactivation for

source plasma. The four IPPIA members are the members of this as well, Alpha, Baxter, Bayer and Centeon and the goal here is to find a solution that would sterilize incoming plasma both for known and unknown risks.

And what does this all have to do with non-human source material or why am I here? And that can actually be read two ways, why am I here from a human source talking or why are you all here talking about viral inactivation? Obviously, there's an FDA interest in this and if the FDA is interested learning from our industry, hopefully can be beneficial. Also I read recently in one of the news reports that a baboon liver transmitted a virus to transplant recipient and this was interesting because all the researchers on this case thought that this was not a possibility and basically the quote from there saying it was quite concerning that an animal virus thought to be species specific could be transmitted. So that should give everyone pause to think that just because you think it doesn't happen, doesn't mean it's not going to. And if there are procedures to put in place to assure safety, then shouldn't that possibly be done?

Next slide. Learning from our experience, implementation with existing processes or

facilities. It has an effect on product costs. It has an effect on efficiency or yield. And it also leads one to question the safety of the products that are existing in market that have been released before these new processes have been put in place, so these are all questions that you need to think about when implementing such technology, but the results certainly for our industry have been phenomenal. We have safe products for the known risks and from the technologies that we have put in place, there's a potential to address unknown risks. For example, if the next HIV happens to be lipid envelope, then I think we're all pretty safe.

That's all I have. I guess we're saving questions for the end, so thank you for your attention.

(Applause.)

DR. WHITAKER: Good afternoon. I'm Barbee Whitaker. I'm Director of Standards and Certification with ABRA. ABRA is the standard setting body and the trade association for the source plasma collection industry and I believe that I was asked to speak today to give you some of our experience in an industry developed standard setting program.

The quality plasma program has been in

existence since 1991 and it's the result of many of the needs that we've been discussing here today, the experience that we've had to try to improve the raw materials that we're making our plasma products with. So we established the program in 1991 and I'll get into the details of what the standards are there, but right now we have actually more than 380, about 390 of them, 410 centers certified, so the large bulk of the plasma centers in the United States today are certified through our program.

That means that most of the donations are collected in certified centers. Most of the donors are donating in certified centers and that's about 11 million liters of source plasma annually.

This program has been supported by the NHF and also we've gotten world-wide recognition particularly in recent years with the -- for example, in the UK with the BPL requiring only source plasma from QPP certified centers to be purchased to make their products. And we've also seen some interest from other countries as well.

So a little bit of historical perspective, the baseline that we use to develop our standards is the FDA guidelines and rules so we have this baseline all the centers must follow in order to be licensed, must follow the FDA requirements. So

what we did was put into position a program that built upon that infrastructure. So our program is trying to raise the quality of plasma, but using additional means that are available as an industry, rather than taking what is required of us. So I'd like to really show here that the industry taking the initiative here has allowed us to raise our standards much higher than would be required and we've had a lot of success with that.

It was originally an mechanism to reward the companies that were out with a leadership position in quality and safety and what has happened with that is it's become a de facto requirement to sell source plasma here and world wide. So it's evolved into quite a good program and it's evolved to a position where everyone recognizes the quality plasma program for source plasma.

And lastly, it does provide a framework for establishing new standards so that as new threats come to the plasma supply we can very quickly marshal the forces and develop something that will be, meet in a very responsive manner the kinds of challenges that we see coming up towards us be it a new disease, be it a new quality guideline that's adopted world wide.

Next. So the basic quality plasma

principles under which we've developed the quality plasma program are the quality donors, high quality plasma from those donors, facilities that reflect professional and medical appearances and standards, high quality and well trained personnel and an industry-wide commitment to continuous improvement.

So the kinds of standards that we do have in place in our program start with employee education and training, a community-based donor population, facility criteria and I'll talk about these in a little bit more detail in a few minutes. Participation in the national donor deferral registry, donor screening and education criteria, viral marker rate standards and to enforce that, a biannual inspection.

