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As an analogy to the microchimerism that we see in allotransplantatiion whereby cells from the transplant migrate and traffic through the recipient's body, and also because we knew that we had patients who were receiving extracorporeal splenic perfusion the day that we were to take the samples, we had to have a strategy to test for microchimerism.

You have already heard about this, but just to give you a refresher course, all pig cells contain PERV DNA, and on average you will see approximately 50 copies of PERV per cell. As you heard also from Gillian before, each pig cell contains approximately 2500 centromeric sequences so that if you did a ratio, you would do a ratio of PERV to centromeric copies and in a normal pig cell it would be approximately 50:2500. If you find a patient who has microchimerism and infection, there will then be more PERV than what you would expect for just a pig cell alone. Therefore, the ratio of PERV to centromeric would be treater than 50:2500, and indicative of infection. Of course, as you heard also, low-level infection cannot be excluded in the presence of microchimerism using this method.

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Here is our DNA testing strategy. PERV DNA was looked for by PCR, and this could be attributed to either a

pig cell or a human cell. If you did not find any PERV DNA, then it would be considered no infection detected. If pig DNA was detected, we would then move on -- and this would be considered microchimerism if you had evidence of pig centromeric DNA, and this was done at GTI, or if you found pig mitochondrial DNA this would be microchimerism. If you found PERV DNA but you did not find any pig DNA, then the patient would be considered to be infected. If you had any evidence of microchimerism, then you would do the ratio which I just mentioned of PERV to pig centromere. Because of analogy to feline leukemia virus where the virus is sequestered in the salivary glands and then saliva can act as a reservoir, in patients who would be found to have microchimerism we went back and tried to obtain saliva to then test for PERV barriers in the saliva by RT-PCR. If you found a patient had microchimerism, we then did the ratio. If the ratio was equivalent to what you would find in a pig cell the patient would be considered to have no infection. If the ratio was larger than what you would see in a pig cell, then the patient would be considered to be infected. For infected patients, we would then go back and test the body fluids for PERV, as well as test the close contacts which we define as persons living in the same household.

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That was to test for PERV DNA, which would be

testing for the latent type of PERV infection. To test for actual viral infection and looking for virions in the serum we went to testing by RT-PCR. If the test was negative, then you would consider the patient to have no infection detected. If the RT-PCR was positive, then you would consider that the patient was infected. Then we would test the bodily fluids by RT-PCR as well as test the close contacts.

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Finally, we have tested for the DNA and the RNA, and for a historical overview of the patient to see if they had been exposed to PERV virions we did the Western blot looking for antibodies to PERV. If it was negative, the patient would be considered seronegative. If it was positive, the patient would be considered seroreactive. For seroreactive patients we would then test the saliva by RT-PCR as well as test the close contacts.

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To give you a bit of more detail as to the actual tests -- you have heard about them already today, at GTI looking for DNA by PCR for PERV they were able to detect 1 PERV DNA copy/half a million human cells, and the false-negative rate for 10 copies would be 0.03 percent.

At the CDC they did 2 PCRs. One was looking for PERV pol sequences, and they could detect down to 5 PERV

copies/150,000 cells, and 1 PERV gag DNA copy/150,000 cells, with a false-negative rate, based on empirical value, of less than 0.02 percent.

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Finally, the microchimerism assay -- at GTI they used the pig centromeric assay, where they could detect 10 copies/half a million cells, and here the false-positive rate was less 0.83 percent. At the CDC they used the pig mitochondrial assay where they were looking for 1 copy/150,000 cells, and the false-positive rate here was less than 0.02 percent.

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In terms of the other assays, the Western blot assay looking for antibodies was carried out at Q-One Biotech. There they used a recombinant gag and if this was positive, they would then test against the whole virus looking for p30. At the CDC they used infected human 293 lysates looking for gag and p27.

For the RT-PCR looking for virions in serum, the GTI was able to detect 400 particles/ml, with a false-positive rate of less than 1 percent. At Q-One, looking for PERV virions in the saliva, the sensitivity of that assay was 1000 particles/ml, with a false-negative rate of less than 1.25 percent.

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The interpretation of these assays are the following: No infection detected we be if we found no PERV DNA nor any PERV RNA in the patients. Microchimerism was defined as if ever we found any porcine genomic DNA in any of these patients. Infection with PERV was if we could find circulating virions in the serum and/or presence of viral DNA not which was not accounted for by microchimerism and/or if there was presence of virions in the saliva. Potential exposure to PERV would be if there was seroreactivity to any PERV antigens.

Now, these assays were quite complex. Although the study was designed two years ago, we have only completed the testing of the last five patients approximately ten days ago. We have had confirmatory testing by the CDC because we knew that this was a public health concern so that if anything had been of concern, this would be known to the CDC and reported appropriately.

We hope that over the summer we will have the report which will then be submitted to the regulatory authorities, and in particular to the FDA. Thank you.

DR. AUCHINCLOSS: Thank you very much. Now, it is essentially 11:30 and our next formal presentation is scheduled for 1:50, and between now and then we have, I think, three things to do. We have questions from the committee to our various speakers; we have questions to the

committee from the FDA; and we have lunch to have. To give you a sense of the schedule that I think we will follow here, we probably won't break for lunch quite at 11:50. That is only 20 minutes. I suspect we will go for about 45 minutes before we break for lunch. Then I won't give you a full hour for lunch, so we will be back here by one o'clock, or something. The discussion of the questions to and from I suspect will amalgamate into one process here, and I will try to make sure that over the course of the next essentially two hours we cover the issues that you have expressed to us. If I haven't by the end of that time, we will come back to them and make sure that we have.

So, what I am going to do is just initiate questions from our committee members, and what I will then try to do is capture topics and keep us focused on individual topics as we go so that it won't necessarily be one person and then another person, and one speaker and another speaker. We will go by topic. Just to get the process started here, I thought I would turn to Jonathan Allan because his list of questions is growing long, although I know lots of people have long lists, and also he is the first in the alphabet. Jonathan, do you want to start the questions for us?

**Ques from the Committee**

DR. ALLAN: Yes, why not? I have several

questions but I will just start with the first speaker, Gillian. As a point of information, you were demonstrating that you were able to get microchimerism with the pig to baboon studies, in the primate studies that were performed. I really liked your real-time PCR that you have done because it is very quantitative. So, one of the questions I had was were you able to quantitate exactly how many pig cells you were finding in the primates and over time, were you able to look to see whether the number of pig cells diminished or were stable? The third part of that question is whether or not different organs gave you the potential of having more numbers of pig cells in terms of microchimerism?

DR. AUCHINCLOSS: Is the question for Gillian Langford?

DR. ALLAN: Yes, for Gillian, wherever she is.

DR. AUCHINCLOSS: Will you come to the microphone, please? We will be asking questions of all of our speakers. So, be prepared to step up to the microphone.

DR. LANGFORD: In answer to the first part of your question, yes, we were able to quantitate the level and the number of pig cells, and we found that in most of the samples we looked at it varied from between 1-20 pig cells per sample. We didn't really find a correlation between the tissue types that we analyzed and the level pig cells, although in some lymph nodes we did find higher levels of

pig cells than in some of the higher tissues that we looked at but it wasn't universal throughout all the samples that we looked at.

DR. ALLAN: Did it vary based on when you took the samples?

DR. LANGFORD: All the samples that we analyzed were terminal autopsy samples so we don't have any serial samples taken from our primates. We are collecting serum at the moment to do a study to look for viral release but they were all terminal samples.

DR. ALLAN: Which is another point for discussion, which is when you take the samples and when you test them. If you test them late after the transplants happen you may get a different result than you would get if you would take them two days after the transplant or a week after the transplant. So, those are things that one needs to consider. If you take it at the endpoint you may get a negative whereas the cells have already gone to tissues.

DR. AUCHINCLOSS: Undoubtedly, that is true. What I would like to do is to take your question about the microchimerism and now focus on the microchimerism issue for a few minutes here, and then we will look for a new topic. All right, is the committee -- and I am looking for comments from committee members here -- is the committee satisfied that you can detect viral infection, should it occur, from

microchimerism with the dilutional assays that are currently being used? Marian?

DR. MICHAELS: I think all of the speakers have actually pointed out that while it is able to detect infection versus microchimerism if you have a greater amount of PERV than you had anticipated based on the correlation of how much there should be of the pig cells versus the PERV to start with. I think low-level infection isn't going to be able to be detected. I think everyone pointed that out.

DR. AUCHINCLOSS: Yes, please?

DR. ONIONS: First of all, I think all of the groups have presented developing assays that are very sensitive and well controlled, and I think that is to be congratulated on, the work they have done.

I think we are probably pushing these assays almost to the limit and I think this is going to raise some problems later on when we start to compare data from different groups. We are pushing the detection of microchimerism to a limit and we are pushing the detection of PERV to a limit. So, we are really asking a lot more of these assays then of conventional assays for HIV or for HTLV.

But my comment would be that I think that it is going to be important that each group actually validates in the background positive, let's say a 293 cell that contains

two or three copies of PERV; that they then validate in those assay systems what level of infection can they detect above that. In other words, we actually do have real data, validated data of what the level of detection would be in various backgrounds of microchimerism because, in fact, some of these assays will have different backgrounds. They are all interesting approaches but they will have different backgrounds. For instance, if you do a cell dilution, that would give you potentially a very discrete signal but its sensitivity is going to be much lower than if you use a whole DNA sample, say. So, I think we need to have those data.

DR. AUCHINCLOSS: Can you expand this for me, what do you mean when you said "we are pushing" these assays to the limit?

DR. ONIONS: Well, it is the very issue of when you start to look at ratios when it is necessary. You either have to look at discrete cell populations or you have to look at ratios with some kind of approach that GTI has used. They are using excellent technology. They are using quantitative PCR technology, the best that is available at the moment. But you are inevitable left between a ratio between two results that are quantitative, each with their own level of variation. So, you are then always going to create a grey area where you are not sure whether you are

looking at microchimerism alone or microchimerism in the background of infectivity. The same sort of arguments apply to the also excellent work done by the CDC. The same sorts of caveats apply.

So, I think what we have to do is actually to validate those systems to actually show in a real sense what infectivity they would pick up in a background of microchimerism.

DR. AUCHINCLOSS: Okay, so there is a general comment that the assays that are currently being used would not detect a low level of infection in the presence of microchimerism, and you are suggesting that it would be nice if we could figure out exactly what that low level would be that we would miss.

DR. ONIONS: It is not quite what I said. It is not an implied criticism. I think we are actually at the limit of what you can do.

DR. AUCHINCLOSS: I didn't mean it as criticism.

DR. ONIONS: All I am saying is that we need to know what level of infectivity we would pick up.

DR. COFFIN: In theory at least it would be possible to improve the situation with some sort of sorting or separation technology. I wonder if any of the representatives would care to address whether they would consider that with the practical issues --

DR. AUCHINCLOSS: So this is a question for either the committee or for the speakers, is there a different technology that would enable us to detect infection versus microchimerism perhaps by sorting cells --

DR. COFFIN: Sorting cells, separating human from pig, for example.

DR. SALOMON: That is a great idea theoretically. The limitations of that, at least in looking at microchimerism in, say, the human transplant situations had been that you really are talking about very few cells in these tissue compartments. So the idea, let's say, of sorting 20 pig cells out of a large population -- and then what you want to do is analyze the tissue without the pig cells. Right? I think that would probably be beyond the technology. Anyone could disagree with that but I would be skeptical.

DR. AUCHINCLOSS: There are more comments on this possibility of other assays.

DR. VANDERPOOL: I just want to ask a question, hopefully that we can keep in mind from the standpoint of where we came from and where we are going. We had a presentation to indicate that the last time we met -- certainly in October of '97 -- there was such concern over particles identifying endogenous porcine retroviruses as to put experiments on hold. Now, as I heard the speakers with

my somewhat layman's ears, even though I am getting more and more educated by the week, I put stars by the presentations that were heartening in terms of former worries we had in terms of controlling and identifying infection.

Now, what I would like for the committee to address at some point is where are the worries, and how big are then? I mean, do we really have worries? And, if we have worries that there are infections that are being produced, what are they? Of what nature? How significant are they? And, I totally agree that we should start with microchimerism, but I want to keep that larger perspective in mind. Where have we come now? Are we in a place of greater comfort? Of very good comfort? Or, do we still need to worry and ask for more things?

DR. AUCHINCLOSS: I think that is a fair comment. Let me go back to the big perspective. What I think we have heard from the morning is that a lot of data is beginning to accumulate that is essentially negative with respect to actual infection of humans having been exposed to pig tissue.

The issue that we are now addressing, over the next hour and a half or so, is how good is that data? Can we trust the data that we have? Are we interpreting it correctly, or are there other kinds of data that we should be looking at? I think that is the issue that we are now

addressing. The answer to your question is no. What we have learned so far reads negative. How good is that negative?

Back for a second now, I saw Jonathan shaking his head about there is no other assay that will detect infection in the setting of microchimerism except a dilutional approach.

DR. ALLAN: Well, the context that I would like to look at is you have microchimerism, and the next part is the infection which is what assay -- RT-PCR versus whatever. Then the third part of that is the antibody-based assays -- are they good enough? So, are the RNA-based assays which detect virus, are they good enough from what we heard this morning? The second part is are the antibody-based assays good enough to detect -- from the negative results you are hearing today the question is are those negative results good enough? Are they truly negative results?

DR. AUCHINCLOSS: That is exactly the question. I want to come to the antibody issue for sure, but any other ideas?

DR. SIEGEL: I didn't mean to interrupt but I did want to address the question and comment that Dan Salomon made a minute ago. With current sorting technology, if you have a rare population, say 1/1000 or even rarer, it can be very difficult to quantitate that population or to purify it because there will be in the general population other cells

that will create noise and that will look like the rare population. So the purity of the rare population, if you were to try to sort it, may be very low. But it should not be particularly more difficult to remove such a population than to remove a more common population providing you have whole cells and you have antibodies that selectively can identify that population.

It seems to me that you may also remove a small number of cells that you didn't want to remove. That shouldn't probably create a problem in this context. It seems to me it is a technology that ought to be looked at. If you get less than 100 percent removal of contaminating porcine cells from a specimen the expectation may be that while you may not totally eliminate the problem you should see, if you were to get 90 percent or more removal, a significant shift in the ratio of, say, centromeric DNA to PERV DNA if there really is PERV infection and you are selectively removing the microchimerism.

So, I am not sure this is something that is feasible to do routinely but it certainly is a testable hypothesis that this can be useful. Much as David Onions was saying that we look at the sensitivity of the test to distinguish between microchimerism and infection, it seems it would be easy enough to also look as to whether that can be improved so that when samples are positive one might

selectively study those samples, assuming they are whole cell samples as opposed to extracted DNA samples. One might then study those samples.

DR. CHAPMAN: Just to clarify a point, my interpretation -- and I ask Onions and Allan and Coffin to correct me if they feel this is incorrect, but I think what you were trying to communicating was not that you think these particular assays that have been presented are being pushed to the limits but that all of the assays and approaches we are using are being pushed to the limits in terms of the competence of detection at the limit we are dealing with, and the confidence we can place in that determination between infection and microchimerism. And, barring a major conceptual or technological advance, we are probably struggling in an area in which our currently available technology, even with refinements on the present efforts, is not going to be able to give us a great deal more confidence than we have at present. Is that a proper interpretation of what you are saying?

DR. ONIONS: I am not quite sure, but I will try to restate it. The limits of sensitivity and the limits of detection have been defined for these assays. So taking the limits of sensitivity as being usually lower, we know from the comments we have heard about quantitative PCR we can detect very few proviruses in the background of $10^5$. I mean,

it depends on which group you go to but we are talking about a few proviruses.

The routine limits of detection are usually put high to allow for statistical variation and various other factors. That is fine; that is understood. But these cannot be refined better than that with our current technology, in my view.

My problem I think is quite simply this one of we have this unusual problem of microchimerism. We have to relate a distinction between microchimerism and low-level infection. Inevitably, however good your assay is, you are going to have this grey area. All I am really just saying is that I think it would be useful to try and actually validate exactly what we can pick up routinely as low-level infection versus microchimerism. That is my comment.

My second comment would be that if that is all we are worried about, we are not worried about a great deal because if that is routinely the levels that we are going to be worried about, that is probably not a good sign. It means that we are not going to be hitting a major problem.

My caveat to all of that, of course, is that the kinds of work that have been done so far are not on the kinds of patients that we are going to be concerned about, which are the ones that are going to have immunosuppression and, on the whole, have not involved animals where we might

not have complement lysis of virus because they are coming out of transgenic animals. With those two caveats, I think the data are very encouraging.

DR. AUCHINCLOSS: Those are three really important points. If we get down to this level it would be kind of reassuring but we are not looking at the patients or the donor organs in the right combinations that really are the test --

DR. HIRSCH: Or perhaps the tissues that you need to be looking at. I mean, we asked a couple of years ago that PBMCs be routinely tested, and everybody came here with presentations about negative PBMCs. On the other hand, we have heard that PBMCs may not be infectable, given the caveat that Dr. Onions said, that under certain circumstances it is conceivable they could be but, nevertheless, we have no data that they are. And, the question is should we be continuing to ask all the companies to rigorously look at PBMCs and be assured, perhaps falsely assured that represents no infection because you can't find it in PBMCs. If you are putting tissues in brains or you are using hepatocytes, then PBMCs, to me, would seem to be the wrong area to look at.

DR. AUCHINCLOSS: This is going to become our next topic of conversation, which is what tissue should be sampled, but before we do that there is a speaker that

wanted to be recognized that I haven't gotten to.

DR. GORDON: Thank you. I would just like to ask a lay question --

DR. AUCHINCLOSS: Just identification, if you could.

DR. GORDON: Oh, I am sorry. My name is Allistair Gordon. I am with the Islet Foundation, in Toronto, Canada. The question I would like to ask is a lay question relating to the whole point of distinguishing between microchimerism and low-level infection. If we have some results in which we have that ambiguous outcome, will the passage of time not resolve it? In other words, will that low-level infection not become a more unequivocal infection over time, especially in some of the patients that were tested that were immunosuppressed? And, in the event that it remains indistinguishable from microchimerism, then does that tell us that we don't have a very virulent infectious agent?

DR. AUCHINCLOSS: Anyone on the committee want to respond? Jonathan?

DR. ALLAN: I think that is a beautiful point, which is if you are trying to decide if it is low-level infection either it will go negative, and that is okay, or it will increase, and that is not okay. If it increases or even if it doesn't increase, if you have active infection in the PBMCs or any tissues in the human you are going to get

antibodies probably. So, even if you lose the detection system for molecular approaches you are still going to get the antibody approaches.

DR. AUCHINCLOSS: And if it is really infection I want to know pretty quickly about it but, never mind, I think we have made the point here.

I promise we are going to come back to the antibody assay. We do want to come back to the antibody assay but right now the topic on the table is are we looking at the right tissues when we look at peripheral blood mononuclear cells? Comments?

DR. HENEINE: Walid Heneine, CDC. I want to come back to your original question, which is how much can we trust these negative data given the newly developed technologies and the limited validation we associate with them?

I just want to point out that the new information we have acquired when we have applied those new assays, for example, the data we showed on detection of viremia in pig plasma -- this is new information that we were not aware of when we applied RT-PCR or reverse transcriptase testing to pig plasma and became aware that there are some free particles that are found in this compartment. Applicability to factors derived from pig plasma, porcine Factor VIII, when we used those techniques, again, we were able to define

PERV in these factors.

So, given the data, one should not take lightly the results we are seeing, the negative results we are seeing. Unfortunately, we are dealing here with several limitations, for example, for the serology assay, and I know we are going to discussion this further, but the problems we have there seeing whether these assays can really detect antibodies in infected individuals is the fact that we don't have any infected person or animal that we can test.

DR. AUCHINCLOSS: That we can use as a positive control.

DR. HENEINE: Exactly. Unlike with an HIV-infected person, we don't have a pool of infected people that we can use to evaluate the sensitivity of the assays of 99 percent, 95 percent or 50 percent. So, keep in mind those limitations that are inherent with us, and we have to live with them and move forward.

DR. HIRSCH: Along that line, could I just ask you a question while you are still at the microphone? You correctly say that there aren't any infected humans that we know of, and we have heard a lot about that non-human primates probably aren't a very good model system, at least the baboons and other ones we have heard about. There was some data in our materials about cats and mink being perhaps more susceptible. Is anybody trying to develop models in

these kinds of animals?

DR. HENEINE: Yes, this is what John Coffin actually touched on in his talk. The discussion at the Banbury Center meeting was focused on really trying to develop an animal model where we can evaluate all these parameters that so far we are not aware of. But there are several ways one can approach that situation other than now looking at xenograft recipients in baboons, and whatever.

One question that was discussed was what if we go and inoculate high virus titers into baboons or other animal species trying to see whether a persistent infection can take place, and then study kinetics of viremia, and so forth.