So how do we develop new standards to keep ourselves in the mode of continuous improvement? It starts with an idea either through the staff of our association or something that has become a threat to the industry or a threat to the blood supply. The association has either the Board approves the idea to develop a new standard or it bubbles up and a functional committee will propose that new standard and some of our functional committees are quality assurance, laboratory directors, medical directors. We have a standards committee which also develops new

standards and these functional committees do the beef of the work. They're the ones who are working and developing out the specifics for the standards, how it would be implemented, how it would be inspected, what are the kinds of operational problems we're going to be dealing with. Then once this committee has developed a good solid proposal for a standard, it goes through what we call our QPP standards committee. And this committee then will talk about much of the operational issues associated with implementing a new standard and what are the kinds of problems we're likely to see, what are the kinds of different situations, since we're dealing with quite a few different members we have things that might be easy for a large corporation to implement, whereas the smaller, plasma collection center, a mom and pop organization might have a harder time. So one of the things that we try to do in the standards committee is to address those issues and to try to make things equitable and yet still move the bar up.

Then finally once the standards committee has finalized a proposal, it goes to the ABRA Board of Directors and if all goes well it is approved and then we have a 60-day comment period for all members and then finally implementation. So in the last year we've had two standards that have gone through this

process and I'll talk a little bit more about those in a minute.

But just to give you an idea, we've also had some standards that have been in existence and that have been upgraded, so not only can we develop new standards as the need arises, but we also can see that once you put a standard into place, you may have reason to raise the bar on that specific standard. So we've added things to our employee education and training standard, updating the training and adding more specific education requirements. With the national donor deferral registry it was reviewed and approved with 510(k). We've enhanced the software significantly. On that note we're planning another enhancement coming up within the next year and we've added the inclusion of other tests, so p24 and PCR or NAT are included in the donor deferral registry now.

And also we've made significant enhancements on the viral marker rate standards, so the viral marker rate standard began with HIV and HBV. We added HCV. We've made several cuts in the levels of acceptable rates for all three viruses and then this year we have made some significant changes in the viral marker standard.

So how do we enforce these standards? First of all, you must be certified by ABRA's QPP

inspectors or inspection process in order to be able to collect QPP plasma. So that's a very potent enforcement of the standards. If you don't meet the requirements when you're inspected, if you don't show evidence that you are following our standards, then you cannot be certified.

In the case that you are certified and have a recertification inspection, then you're -- and for some reason or another you do not meet a standard, you must provide corrective action and evidence of that to ABRA. And if there are -- this is sort of the bigger and bigger stick, if you don't -- if you are certified and you show evidence that you are no longer worthy of being certified, we can push it up higher and higher until we remove the certification from a center.

Next one. So the standards that we have in place are established standards which are the qualified donor standard and that is specifically that a donor must pass two batteries of viral marker testing and donor screening prior to being accepted for use in a plasma pool. So that plasma cannot be sold and used by fractionation, in the fractionation process until the donor is qualified.

We have a community-based donor population. That means that you must reside within

125 miles of the donor center and you must provide proof of that residence before you can donate. The national donor deferral registry is a national data base into which all repeat reactives for any of the -- - for HIV, HBV and HBSAG as well as PCR positives are put into this data base and so that any time a new donor comes into a center, they're checked against the NDDR to see whether they've donated before and possibly have been positive. So centers are required to reject donors who are in the NDDR.

We require drug screening for drugs of abuse. We, as everyone, exclude high risk donors, but we also provide, require an assessment of donors comprehension of those high risk questions, so some companies use a quiz. Some companies use a video. There are a couple of different interview techniques that we try to make sure that the donors really do understand what they're answering.

The personnel training requirements and then facility criteria which go into ventilation, floor and counter surfaces and things like that.