DR. ONIONS: Maybe I could just comment on his last point. I think he is right. I think when we look at the permissivity of a particular species for infection, it should always be borne in mind that the infection in vitro does not always mean you can infect in vivo. I think Karen, in her excellent summary of this meeting, pointed out that you can give chemografts into rat cells in vitro but it has not been possible to infect rats, as far as I am aware, in vivo.

On the other hand, we do have some primates now that Gillian Langford referred to, and both in collaboration with her and independently we have shown that rhesus cells

can be infected. So, I think I think we need to look for a primate model. That might be worth investigating because, to my mind, it would be better to have a primate model because of all the other associated factors, including complement-dependent lysis.

So, I think there are models that can be pursued, but I have some reservation about whether they are really going to give us the kind of data that would be helpful.

DR. AUCHINCLOSS: We now have actually two topics on the table, which is fine. We will have more than that. One is which tissue do you want to sample in and now we have shifted over a little bit into which species do we want to look at and which species is worth looking at.

Can somebody summarize for me? Yes, baboons can be infected with this virus; yes, rhesus cells can be but so far no animals? Is that correct? Does everybody agree that the baboon can be infected with this virus?

DR. HENEINE: Baboon cells.

DR. AUCHINCLOSS: Baboon cells, thank you. Chimpanzee, yes, also.

DR. HIRSCH: Is there any evidence of baboon?

DR. ONIONS: I am not aware of baboons being able to be infected but maybe someone else in the audience could correct me if that is the case.

DR. AUCHINCLOSS: Sorry?

DR. ONIONS: As far as I am aware nobody has shown infection of baboon cells in vitro. There adverse event data on the gorilla, there are data on the chimpanzee, and there are data on the rhesus that show that you can infect certain cells, but I would again add the qualification that in the case of the rhesus cells, in our hands, to do that we actually concentrated the virus very significantly before we actually infected the cells.

DR. HIRSCH: Did any of the sponsors say yes to baboon cells?

DR. ALLAN: I mean, I think you have to consider whether or not, or how seriously people looked at baboon cells lines and how thoroughly they have investigated that, and I don't really know at this point.

DR. ONIONS: I think Jonathan's point is very good -- perseverance, how much you use and whether you have luck, and it is probable that you could infect other primates.

DR. COFFIN: It is also important to keep in mind in that kind of analysis that these cells lines are all different from one another. The cell lines used in humans in no way parallel the one that you might have from a baboon. You take what is on the shelf basically, unless you are looking at fresh tissue, which there hasn't been much result with. This whole effect could just be a difference between one cell line and another that might have nothing to do with

the species of origin. So, we still have to keep that in mind when we are talking about negative results.

MR. BENEDI: It is a non-medical question, and maybe it is a silly one but I had it written down and you are touching on it now. The baboon and other non-human primates that have been infected, are we not creating something that we haven't introduced to the human cells yet but have introduced to baboons that could go from the baboons to humans instead of pigs to humans?

DR. AUCHINCLOSS: Your question, I guess, is by doing the baboon experiments are we setting up --

MR. BENEDI: Are we creating something new that we haven't tested human cells with in the baboon that has been developed through the pig?

DR. AUCHINCLOSS: I guess it would be theoretically possible but there are enough humans that have been exposed directly that the question has really been addressed even in the human directly.

DR. SACHS: I think Dr. Coffin's point is extremely important. How many different cell lines of humans were looked at before some good ones that get infected easily by these viruses were found? Was it quite a few?

DR. ONIONS: Well, in our initial screening we did about 20 cells lines of which we got two infected.

DR. AUCHINCLOSS: I think what we are hearing is

that if you look hard enough for a cell line from any species you will find cell lines that can get infected under the proper circumstances in vitro with virus if you have enough virus. And, I think the importance of it is that the baboon, the rhesus, any other primate as an in vivo model is reasonable to be looking at in terms of screening to find out whether or not any infections occur, regardless of whether the data are identical on the cell lines.

Well, let's put it in practical terms, and the practical terms are that in non-human primates you can do the experiment with transgenic organs; you can avoid complement; you can give them lots of immunosuppression; you can biopsy them at lots of different times; you can get lots of different samples from different places. So, you have all those things that you can do. But the disadvantage is that when you have done all the experiments and you get a negative result --

DR. SACHS: Well, it is the same as saying two different humans. I don't see that there is any real difference. There is no evidence that there is a real difference between what is going to happen in a non-human primate and what is going to happen in a human when exposed to a xenograft.

DR. AUCHINCLOSS: So, in the face of a big negative, which is what we have, it is worth doing these

experiments even though the result may be negative also.

DR. KASLOW: I was just going to follow-up on an earlier point from Dr. Wilson's presentation. You made it sound like, if I remember correctly, that human lung cells were the most favorable in terms of your infectability. Did I misunderstand that?

DR. WILSON: The 293 cell line is actually in kidney.

DR. KASLOW: I thought you mentioned lung cells as well when you were going over the various cell types that you had tried to infect or that somebody had tried to infect. Not true? No? Sorry.

DR. MICHAELS: I think that was Gillian's comment, and Gillian correct me -- the porcine lung had more PERV. High levels of PERV came from the lung from the pig.

DR. KASLOW: Thank you.

DR. HIRSCH: I am not sure I agree with David's comments that a baboon model is necessarily as good as a human model, if I heard him right, and that just because you haven't infected baboon cells doesn't mean that the baboon can't be infected. I think the onus has to be to prove that the baboon can be infected. If the baboon can't be infected in vivo, then it is not a good model system.

DR. SACHS: We have no evidence that a human can be infected in vivo.

DR. HIRSCH: Well, I am not saying a human can but I am just saying that if you are trying to see what the effects are of pig endogenous retrovirus and using a baboon as a negative model, and you don't have to worry about it because you haven't seen it in a baboon, until you can show that a baboon cell or a baboon in vivo can really be infected then you can't say it is a good model.

DR. ALLAN: That is the model though, the model is to determine whether or not that species can be infected or not. So, that is really the model. So, even when you get a negative -- that is why you are doing the experiment, to see whether the animal would be infected or not. So, it is reasonable to do that with high levels of virus in several different species. If you get a negative, like what we are seeing, it doesn't tell you that you are going to get a negative in humans but it gives you more information. So, I think we are saying the same thing; maybe we are not.

[Laughter]

DR. AUCHINCLOSS: There are three or four hands up. Let me start over here.

DR. COFFIN: I think in many respects the best animal model is the one that works, even if it is the mouse. I mean, you can get a lot of information on what could happen and what to look for in a virus infection with any species, any mammalian species into which you can get any

infection at all. Obviously, you want to be as primate as possible, and you would like to learn that the infection can't go in certain species, but I think it is worth working right on down until one finds something that is infectable because of the amount of sort of basic information and information on what to look for in people that could be generated.

DR. HIRSCH: John, wouldn't you say even though in vitro and in vivo are not necessarily parallel, but if you have data to show that pig viruses can easily infect cat cells that would be a model that you would be interested in looking at.

DR. COFFIN: I would start there, yes. That would make, I would think, a sensible starting point. If that doesn't work you would just keep on going.

DR. COOPER: This discussion about the model makes me think very closely because in vitro would be one thing to show that the cells could be infected but what we really want is an in vivo model and, as mentioned, we want a primate model. Now, a big problem with a primate model is that the primates, the Old World monkeys have these antibodies that destroy these pig tissues very quickly, and even though we have talked today about sort of long-term follow-up, we are actually talking about a very few cells surviving or we are following up an animal that has rejected

its organ within a few days or weeks in most cases, often earlier than that.

Have we thought of looking at New World monkeys which do not have antibodies against pigs? They have the sam gal expression on their tissues. So, the whole business of antibody complement-mediated rejection would be overcome, and if we could show that you could infect a New World monkey cell in vitro, then you would have an in vivo model for a least putting in cells. New World monkey happen to be rather small, but at least you could put in pig cells in large numbers and see if they became infected.

We know that this would be rather like a concordant xenograft with the antibody problem, and we know that in other models of concordant xenografts in primates, for example monkey to baboon, with the standard immunosuppression of today we can have survival of over a year. So, we would then have follow-up of living pig cells in a primate for more than a year, which would give us some idea perhaps in vivo whether these monkeys can get infected.

DR. AUCHINCLOSS: From the experts, is there enthusiasm for going to a species that doesn't have a lot of anti-gal to do these experiments?

DR. COFFIN: Well, as far as infection is concerned, I don't think it is such a serious problem in any species because one passage through a primate cell will take

care of that. So, virus produced by 293 cells, for example, will now be the same as virus that was produced after one round in a human. So, as far as that issue goes -- there may be other perfectly good reasons for doing it but I don't think that necessarily is the best reason for it.

DR. LERCHE: The last speaker covered the issue I was going to bring up, namely that with regard to many retroviruses non-human primates are not created equal in terms of susceptibility to these things, and I think a systematic approach is necessary for doing this, and I think the New World species are worth looking at in the grander scheme of things. So, in the interest of time I will just leave it at that, but there are other primates that haven't been looked at that may offer at least some comparative model systems to gain some more information.

DR. CHAPMAN: My point has been partially made but I will try to underline it with more clarity than I did the last time I spoke. As long as there is not <u>in vitro</u> evidence that you can infect baboon cell lines I would argue that it is foolish to put efforts into <u>in vivo</u> models using baboons to ask the question of whether PERV can cause infections.

Now, given that the baboon is a good model for other aspects of things people are going to be looking at, like the physiology of whether the organ will function and perhaps immunology -- I defer to people more expert on that

than I, it is reasonable and it is probably prudent to continue to study baboons who are exposed to porcine xenografts for other reasons for evidence of infection because evidence of an active in vivo infection with PERV in the baboon would be helpful. But as long as you don't have in vitro evidence you can infect any baboon cell lines I would argue that we cannot put any confidence or any weight on the absence of in vivo evidence of PERV infection in baboons and make decisions of the safety of xenografts.

DR. ALLAN: I wouldn't say it is foolish to go in vivo into a model system when you haven't -- I mean, how many baboon cell lines have been looked at? The problem, as John pointed out, is that every cell line is different and if everybody has looked at one baboon cell line or three baboon cell lines from one animal it makes it very difficult.

I agree with you that negative data is going to be very difficult to interpret in the baboon, but I think it is not that difficult to take a couple of baboons and bolus them with virus and see what you get, regardless of the in vitro data because it is worth doing.

DR. AUCHINCLOSS: We are bumping up the problem of how you go about dealing with a bunch of big negatives. There is a comment down here and then a comment from the floor.

DR. PAUL: I think there are numerous examples of viruses that don't grow in vitro and, yet, replicate in vivo. So, I would say that there is a merit in doing in vivo experiments. In addition, there is the possibility of developing standardized reagents for antibody assays.

DR. DINSMORE: Jonathan Dinsmore, from Diacrin. I would agree with Dr. Coffin about if you are looking for an animal model you want one that is permissive for infection. I would directly answer Dr. Hirsch's question about the tropism of this virus. There is a basic biology of different cell types, different organ types that is very similar across species. So, you could look to see if it appears in PBMCs, whether it has the specific tropism for a specific organ because you can dissect that quite well in an animal model. And, I would actually go back to the work done with the cat virus that jumped dogs because there you know what the history is of that virus in cats. You also know what the history is in dogs, and you can see if it followed the same course. So, I would vote for looking for a permissive animal model to look at these viruses rather than continually generating negative data.

DR. SACHS: The reason I asked how many cell lines had been looked for before you found some that got infected was exactly that issue. If all you are really looking for is to have a cell line that will show an infection, the

evidence is that if you keep looking hard enough you will find it.

DR. HIRSCH: Not necessarily. I mean, there is species specificity for a number of different viruses.

DR. ONIONS: I concur with the last statement. For instance, feline virus has been very extensively looked at by all sorts of technologies, including serotype marking. For instance, say, in guinea pig there is not a receptor that that virus will use, whereas in closer related species there are receptors. So, you can't make that assumption.

Personally, before investing resources in this kind of experiments -- the ethics of using animals anyway, I would not do that until I knew that you could infect the cell in vitro. Infection in vitro does not necessarily mean you will get infection in vivo but at least it is a start. For instance, now we know the rhesus can be infected in vitro, certain cells. That would be the species I would take as the species of choice to do the kind of bolus infection that has been suggested here already. That is, perhaps infecting those animals with high titer virus and 293 cells.

DR. SACHS: Can I answer that? I certainly agree with what you are saying. That could be, but I was just asking the question has it been done in a statistically meaningful way.

DR. ONIONS: No.

DR. SACHS: Has one looked at a similar number of baboon cell lines as the human? If the answer is you have and there is a difference, then I would agree with it. But, if it hasn't been done, it is silly to be speculating that there is a species difference without having done that study. That is what I was asking.

DR. ALLAN: The problem is that there are so few monkey cell lines to begin with and there is virtually baboon. We have generated some in my lab and we have sent them to Carolyn to look at, and maybe we will get some data on some of those cell lines. But the problem is deriving cell lines from monkeys. That is the problem.

DR. AUCHINCLOSS: I am going to try a brief summary of this portion, the animal species to look at, in the following way: that we have a lot of negative data, and with negative data it is hard to have confidence. Some negative data is less reassuring than others because if you can't even infect the cell line in vitro, then you are really not impressed by the negative data. Therefore, most of these animal models should not receive targeted resources for this kind of experiment but, on the other hand, it would be equally foolish -- I think I heard everybody say -- not to look at those animals that were being used for experiments, anyway -- pig organs going into baboons, or whatever. But if there was one species amongst the non-human

primates -- I thought I heard rhesus as a species that you might use for further investigator of PERV in non-human primates.

DR. VANDERPOOL: I want to ask one question off of that good summary, and that is, we said we were reassured by the data we have heard, and then we said there are two caveats. Number one, we are not looking at the patients and, number two, the donor organs that are going to be put in these patients. Then we shifted to this animal model discussion. Are we saying by your comment that this committee feels that an animal model should be looked at as a prerequisite to clinical trials, or are we saying something else?

DR. AUCHINCLOSS: That is a good question because it is the ultimate question that the FDA will be asking, is this a requirement?

DR. COFFIN: I don't think it should be a requirement, but I absolutely think it should be done in parallel. First, did I hear you say that all data are equal but some are more equal than others?

[Laughter]

DR. AUCHINCLOSS: All negative data are equal, and some are more equal.

DR. COFFIN: I wanted to point out that retrovirology has actually become a fairly advanced anti-

retroviral. So, with a lot of viruses we have a very good understanding about what makes cell lines permissive or not. So, I would put a plug in here for also doing a lot of basic studies, like trying to identify the receptor for these viruses. Then we could find out very directly whether the receptor was present in the species or not and saves ourselves a lot of work. We could find out if permissivity or non-permissivity at some other level, for example how efficiently the LTR works in certain kinds of cells -- all of that stuff is very well charted out with experience with HIV and MLV and lots and lots of other retroviruses, and I think deserves a lot of support.

DR. AUCHINCLOSS: I think that is a really good point. One can separate from the FDA's point of view a requirement for testing -- I thought I saw around the table sort of a lot of people going no. So, I am going to suggest that that is the committee feeling, that is not something we are suggesting should be a company requirement. On the other hand, good basic research in this field should be.

Then I am going to suggest that we probably ought to break for lunch. But we still have a lot of questions to cover before 1:50. So, we are going to come back here at one o'clock and keep working as hard as we can. Okay?

[Whereupon, at 12:16 p.m., the proceedings were recessed, to be resumed at 1:00 p.m.]

A F T E R N O O N P R O C E E D I N G S

[1:10 p.m.]

DR. AUCHINCLOSS: We will get started and resume our conversation at the table here with contributions from the floor or from the sponsors, with questions to the sponsors if the committee members like.

We have a 1:50 talk scheduled. That speaker has a plane to catch afterward so we are going to be on schedule and starting at 1:50 and we will break this discussion, which is not to say that it will necessarily end. We have another day's worth of meetings.

There are at least four questions that still remain on my list that we should be addressing including what tissue should be sampled, quality-control issues, the development of an antibody assay and timing of sampling. These are questions that have been put on the table and I am happy to hear any other issues that anybody wants to put on the table for us to discuss as well for advice to the FDA.

Let me go ahead and go back to the "what tissue" issue. So far, sampling has been in peripheral-blood lymphocytes or mononuclear cells. Does anybody on the committee suggest to the FDA that we should, in fact, be looking at some other tissue. I will open it there.

DR. SALOMON: I think the idea of looking at peripheral-blood mononuclear cells has got two problems.

One has already been demonstrated. The other one is that, and Carolyn, please correct me if I am getting the spin on this wrong, but the actual data you presented when you looked at the pig PBL as a source for PERV, these were activated cells whereas a lot of these studies have been done without activating them.

In some cases, we are doing DNA PCR which would show integrated pro-virus. So that might be okay but, in other case, probably a better marker for infection would be either the release of infectious virions or messenger RNA at least by RT PCR. In both cases, you might have to activate the cells.

I would also point out that other cell types have to be considered in terms of infectious profiles. I think the cell that everyone keeps coming back to is UT93. This is a human kidney epithelial cell line. It would appear to have the receptor. I think John Coffin made a key point. We don't know the receptor yet. I hope that that will come out in the next year or so. I know there are a couple of groups working on it but, in the absence of that, pig cell lines that you know have it, if we are talking about epithelial cell lines having it, it would, I think, be remiss not to test at least several epithelial cells from primary tissues as part of a profile for looking for infection in animal models or in human patients.

DR. ALLAN: We have the opportunity, if you are looking at the pig-to-monkey studies, you may not have in humans which is to sample lots of different tissues. I think, obviously, everybody would suggest that you have to do that.

I don't think I am hearing you say that we shouldn't be doing DNA from PBMCs on patients. We still should be doing it. It is just that it is not going to give you all the answers. But the thing is, even if you get a very small amount of positive results and it is like microchimerism or something that we have heard earlier, if you continue to sample a patient, the real information comes if that varies, if it, all of a sudden goes up, with or without any RNA.

So the RNA may be intermittent. Depending on the type of retrovirus, you could only have intermittent viremias and you may miss them when you are sampling.

DR. SALOMON: Or you could also activate cells, is my point, or stress cells. You could do the same thing with epithelial cells. I also point out with humans you can do kidney biopsies, you can do skin biopsies, you can do cell, corneal epithelial, lung biopsy. Those are not horribly difficult things to do, actually.

DR. AUCHINCLOSS: Can do, but should the FDA be asking for them?

DR. MICHAELS: I actually agree but not completely to the degree that you are suggesting in terms of the other types of tissues. I think that when the other types of tissues are available that the FDA should ask that those be sampled as well, but I think to ask for a lung biopsy or a kidney biopsy if there is not another reason to be doing it--I don't think you were suggesting that.

DR. SALOMON: I wasn't suggesting that.

DR. MICHAELS: However, things such as the Diacrin studies where we have brain tissue available on the patient that had passed away that, perhaps, that tissue could be looked at if it hasn't been looked at already.

The same thing with patients that, perhaps, are on hepato-assist devices and then go on to have an allotransplant and have the native liver removed, we could, then, look at those tissues or when the abdomen is opened, take some of the lymph nodes.

DR. ONIONS: I am still a proponent of, actually, looking at peripheral-blood mononuclear cells for several reasons. One is the reason I adduced earlier and that is that, in animal systems, the root of infection of the cells in the peripheral blood is not necessarily through the cells in the peripheral blood, it is through a stem-cell precursor that is miotopically active. That is my first comment.

My second comment is that what we are really

concerned at here is, to a large extent, a public-health issue and, therefore, the other tissues that you might think of looking at or at least fluids, body fluids, that are produced by the patient. Saliva is a very obvious choice because that is the known means of transmission of some of these viruses.

Whether or not you put that in as a routine is, perhaps, a matter of conjecture at the moment but, certainly, I think most of the people who consider the matter have that in their option program that if they have equivocal signals from peripheral blood, that those, at least are examined.

I am a clinician, but I am very cautious about overinvasive procedures on patients without any rationale for it. So, for the moment, I think peripheral-blood mononuclear cells are still one of the best sources we have to examine.

DR. HIRSCH: I am a clinician and I certainly would second that, that to go on a wild goose chase to look at a variety of different specimens with no real rationale that it is going to be there doesn't make any sense. I think if you are talking about putting tissue in brains, certainly looking at spinal fluid would be reasonable. If you are doing kidney transplants, looking at urine might be reasonable.

But without a good rationale to look at saliva or lung biopsy or salivary-gland biopsy, whatever, doesn't make a lot of sense to me. I would certainly put most of my money looking at antibody and developing a good, reliable antibody test. It sounds, from what we heard this morning, that people are well on their way to doing this.

If you can find no antibody in humans who have gotten various xenotransplants, I think going on a wild goose chase looking for lots of different specimens doesn't make much sense to me.