So those are the established standards. Within the last year we've developed and implemented two new standards and I guess I shouldn't call the viral marker standard a new standard, but it's such a significant update to what we had before that we

really do consider it the new viral marker standard and then also the QA program.

So to begin with the viral marker rate standard in 1991, we required that all centers report their HIV and HBV rates on repeat reactives, repeat reactive rates for both applicant and qualified donors, all their donors to ABRA for a six month period prior to certification. So our standard way of enforcing this standard was to review their data for their six months prior to their application and then if it met the criteria, our cut off, then they were accepted on that standard.

The standard was based on the industry mean plus two standard deviations. In 1993, we added HCV. We lowered the rate for HIV and HBV in 1993 as well and in 1995 we did it again. And then this year we came out with a standard based on qualified donors. So those are donors who have donated at least twice with negative test results on those donations.

So this standard that's in place now is the qualified donor standard and it's confirmatory testing. So as I said before, our previous standard was based on repeat reactive results and this is confirmatory testing. So it takes out a little bit of the false positives.

It's based on collection center volume, so it's more fair to a small center than -- or equally fair to small and large centers. The previous standard of a very large center would have an advantage over a small center because one positive would mean a much lower increase to their rate than with a small center.

So that was one of the things that we did. We believe we made it more equitable. The assessment here is on-going. So in the past we would take six months of data prior to recertification or certification and now we require that centers report in on a monthly basis. So we have much better control and knowledge of what's going on in the centers.

We use a reference rate which is the industry average and then we apply a Poisson distribution to that so that we can develop alert limits that are based on collection center size.

So the requirements for passing the viral marker standard now, 1999, centers must -- companies and centers must participate in the viral marker data submission process so they should be sending us on a monthly basis their test results. And then they must be below our alert limits which are based on the size of the collection center and then in the center they

must have a mechanism for handling corrective -- providing for corrective action should they exceed that alert limit. So they should be thinking about what am I going to do if I'm out?

So this is just an example of what we, the way that we implement the standard will provide some information for the center so that they can see -- we have a one year, let's see if I can do this. We have a one year period here. Our review periods are six months. We have a three month interim period and we provide feedback to the centers, to the companies, so that we can insure that the data that we have in our data bases is accurately representing what their situation is.

So at the end of six months we close the data collection. We do an analysis to see who is at the alert limit, who is -- which centers are in jeopardy and then so on. This is the standard review cycle so we go through six month period and we do reviews and we communicate with centers.

And the next slide, you can see this is what would happen if you had a center that was out so that -- let's say a center was -- did not meet the alert limits. They would be required to provide a corrective action plan within 30 days and then have six months within which to get themselves back in

order and below the alert limits.

If they were not to do that, then we would revoke their QPP status for certification.

So the second of the two recent introductions of standards or programs is the QA program. What we've done here is to try to define for our own industry what current good manufacturing practices are. What we found is that as we fit in with the blood industry as well, there are some things that we do differently and that sort of makes our current GMPs a little bit different than blood establishment current GMPs, so what we have done with the quality assurance committee of ABRA is to develop GMPs for plasma centers. And we have specific definitions that we've managed to iron out so that we all agree on the same terms which was actually, took quite a bit of time and then we defined certain requirements and pretty much worked on what can we do that will allow people a fair amount of flexibility and how they implement their QA program, but still meet the sort of higher ideals of what QA should be. So that's what the goals of the QA program are.

So primarily there's independence of QA function. Then we have developed a checklist specific to the source plasma collection industry so we took the ten areas of quality assurance from the

quality assurance, the FDA quality assurance guidelines, SOPs, training and education, and so on down to QA and internal audits and we defined it specifically for our industry so that I think that when we -- when centers receive this standard, they get a good idea of what the industry things for itself would be a good quality assurance program.

So right now we're in the introductory phases of this and we're seeing that in some areas, of course, we're in great shape and other areas we're trying to refine our definition so that people have a better understanding, particularly validation. That's an area that we're working on right now.