DR. ONIONS: Could I just come back very briefly on that last one. I agree almost word-for-word with that except one comment, one I keep coming back to, and that is we are working towards better antibody tests. We have heard presentations from several groups about that and they will get better.

But I think we must always remember that the probability--and it can only be a probability at the moment--is that antibody in these groups of patients is likely to be indicative of exposure and possibly of recovery. The patients you worry about are the ones that don't develop antibody because the cats that develop life-long infections, the gibbons that develop life-long infections, the primates that are infected with alphatrophic neuron leukemia virus that develop life-long infections, don't develop antibody.

Those are the people that you are worried about.

But there, you get an unequivocal pattern of infection because you can pick up virus, the pattern of viremia, and you can pick up virus in all sorts of other sites including peripheral-blood mononuclear cells. So antibody is an important adjunct to the test we need, but you need all of these tests because, only by having each of those tests, can you put the patient into the correct profile.

DR. AUCHINCLOSS: I want to come back to this point but just let me close off the discussion. What I heard for the FDA was pretty resounding silence to the notion that there was some other tissue that you should be actively seeking. Is that what you heard?

DR. SIEGEL: I guess what I would ask about that hasn't been addressed in the last two minutes but was raised earlier would perhaps be a little more discussion about plasma. It was noted earlier that some retroviruses have an early plasma-viremic phase. Obtaining plasma certainly doesn't add any move invasive nature than obtaining lymphocytes.

Should there be more routine screening of plasma? Should there be more occasional screening or is plasma not a high-yield place to be looking.

DR. AUCHINCLOSS: So we are about to come to

antibody assays but, before we go there, we are talking, now, about other tissues and we are on the question specifically of plasma. What do the experts say?

DR. HIRSCH: I would certainly go for plasma. I think plasma, given what we have heard so far, makes at least as much sense as PBMCs because it reflects not only what is in PBMCs but what may be elsewhere. Certainly, I would go for that. It is an easy source of material.

DR. ALLAN: It is also much more informative because you are looking for RNA expression so you are looking for virus expression. So it is much more informative.

DR. ONIONS: I would agree with Dr. Allan's last point. I think you need all of these. Again, I would just reiterate, I don't think any of these should be done in isolation; that is, you need to look in plasma for plasma viremia, you need to look for latent infection or probable latent infection in peripheral-blood mononuclear cells and you need to look for antibody.

Each of those gives you a different piece of information which, in combination, gives you a good snapshot view of what that patient is--the status of the patient at that time.

The comment I make about looking in plasma is that I mentioned earlier using RT PCR which you can do and we

have done. But that is probably not the ideal test where you are putting in, or where you are potentially putting it, solid organs because you will get potential leakage of messenger RNA from the cells and, whilst there are techniques for distinguishing between messenger RNA and virion RNA, that is not, perhaps, the best methodology.

The best methodology is probably using reverse transcriptase-enhanced PCR, RT-PCR, because that will detect virions down to at least down to $10^2$ virions. So we can detect low-level viremias using that kind of technology.

DR. COFFIN: David, I assume you meant PCR-enhanced RT, not RT-enhanced PCR.

DR. ONIONS: So sorry. Thank you, John. That is exactly what I meant.

DR. AUCHINCLOSS: Did the FDA get what they are saying?

DR. SALOMON: One point I wanted to emphasize, though, in my comments, is that I think that, oftentimes, in these discussions we get so focussed on vascularized organ transplantation that we don't think enough about the implications of cellular and tissue transplantation.

I realize I set some people thinking that I meant that you should go around biopsying all those organs. That was the last thing I meant. For example, if you do pig-islet transplants, which is right on the horizon, and you

inject them into the portal vein, therefore embolizing the liver, then I don't think it is unreasonable to request a liver biopsy.

If you do porcine stem cells, hematopoietic stem cells, into a patient in the tolerance-induction strategy which is not that far off, then I don't think it is unreasonable to do a bone-marrow aspiration. So that is actually what I was specifically referring to.

DR. ALLAN: I wanted to come back to what David just said. I want to make sure I understood what you are saying. When you are asking for an RT-based assay versus a PCR-based assay, what you are saying, basically, is that it is more sensitive, therefore it is a first screen. But you are not saying not to do the RT PCR because you need to know that what you are looking at is really a pig virus and not some other retrovirus.

DR. ONIONS: Our RT PCR assays and the assay that Walid calls AMP RT--in other words, a reverse-transcriptase that has a PCR input--those assays, in our hands, give very similar sensitivities. In fact, if you actually take the limit of detection--that is down to literally 10 virions--but--the limits of sensitivity you put higher because you need a reproducible assay.

So you still are talking there about 100 virions in either assay system. I prefer to use the reverse-

transcriptase-based assay because you can detect, I think, a message that is creeping back into plasma. Because if you look for a housekeeping message, then certainly some of the primates that we have been involved in looking at, you can pick up those messages.

DR. ALLAN: I understand that. What I was getting at was that if you use an RT-based assay, it is not telling you what virus you are really looking at. So, to me, it is a first screen but you still have to go back and do something like RT PCR to know that it is a pig virus.

DR. ONIONS: Absolutely.

DR. AUCHINCLOSS: You are allowed to have some jet lag, Dr. Onions. This is what, your third trip in six weeks to the United States to help us out? We really appreciate your coming.

DR. HENEINE: I think the point that David was trying to make regarding the RT PCR analysis of PERV RNA, that in the pig plasma testing, sometimes you also detect PERV RNA that is not particle-associated that is coming from the cells. That would interfere with the interpretation of the data. Therefore, you should be aware of that limitation.

What we don't know of is whether this situation will be present in xenograft recipients, cellular xenograft recipients or maybe it will be present in organ xenograft

recipients. But I guess this is what he wanted to highlight, that there could be a limitation in that. That is not to say that we need not use this technology for diagnosis.

Again, I fully support also the other point that we have technologies available to look for reverse-transcriptase testing which are markers of particles. And we could also use those.

DR. ONIONS: You interpreted me accurately. Thank you.

DR. COFFIN: It is not real hard to centrifuge the plasma and see if what you are looking at is a particle or not.

DR. AUCHINCLOSS: Any other comments on plasma? I think I am hearing a loud "yes" there.

DR. DINSMORE: Correct me if I am wrong here, but difference between an infectious endogenous virus and a noninfectious endogenous virus is just that it could go on and infect another cell. So you could actually have an endogenous virus that has malfunctional envelope protein. You would make perfectly good viral particles but they would be noninfectious. That could be picked up in this sort of assay. So, again, we have to be careful about those interpretations.

DR. COFFIN: But, in most of the models, we know

retrovirus as the production of those is very low by comparison because you get so much amplification on infection of other cells. It is not at all clear whether you actually see that in any retrovirus in an animal.

DR. AUCHINCLOSS: Okay. I think it is time to move on to the question of do we now have an antibody assay which, last time we met, was the big missing piece. Everybody said that when we had it, we would feel much more comfortable.

Let me come back to the point that David Onions was making that patients with persistent infection may not--but that is not, necessarily, the issue that we--at this point, we are still asking the question, can infection occur and so, even if you miss 80 percent of the people who are infected, it is still an excellent way of determining the answer to that question.

Is that a fair statement?

DR. ONIONS: That is exactly a fair statement. Could I maybe make a comment about the serologic assays. There are several people, groups, systems, out there. We have heard from the Nextran group, from John Logan, from Walid, from work carried out on behalf of Immutran using recombinant P30. There are a number of approaches and I think all of these are interesting approaches.

I think one comment I have to make about them at

the moment, and I think it is a criticism that Jonathan Allan has raised and I think it is a well-made one, and that is that it relates to this problem that we don't have a patient population--somebody else may--that we know we can look at the patient population and follow it through.

We don't have that so the interpreting the significance of the data is difficult. The second comment is that, at the moment, we are looking at reactivities to maybe one or two viral proteins--sorry; we are looking frequently to inactivity to one viral protein instead of P30, although we are usually following up with, say, whole viral proteins as a sort of an adjunct.

Normally, when looking at serological responses, for instance, the FDA would require in an HIV test or an HTLV1 test, that you have reactivity to at least two viral proteins. I think those kinds of criteria are going to become necessary to be sure that we are looking at true serological responses to virulence and not to crossreactive antibody.

What we can be certain is that, at least I am confident from my own data, that there are patients out there, people out there, with antibody that recognize epitope on recombinant P30. Whether or not that is antibody elicited in response to a virus, I cannot say and I do not know.

What I think you, therefore, need is, as a minimum criteria, response to two viral proteins to be sure that you are actually looking at a response to a virus-induced protein response.

DR. SIEGEL: I have a question about that comment. This issue of crossreactive antibodies has come up before. In this particular case, at least as we move prospectively from here, we know, unlike in the diagnosis of many diseases, the absolute time of exposure and we are able to obtain pre-exposure serum.

Shouldn't the existence of pre-exposure serum give you a pretty good handle as to whether it is a cross-reactive antibody or a newly arisen antibody?

DR. ONIONS: Absolutely. That is a very good point. Unfortunately, in the particular patients that have been positive, those sera do not exist.

DR. SIEGEL: But prospectively, obviously--

DR. ONIONS: Absolutely. No, no; that would begin to sort itself out. I absolutely agree.

DR. AUCHINCLOSS: For my sake, can you elaborate a little bit more about--there are people out there with reactivity to at least one epitope on P30. They were people who received pig tissue or they were people who you just screened in the population?

DR. ONIONS: We have a group of people from

different studies who was to have antibody to P30, and we are confident they have antibodies to P30, because we can show the purity of the antigen by mass spectrometry. We can also show you in the Sofigen system that the actual moiety that is binding to the P30 has the mass spec characteristics of human IgG.

So, I think, to that level we are confident there is antibody in these people but recognize an epitope on P30. But, again, I will restate that we do not know the incidence of that antibody.

DR. AUCHINCLOSS: But they were people who received pig tissue?

DR. ONIONS: Yes; they were.

DR. AUCHINCLOSS: Do you find any of the rest of us that have this antibody?

DR. ONIONS: That is an interesting question. In a validation study--I have to be very careful what I say here if it gives away something, in the validation study, we did look and we did find two reactors. Those reactors were exposed, at it turned out, to porcine material.

DR. AUCHINCLOSS: But most of us are.

DR. ONIONS: Yes; they were exposed in a very particular way.

DR. AUCHINCLOSS: Antibodies?

DR. ALLAN: I wanted to just add a little to what

David said which is if you are looking--and I think people have done a very good job of developing antibody-based assays in monoclonal antibodies and recombinant antigens. If you look at the established assays, either for HIV1 or HTLV, which are two human pathogens that are retroviruses, you typically need to have more than one antigen in your assay system to validate it.

There are a lot of problems with using one antigen. If you look at the HIV system, you look at SIVAGM which is a virus found in African green monkeys, those monkeys don't may antibodies to gag, not that you wouldn't make antibodies to PERV, but it is possible that you might not make antibodies to one particular antigen or that it is not as sensitive as another antigen.

I don't know that it is that difficult. It may be--to produce enough virus to make either a whole virus assay, Western Blot or ELISA. That is typically what is used for HIV. With HTLV, it is a little different, because it is a cell-associate virus. But I would suggest that you try and stay away from those assays in which you use cell lysates because of the dirty nature of those things.

If you have a choice, and I think you have been developing them, but whole virus assays.

DR. ONIONS: I absolutely agree with you. I didn't want to compare one assay against another. I don't

think that is very productive. But my own view is exactly yours, that you don't use cell lysates.

What we do is we use recombinant P30 as a cell lysate, but we do as a secondary is we use whole virus that has been sucrose-banded so this is just virulence, not cell lysates. So it is equivalent to looking at all the viral proteins in that secondary Western blot.

DR. ALLAN: But should that be the first--

DR. ONIONS: I am inclined to agree with you, actually. Yes; I think it probably should be.

DR. HENEINE: The data we have speak to the fact that cell lysates are good antigens and there are high-level detectable antigens there and are seroreactive with antisera, the antisera we use and with diluted antisera. So, from these data, I am not convinced that these are not adequate antigens.

DR. ALLAN: The only thing I would say is we don't have any positives, so you really can't know how good your assay is because you don't have a positive.

DR. HENEINE: That is problem we are all faced with. You don't have a group of 100 infected people where you can use their sera and define criteria for positivity like we do with HIV and

DR. AUCHINCLOSS: This is the theme of the meeting; how confident are you with negative data when you

can't find any positives?

DR. ONIONS: Let me just address this question. I think this is going to become a real issue. There are very good labs out there with slightly different assays and I think you will find, as in all new areas of research, there are going to be differences of results. That is inevitable.

One point I would make earlier, that I didn't make earlier, about the PCR-type assays is that because people are pushing these to high sensitivity, we are going to get into stochastic things where you are almost at the Poissonian distribution of whether one sample taken from a patient is positive and then the second is, maybe, negative. So those kinds of conflicts are going to occur.

There are also going to be some problems over different methodologies and whether they be for serology or for PCR. What might be very useful for all of the groups involved is if we had a set of standards and those standards can be multiple. They could be, for instance--they might have to be polyclonal antibody raised against virion. They might be antibodies raised in a primate against recombinant P30 as a standard--such the we have a number of standards that can be used between us for inter-lab comparison.

I think that is going to become necessary to get some feel for the sensitivity and specificity of some of the assays that are around because I do see that as a problem

that is emerging. But I think there is good will on all sides and I think that can be sorted out.

DR. AUCHINCLOSS: I want to take that comment to go in two directions. The first is the question of what the FDA should actually require of potential sponsors. Are the antibody tests sufficiently well-developed, useful and available that that should now become a part of a requirement for any sponsor coming to the FDA, that they can track their patients with some form of antibody assay.

DR. ALLAN: It looks to me as though we are in the development phase of our antibody-based assays and there needs to be some direction in terms of what we are really going to use to validate the assay and what assay it is going to be. It is going to be an anti-gag assay.

DR. AUCHINCLOSS: That was the crucial word, and I saw nods around the table. But if the FDA wants to hear more about this--what I heard was still in the development stage and not at the point where antibody--but, now, let's come to the second direction that I wanted to go which is actually part of a larger question that Dan was bringing up which is quality control and sharing of tissues and assays between the different potential sponsors which is a potentially tricky area.

What can the FDA do to foster interactions between the different companies, get good things to happen so that

the assays are done the same way, that we know each company is doing this assay as well. Is this an issue that you want to go into a little bit here?

DR. SIEGEL: I think that as long as the committee is advising that we not require the assays, it is somewhat of a moot issue as to how we can get the companies to do the same assay.

DR. AUCHINCLOSS: But what I am hearing the committee say is that the assay is not well enough developed to be a requirement at this time but still a high priority to develop antibody assays that are of good quality and that standardization between the different groups is important.

DR. ONIONS: I wonder if I could take a little bit--slightly reply to Jonathan Allan and slightly to extend the comment you just have made, Hugh. Jonathan Allan is right. I don't think we yet have assays that we can totally rely on because first of all I think we will find that there are going to be some differences between groups. That is one issue.

The second issue, I absolutely do agree with him that a good serological assay should have a system for detecting more than one viral protein. I would suggest, however, that, perhaps because of the speed of presentations, that has, perhaps, gone by the board. The assays that have been used by most groups actually do detect

antibody responses to multiple virus proteins. That is the first comment.

The second comment was they have actually been validated in the strict FDA sense of the word validation; that is, against sera from patients who are clinically normal, from patients with HIV infections, patients with HTLV infections. So those kinds of formal validations have been conducted.

We have also heard from John Logan and his group that pig veterinarians who have been exposed to virus who are doing very similar study at the moment in collaboration with a group in The Netherlands.

So that kind of work is ongoing and, I think, very near completion. So I accept the partial criticism but I think it is only a partial one. I think, of the point of view of standardization, that is a very simple thing to do. I am very happy to hand out recombinant P30 to other people. That is not a problem. I think what we need are a few standard sera that we could use between labs. That is the usual way of doing the kind of interlab comparison.

DR. KASLOW: That is exactly what I was going to suggest. At the very least, what you would have is a common panel that are available to everybody who is developing an assay so they can compare internally what they are doing and externally.

DR. WILSON: I was wondering if we could just back up a little bit and ask that same question about looking for plasma viremia since I think I would value the committee's comments on whether or not assays for that type of analysis are better standardized and further along in terms of development.

DR. ONIONS: Could I just comment because--Walid expressed more elegantly than I did what I was trying to say. Both Walid and I see the same kinds of data as has Jimmy Langford and that is that if you look in the plasma of pigs, you will find--by RT PCR, you can certainly find RNA of PERV.

But the problem is if you also look for a control RNA, globin, you will find that so that you cannot distinguish whether that is really associated with a virus or whether that is just because you are looking at cell breakdown. So that is probably in the context of the whole solid-organ transplant into a patient not the methodology of choice for the initial detection. Probably that ought to be based on a particle-associated assay which John Coffin has emphasized is using reverse-transcriptase.

Using very sensitive PERV-type assays, you can do down to $10^2$ or even lower in terms of the number of virions you can detect and we are sure of that because we can take virus particles actually and count them under EM and take

the dilutions out. You can validate that that is the number you can detect.

DR. WILSON: But I guess the question you had asked regarding antibody assays was whether or not the FDA should require that and whether or not the same question would apply in terms of, for example, looking for RTX in these sensitive assays.

DR. ONIONS: My position is very simple. It has not changed for two years. I actually think that if you are trying to determine the status of a patient, that you really need three separate pieces of information and those are the peripheral-blood mononuclear cells PCR-positive, is there evidence of plasma viremia by an appropriate assay which could be infectivity if we had a good one, and we don't, so the next best assay, in my view, would be PERV. And the third criteria are the patients antibody positive.

So I think if you have those three sets of data, those are the data that are likely, in the most cost-effective fashion, to give you a picture of the status of that patient.

DR. ALLAN: From my perspective, there was some suggestion from, I think Jay, that said, "Well, I guess we are not going to require an antibody-based assay." I would step back and say maybe not the assay that some people are using, but I think that you do need to have an antibody-

based assay.

The only reason we didn't really push it last year was because there wasn't anything developed. Now there is something developed. So I think that you can require antibody-based assays. The only problem, then, is which assays.

DR. SIEGEL: I don't think I was expressing what my thoughts were. I think I was expressing what I heard Hugh say, that it is not something the FDA should--

DR. ALLAN: I would say just opposite.

DR. AUCHINCLOSS: I didn't mean it to be--I meant it to be sort of a trial balloon to see what the committee was going--

DR. SIEGEL: So you have some consensus on that.

DR. HIRSCH: I would think the FDA should require, certainly, the collection of the specimens and testing by an appropriate assay as soon as a group determines there is an appropriate assay.

DR. VANDERPOOL: I agree with the comments of Richard and Martin and a number of you. Isn't there agreement on this issue of required standardized assay. If so, I am not sure the committee, at this point, can spend its time determining which one that is but could, quite easily, get together a group of very experienced individuals who are using different types of assays and determine which

of those is the best for the FDA to require. Perhaps you would want more than one.

But I am not sure that this group can decide which one that would be that would give the best information as a requirement, not that others couldn't also be used by different parties.

DR. HENEINE: Again, we are dealing with investigational assays that we have some experience with. We don't have experience enough that the FDA can approve them for screening the blood supply like some HIV HTLV tests, but I think we can learn much by using those in these prospectively done clinical trials.

Imagine if you could be able to demonstrate seroconversion in a patient and persistent antibody production using those assays. That would be very informative to know the status of the infection in that patient.

Regarding viremia, too. We have two tools, like David was saying. You could look at PERV RNA by RT PCR and you could look genetically for reverse-transcriptase activity. Again, if we can demonstrate the presence of the particles using these two tools, why not? This is evidence of productive release of virions.

So we were not where we are today, where we were a year ago. These investigational tools are available and we

would like them to be validated more. Unfortunately, we cannot. All that we have is a panel of maybe five to ten antisera that are produced in several labs. What we could do is pool these antisera so people can exchange and test sensitivities and specificities of these assays.

Regarding just one comment with David, the RT PCR PERV RNA, I don't think we need to discount its utility because of the potential problem with mRNA of PERV of cellular origin. We don't have, yet, evidence that in people that are exposed to vascularized tissues that we will encounter that problem like we do in some pig plasma.

So, again, we will keep that thing open until we have that evidence that PERV RNA can be limited by that limitation.

DR. ONIONS: I agree with you.

DR. DINSMORE: I would like just like to make one more comment about the viruses in follow up with you. If you have a xenotropic virus, it could be a reservoir of pig cells that are producing it and you would have viremia that would not be infective. So, therefore, I would encourage any of these assays to be coupled with something which shows infectivity, some form of infectivity assay.

That goes along with the antibody base test, too, because one has to know how to interpret the data that one gets and you should put some thought into how to interpret

the results and what tests should be tied together.

Finally, I don't see why there has to be one antibody test so long as you have a protocol for validating anyone's given assay. You could have ten different assays all validated to the same criteria and they would all be viable assays. So I don't think you necessarily have to have every company using the same antibody.