So as I said before, part of the intent of QPP is to have an eye toward continuous improvement and continuous expansion as well. Right now we're working on a QPP for Europe and there are other geographies that we've been discussing what the process would be for going into -- for developing QPP in their geography. And then we've had quite a bit of interest particularly from Europe in the development of QPP for plasma from whole blood or recovered plasma.

And so lastly, I'd like to bring us back to the quality of plasma principles. And these are the things that we as an industry have defined as

critical although they're fairly general. They are critical and every one of our standards goes towards meeting one of these five plasma principles: quality donors, quality plasma, professional medical facilities, high standards for personnel and the commitment to continuous improvement.

Thank you.

(Applause.)

CHAIRMAN HEINTZELMAN: Well, I believe that bring the speaker sessions to a close. If I could ask the speakers to come up front and join us at the table, we can have a brief open public discussion if anyone has any questions or needs further clarification.

If everybody gets up and goes, we just won't do this. So if they would, would you please come back to the table?

(Pause.)

While that's happening I'd like to thank everyone for their participation here. This is a good format for us to hear not only from the speakers and how the North American continent and European continent is progressing, what the industry initiatives have been here in the United States, but it's a good opportunity now for us to hear from the participants in the audience if there are issues in

particular you'd like to discuss. We've been fortunate, in my opinion, in that we have seen some very impressive methodologies that have been developed, looking at the historical problems that were first recognized and seeing the regulatory responses that were put in place to prevent transmission of disease. Dr. Lynch and Dr. Snoy talked extensively regarding technical requirements for these products and capabilities, be it the quality of the starting material or the technical abilities that you have to inactivate. The concerns of the National Hemophilia Foundation as a special interest group are, I'm sure pertinent to all of us and having IPPIA and ABRA speak to demonstrate the areas that they've been able to drive human source plasma to a higher level of safety too. It's really quite impressive. So at that time I would invite anyone that has any questions or anyone on the speaker table that would like to make any further comments to please feel free to speak up. If there is anything you'd like to say I encourage you to please use the microphones and identify yourself.

MR. LYNCH: Actually, I have a question while people are moving.

CHAIRMAN HEINTZELMAN: It's not often the panel gets to question the audience, but that may be

the outcome.

MR. LYNCH: It occurs to me that there are a number of programs including those under the purview of the World Health Organization for tracking and surveillance of human diseases and I wondered if there's any veterinary cognate of those programs that Phil or Laura may know about or any of the members of the audience. In other words, some sort of bellweather system for new animal diseases that may affect production animals.

DR. SNOY: Well, I don't know about tracking of new animal diseases, but there are certainly reportable diseases to the USDA in this country, blue tongue virus in sheep is a good instance plus there's TSE certification programs in this country which the USDA oversees, so there are certain diseases in this country that are reportable to the USDA, but outside of that, I'm not aware of any tracking systems outside of those.

DR. WILLKOMMEN: Yes, there's also a European system available that is located in Brussels and they prepare reports also and distribute them and I mean there is really a system in place and I mean that it is also associated with the WHO, but I'm not so sure about it. I know that there is also a system in place in Europe.

CHAIRMAN HEINTZELMAN: If we had a representative from the CDC here, they too might be able to contribute to that.

MR. FRAZIER: Just briefly and it's not much of a note, but I found on the internet something called ProMed which reports various human and animal disease reports and it's sort of unedited, just reports from clinicians flying back and forth, but it does sort of serve to bring up an awareness of what's happening where. Pig viruses in Malaysia, the latest serological testing of West Nile viruses. ProMed is the only name -- I just found it last week, but I've gotten 44 notifications over the past week of various things. So there's a potential extra source of information.

CHAIRMAN HEINTZELMAN: Could you identify yourself, please?

MR. FRAZIER: I'm sorry. Douglas Frazier, Division of Hematology, CBER.