DR. SIEGEL: I guess somebody put to the FDA whether we had a role in providing a standard or helping validate them. I wonder, though, given that we don't have any animal or human model with infection, what is the standard that one would provide to validate such assays?

DR. SALOMON: I have been concerned about that all morning; really, you have ten different companies with three different, four different, assays, maybe more. We all know that when we set up these assays in our laboratories, we never do them exactly the same way. We have also all been at meetings where someone has taken a very similar assay and gotten very different results.

So it makes me concerned. I can handle that in a scientific meeting, but I have more difficulty when that is part of a regulatory process, the end of which is at least a perception by the public that there is a risk here. So I don't think that the FDA, at least this is my personal opinion, now--I don't think that you can avoid this issue,

Jay.

I think you are going to have to establish validity and that validity may change from this year until next year as the thing evolves. I would finish by saying that I am sitting here thinking that maybe, after the first of the year, we ought to be getting a group of us together and having a workshop where people would agree--there is a precedent for this, and that is the MHCHLA international workshops. This has worked very well for defining validity of tests from typing. I don't see why we can't do something similar like that collegially in PERV.

DR. SIEGEL: I am not trying to avoid the issue. I can visualize well how one might establish standards and help validate and cross-validate tests, say, for viral DNA or viral RNA. It is just hard to imagine how to do that for antisera when we don't know what the antiserum of an infected animal would look like, what antigens it would respond to and what sorts of antibodies, because we don't--I mean, what is the positive control.

DR. CHAPMAN: Not a comment, but a question. It sounds to me like what is being laid out here, or I will ask it as a question--is what is being laid out here actually an argument that the best approach and the fastest progress would come not from having FDA identify and sanction one serologic assay as the best for testing but rather an

environment in which multiple assays were tested against the same specimens repetitively which would be either a requirement for testing in one lab and confirmatory testing in a second lab, or a panel of assay testing, until we get to the point where we have better development of the field.

DR. AUCHINCLOSS: I don't know that this is a fact but I imagine it is a fact. One of the concerns that companies have is letting their tissues out or their samples out to somebody else for fear that somebody else comes up with a positive that they don't have a chance to validate before it suddenly is public news.

Are you suggesting that it be a requirement that everybody make their serum from their patients available to everybody else so that these kinds of assays can be done--

DR. CHAPMAN: I don't know that I would want to suggest that because I haven't had time to think through all the implications. But I think, from a practical point of view, the situation is that we have multiple people or entities with experience in developing diagnostic assays who are developing serologic assays.

Those assays have been validated, different assays to different extents, but reasonable well considering that you are working in the field where we have no known positives against which to test them. Ideally, you would want to test each assay against a couple of thousand known

positive infected humans and a couple of thousand known negative infected humans.

Instead, we are working in a setting where you can test them against presumed negative people and a variety of somewhat artificial positive controls. And you can also assume, the limits of science and humanity being what they are, you are going to have false-positive and false-negative results and cross-reactive results.

One way to clarify the significance of results in any assay may be to test multiple assays on the same sample and then use the discrepancies in results to target your further R&D activities on any or all of the assays.

DR. ONIONS: Just a comment. I think Louisa's last point is exactly the one. I don't think this is a heavy-duty issue. I think it is a normal thing that you do in developing assays, and that is that you exchange reagents and compare results. It is a very simple thing.

DR. DINSMORE: Again, the foundation of scientific investigation is that it can be repeated in multiple labs by multiple methods. So, in fact, all of these different assays, I think, rather than providing weakness show strength in that, by many different assays, by everyone's coming up with the same results and, therefore, it is less likely to be due to an artifact in one given lab's hands.

DR. AUCHINCLOSS: Jay, what are you hearing from

this? Are you getting the information that you want or should we phrase the question differently?

DR. SIEGEL: I certainly concur with the committee that there is a need for standards, standardization and for validation of sensitivity. It came up before, I guess, with Dr. Onions' comment about the sensitivity of detecting virus against microchimerism. Standards are very important for comparing across assays.

The FDA has played a substantial role both in in-house testing and distribution of standards, for example, for HIV test that is for the blood supply. There is no question that that is valid. As to whether we have the resources and wherewithal to play such a role for this particular virus is something that we need to check into. But I certainly hear the committee saying it ought to be done and I agree.

DR. PAUL: I think would should give some thought to some virus neutralization which may be a very simple and an old method but, nevertheless, if there are samples that come up to be questionable, at least to look at virus neutralization as a backup test.

DR. MICHAELS: Not to put David on the spot, but I was just curious with you all, the discussion going on, on the samples that you have on patients, and I know all the qualifications that you have made in terms of not sure if it

is crossreactive, have you sent it to any other groups that have, perhaps, different assays to have them look at it as well, yet, or is that something you are considering doing?

DR. ONIONS: I have two completely different sets of samples from different origins that are positive. One set has been compared with another laboratory. The other set has not. I would rather not comment further at the moment partly because some of this data is about to be published or submitted for publication and the other set of data, again, is under a sponsor who doesn't, at the moment, wish to go further with it.

DR. VANDERPOOL: Just a quick comment. I think that we let the genie out of the bag when we encouraged the FDA to have all those who are doing xenotransplant research to develop assays of various kinds for testing. Now, the genie that got out of the bag was different people have done different things, developed different tests and surely have proprietary interests in quite a number of things done.

I think, in the spirit of what Louisa said, the question is for the people to get together, not necessarily choose just one thing, but at least find uniformity of test results between the various assays that are being set forward.

It may mean that one or two would be shown to be inferior. But plurality is certainly possible within the

general framework of being able to cross-test between the various assays that have been developed at our urging.

DR. NOGUCHI: I think what we still would like the committee to really give a strong signal on--we have heard that, ideally, there should be three types of assays; peripheral-blood mononuclear cells, serology and plasma. But the real question is we required everybody to develop the PCR assays and we wanted everybody to develop the antibody assays.

Are you now ready to say we need to have everybody doing the antibody assays or not, because I think we heard a little divergence of opinion here. We certainly can make it so that every clinical trial from this point on needs both the PCR and the antibody assay, not specifying the particular type of antibody assay, or are we truly still in a developmental field.

Basically, is what we are doing adequate requirements for continuing a trial at this point or do we need more based on what we now know?

DR. AUCHINCLOSS: I thought that the answer I heard was bank serum don't require an assay because the assay is still developmental, or in the developmental stage, but push everybody to develop it together.

DR. COFFIN: I would take a slightly stronger position that I think studies should continue to be done. I

think companies should still be required to continue to participate in this development of antibody assays. I would be very uncomfortable right now just telling them to bank sera against something in the future. I think continued research and progression--

DR. AUCHINCLOSS: I put it that way to kind of provoke a response from the committee.

DR. ALLAN: It seems like most everyone has an antibody-based assay because they have been told to develop them.

DR. AUCHINCLOSS: Everybody should have an antibody-based assay that they are working on.

DR. ALLAN: Or should use one through whoever. There are companies, I think, that are developed, not just Q-One Biotech but other companies that have developed these types of antibody-based assays. So I think you can require the clinical trials to use antibody-based assays.

Obviously, you can't tell them what assay they have to use, but it is just like with HIV when AIDS first came around. Whatever we have got available, let's use it. Let's not wait because you know what happened when people waited to test assays. It can backfire. So whatever resource you have available, I think you should use it.

DR. HIRSCH: But the smartest thing, I think, would be, as was done with HIV, that you use the

contemporary assay but you also bank material so that when you have a better assay come along--

DR. LERCHE: I would just like to second the comments of John Allan and John Coffin and probably others. I think the antibody testing should become an active, ongoing component of follow up. I notice this morning, in one of the presentations, that there was a fairly large body of patient samples or a population of patients that were not monitored by serology, at least in the presentation, and that represents a loss of a lot of information.

I think the more experience, the more numbers of patients that we can look at with these assays, the faster we will get to where we want to be.

DR. AUCHINCLOSS: Phil, I am glad you got us to clarify that because I think what we just heard in the last several comments was a little bit different from what I thought I had heard before.

DR. NOGUCHI: No; thank you for that because that has been a very critical reason for bringing this up before the committee.

DR. AUCHINCLOSS: We may need to come back to some of it, but I did promise our next speaker that, at 1:50, she would be allowed to get going and make her plane. Dr. Khabbaz from the CDC.

**Guest Presentation**

**Nipah Virus Outbreak, Malaysia**

DR. KHABBAZ: Good afternoon.

[Slide.]

My talk is an informational talk so members of the committee can relax. There are no questions and no vote. In fact, if any of you is a subscriber to ProMed, you might even want to consider taking a nap because you have probably heard it all.

What I have been asked to cover is the most recent emerging viral zoonotic infection we have been involved in or are assisting in investigating and that is the nipah virus outbreak in Malaysia. It has affected a large number of humans and pigs in that country.

[Slide.]

Before I talk about the nipah virus investigation, to frame it, I thought I would take you back to 1994 where a cousin of nipah virus, basically what we call now the hendra virus, first appeared. This virus caused an explosive outbreak of respiratory infections in horses, in race horses, in Brisbane. Thirteen horses died. Twenty became infected. Two humans became infected including this gentleman here, a trainer of horses, who was reported to actually have put his arm all the way in the mouth of an infected ill mare to try to help relieve respiratory secretions. He became infected and died.

The title here suggests that the virus mutated. That outbreak made headlines. The virus did not mutate. It was a new virus. What we have learned since then about this virus is initially it was called equine morbillivirus. Morphologically, unlimited sequence information suggested it may be a morbillivirus, but it is not. It and nipah seem to members of a new genus of paramyxoviruses I will show you.

In addition to the explosive outbreak in 1994 in Brisbane, a year later, was discovered another smaller cluster, if you will, Mackay. Two horses had become infected and one person who had handled tissue from-- actually assisted in an autopsy of one of the horses developed mild meningitis and recovered and, a year later, went on to develop meningoencephalitis and died. Hendra virus was recovered from his brain so a latent infection.

[Slide.]

The Australians have done quite a bit with hendra virus. They have been able to experimentally infect horses and cats, guinea pigs and black fruit bats. With horses and cats, they were able to reproduce a respiratory infection and these animals experimentally infected, died. Guinea pigs, a generalized infection, also fatal, clinically ill and fatal.

In fruit bats, it was a latent subclinical infection. Nevertheless, in all these animals that were

experimentally infected, pathology showed endothelial-cell tropism and formation of syncytia in blood vessels.