DR. BAYER: Joanne Hotta Bayer. I just have a question regarding production facilities. If you have a qualified animal program where you can monitor the herds for transgenics and you can demonstrate at the lab scale that the manufacturing process that you develop for these transgenic animals can clear viruses, would you have to build a separate

production facility to purify these transgenic proteins?

DR. WILLKOMMEN: That's a question for me, yes? I think -- I'm not sure I understood right your question. I mean it is really a general question. I think that it has to be shown or the flock has to be controlled for infectious diseases, of course, and they have to provide in the report about what they do, what's a control, what is the testing and so on.

On the other side, we think in Europe, we think that these are two things. One is the safety of the source material. The second point is the safety of the final product. That means that the manufacturing process would contain all the steps which are effective for removal or inactivation of viruses. And so -- and you know, you have to see it a little bit, case by case, and you have to look at the product itself. But in principle, it is. But I'm not sure it was your question.

DR. BAYER: Yes, we process human source material and with the animal source material we produced at a separate production facility from the human source material.

MR. LYNCH: I think our standards wouldn't preclude that, but there would be a very

high hurdle to leap in terms of establishing the change control procedure, eliminated any risk of cross contamination. There's a bit of a dilemma here in identifying which material is risky compared to the other, whether you're worried about contaminating your transgenic product with your human material or vice versa. I'll leave that aside for wiser heads, but the cross contamination issue should be addressed via change control or via segregation.

MR. PIZZI: Vinn Pizzi from Milpor Corporation. It's been mentioned a few times about transmissible encephalopathies and having to do with the assay system, having known a few companies in the industry, the validation companies that is, offering a Western Blot type of assay and is this an adequate mechanism to prove that there is adequate clearance as opposed to the animal model or the bioassay being used as well?

MR. LYNCH: I think the Western Assay for the proteinase resistant core of the PrP protein has proven to be very useful, but perhaps not a definitive assay for infectivity, particularly as applied to clearance studies. One example of a productive use of that assay would be to do a preliminary screen of a multi-step production process to look for promising manufacturing steps that might

be effective at removing contaminating prion proteins more than others and then going back once those most promising steps have been identified and confirming the clearance of infectivity via more conventional assay, again it's a dilemma of establishing a strict correlation between the biochemical measure and the -- and infectivity. Now I'm not sure they're published, but I know some reports where people have been able, at least to partially segregate the biochemical marker from the functionally infectious material, much like one could have a mixture of naked nucleic acid which is non-infective, plus intact virus and a PCR signal might give misleading results.

MR. PIZZI: Thank you.

DR. WILLKOMMEN: We had a discussion about this point in the last year in Europe and -- it's the beginning of this year in Europe and we think -- the question was is it necessary today to require validation studies and the outcome of the discussion was that it would be helpful to continue with performing studies because we would better understand what the behavior and the properties of this agent is. But nevertheless, there is a problem with the spiking material and it is not good or we don't know at the moment what would be the best material for spiking because the nature of the

spiking material would influence the outcome of the study and therefore it is a very important point. And we think -- I know that at the moment there is a European research program underway comparing the different spiking materials as I have heard promised. It is underway and we don't have the results.

With regard to your question to the best system for the detection of these agents, I think it is also clear so far, it is so called gold standard is infectivity assay, but it could be shown already that the immunoblot or assay techniques gave results which are comparable to the infectivity assay and I mean that we need more data, more information, more knowledge about it and it cannot be finally decided what's the best way would be or what is to recommend in order to perform such studies.

I think it is from my knowledge the situation at the moment.

CHAIRMAN HEINTZELMAN: Does anyone else have any comments they'd like to make? Well, I want to thank everyone for coming. It's been a very beneficial time. The speakers, in particular, I want to thank you for sharing your thoughts and ideas and allowing everyone to hear what the concerns have been.

Thank you very much.

(Whereupon, at 3:36 p.m., the workshop was concluded.)