The Australians have also been successful in identifying the natural host for this virus, fruit bats, flying foxes. Four species of flying foxes appear to be infected in the wild. About 40 percent of some of these flying foxes have antibodies to this virus and the virus has been isolated from fruit bats and sequences are identical to virus from infected horses and people. The virus belongs to the paramyxo family of viruses.

[Slide.]

This is the natural habitat for fruit bats in Australia. I will show you two--

[Slide.]

This is the spectacled flying fox, one of four species that harbors hendra virus.

[Slide.]

This is the grey-headed flying fox. The other two are the red-hooded and the black flying fox.

[Slide.]

Now we forward to Malaysia, 1998, 1999. I put up this map to try to orient you to where the action in Malaysia occurred. The outbreak appears to have started in this area. This is Ipoh. This is 200 kilometers north of the capital, Kuala Lampur, here, the State of Perak.

Infections in pigs and humans appear to have started here, as I will tell you.

A cluster was recognized in the fall of 1998. In December of 1998, another cluster of infections both of illnesses in pigs and humans was recognized in Sikamat. It is a town in the State of Negeri Sembilan. The largest cluster was actually near a town of Bukit Palandok here in the State of Negeri Sembilan.

I don't know if you can appreciate these blue marks. There have been clusters and cases and illnesses in other parts. The Bukit Palandok area has the largest concentration of pig farms in Malaysia. There have also been cases of human illness in an abattoir worker in Singapore who had handled pigs imported from Malaysia, not recognized to be ill pigs.

[Slide.]

In terms of the chronology, now, retrospectively, what we have been able to reconstruct is that--the story starts in January, 1997 so more than a year before a cluster of illnesses were recognized, the patient, a pig farmer, in Ipoh, developed encephalitis and survived. Basically, that person is IgG positive for hendra and nipah and the viruses cross-react and, between the standard, clearly have been fatal and nonfatal encephalitic clinical illnesses, going back, reports of illnesses in pigs.

October, '98 seems to have been an amplification in pigs. A lot of pig disease and human disease recognized and the initial thought was that this was Japanese encephalitis. Some diagnostic testing in Malaysia and Japan suggested that some of these illnesses may have been Japanese encephalitis.

As I mentioned, the outbreak moved to the Negeri Sembilan area. I will show you an epicurve and you can appreciate the number of human cases there.

There were some things that didn't fit with this all being Japanese encephalitis in that predominantly illnesses in humans were in adult male pig farmers, mostly, primarily, Chinese ethnic. Why would a vectorborne infection select adult male of one ethnic group is not completely consistent with what we know of JE.

Also, the deaths in pigs was inconsistent. So, in March, basically, Dr. Chua, working in Dr. Ken Lam's lab at Malaya in KL got some specimens, brains and CSF material, from ill patients and attempted cell culture and were able to isolate the virus. Actually, the virus was easy to grow in a number of different cell lines and produced syncytia.

Dr. Chua, by mid-March, had done a couple of things there and, by mid-March, came to the CDC, first came to Ft. Collins and brought with him serum samples and tissue-culture material. Basically, the JE serology was

negative in 12 out of 13 fatal cases.

[Slide.]

He contacted us in Atlanta and arrived the next day with samples. EM was suggesting a paramyxovirus. I have to say, in hindsight, there was a good investment following the hendra virus for us to work with the Australians and get some samples and develop some reagents. Hendra is considered at BSL-4 agent and so the work was done in our BSL-4 lab by Dr. Ksiazak and others.

So we had some reagents and were able to stain the slide that Dr. Chua had brought and, basically, reacted for IFA to hendra. Also, we had some PCR primers and they amplified a paramyxal sequence. Immunohistochemistry using hyperimmune antibodies to hendra lit up as well in tissue samples.

We notified the Malaysian officials and they requested that we assist in investigating and helping control this outbreak. And then the sequence the next day, we had sequence information that this was hendra-like. But, as I will show you, it is not exactly hendra, actually. It is 25, 30 percent nucleotide different from hendra virus.

Serology; I mentioned that 12 of 13 negative for JE. They turned out to be positive by an ELISA capture assay, IgM positive. The Singapore cluster, as I mentioned, we also got samples and confirmed the same nipah virus. The

viral isolate from Singapore and Malaysia was the same.

[Slide.]

This is to show you a nucleocapsid of this virus, negative strain, by EM. The virologists amongst you can appreciate, if you can see that, basically EM on the tissue culture showing here particles with a nucleocapsid. And, in here, nucleocapsids budding on the plasma membrane tissue culture.

[Slide.]

This shows a comparison of the genome of hendra and Ipoh morbilli and parainfluenza and nipah and hendra as I will show you on a tree constructed by Paul Rotha and Bill Bellini in our division appears to be 25 to 30 percent, as I said, about average nucleotide difference. They have sequenced, I think, by now, a large part of all the nuclear protein and another of other genomic areas as well.

[Slide.]

This is a family tree. This is a genetic tree based on the nuclear protein sequences. The nipah and hendra virus here appear to be on one branch of a tree and the suggestion is that they may represent a new genus. They are close but different from a morbilli virus and the other viruses.

[Slide.]

This is the epicurve. This is the outbreak

cluster started in 1998 in Ipoh area. These are the human cases. You can see here the large numbers of cases in Palandok, in green. The total number of human cases were 258. This is the official number of cases. There have been some cases, a handful of cases after that, additional to the 258 and there have been 101 deaths, over 40 percent case fatalities.

[Slide.]

What is this disease like in humans? For the most part, patients presented with febrile encephalitis, fever, headaches, myalgia, within 24 to 48 hours of presentation to hospital as they progressed to coma and needed respiratory assistance. Pathologically, it appears to be a multisystem involvement, and I will show you some of that, with vasculitis and syncytial giant cells.

It is noteworthy that the respiratory symptoms have been generally uncommon in patients in Malaysia. I will have to add that of the 11 abattoir workers seen in Singapore, there was one fatality. So, clearly, the case fatality there is different than whether it has anything to do with different exposure to the virus. We don't know, but two of the 11 were respiratory and were pneumonia.

In the brain, what you see is a diffuse foci of necrosis and neuronal regeneration, and this is of brain or from a fatal case, and I don't know if you can appreciate

the area of foci of necrosis.

[Slide.]

This is, again, showing that.

[Slide.]

This is the giant syncytia cell with immunohistochemistry staining in red, here, for the hendra/nipah virus.

[Slide.]

This is from immunohistochemistry on brain tissue. Basically, the antigen is found in neuronal cells, glial cells and other--endothelial cells.

[Slide.]

This is a blood vessel and you can see the staining. Endothelial cells.

[Slide.]

This is to point out that, as I said, it is multisystem. And renal tubules have stained with antigen as well, shown here.

[Slide.]

You concern yourself with animals and pigs. This is what is the story in pigs. This is one of the farm stalls in Malaysia. You can see that pigs are kept at large concentration in stalls. In pigs, the presentation is different in that there appears to be a significant respiratory component.

Pigs are described here with rapid, labored breathing. A characteristic explosive nonproductive cough was described. "Very loud," is what we are told. Pigs developed neurologic changes as well, lethargy and aggressive behavior and some focal symptoms as well.

[Slide.]

In terms of the pathology, it is primarily a pulmonary pathology and viral antigens have been seen in the epithelial cells lining the upper airway. But it is, by no way, only pulmonary in that it is also renal, heart, brain and other tissue.

[Slide.]

This is showing a bronchi, trachea, of a pig, again showing there is some free vascular filtrate as well.

[Slide.]

Same here. Red is the--it shows the slide that shows you immunohistochemistry lighting up of the antigen.

[Slide.]

In terms of the epidemiologic investigations that were carried out, one concern was whether this viral infection could transmit from person to person. The concern was with the health-care workers and pathologists and clinical microbiologists. Investigation of this group was undertaken. I think over 300 people were tested. There were no cases of illnesses in any health-care worker or

pathologist or laboratorian.

In terms of the serologic testing, I think I can say with confidence, there is no evidence of acute infection but some of this testing is being repeated with nipah-specific tissue at this point.

The case-control investigations, looking for specific, clearly predominantly over 80 percent were pig farmers, also people exposed to pigs and abattoir workers in other areas, looking to see what specific exposure to pigs may be associated with this illness.

The cases; over 65 fatal cases are positive for nipah in tissue and others. I will say that another of well, or control, pig farmers enrolled here turn out to be positive and some of them have had minor illnesses suggesting that the spectrum of disease with this virus may be broader than suggested by the encephalitic fatal illnesses.

We have also investigated other groups exposed to pigs, abattoir workers, soldiers, veterinary health-care workers and others. I mentioned the investigation of encephalitis cases in Perak and a number of the sera are still being run. So I don't have the results on those. I would just say that we saw fatal illnesses in soldiers involved in culling of pigs. They ran out of protective gear so some of this was done without protective gear.

There have been a couple, maybe a handful, of cases with no documented exposure to pigs.

[Slide.]

In terms of control measures, again, killing of pigs makes the headline but that was part of a number of efforts. One was basically to try to prevent movement of pigs from farms with illnesses to others. That, apparently, turned out to be very hard. There was quite a bit of illicit traffic of pigs and a farmer who had illnesses would try to recover rather than let the authorities know that you had illnesses.

Also, try to educate farmers and others in terms of protective gear and protected ways of exposure to pigs was undertaken.

[Slide.]

But the most sensational, and I think a major part was with killing pigs, culling as we refer to it. Basically, what was done is an area of about five kilometers around the area of farms with activities was kind of defined and quarantined. Within that five-kilometer area, all pigs were killed.

[Slide.]

This is just to show you some of the massive killing of pigs. Holes were dug and pigs were just walked into it and shot, as you will see here.

[Slide.]

This is some of the protective gear used.

[Slide.]

As I said, they ran out of it and so this was probably the better of the pictures I am showing, and covered with lime. I have to say that the killing of pigs was effective and that it did, as I showed you on the epicurve, brought us down on the curve of illnesses.

[Slide.]

This is the killing of pigs. Over, I think we are told, 2 million is the number of pigs in Malaysia before. And half of those pigs have been killed. The efforts have not stopped there and cannot stop there. What the Malaysians have instituted is a surveillance system of pigs going to abattoirs and surveillance systems of farms where they test a limited number of pigs every 90 days and farms that have, I think, more than three infected pigs with serologic testing, then they cull the pigs and offer compensation for the pigs and also surveillance for human illnesses and activity.

I have mentioned a couple of foci as being two or three farms with illnesses in pigs and humans. We think it is because of the traffic of pigs outside of those areas.

[Slide.]

An important aspect and question that came up was

whether the virus has infected other animal species. The reports were that yes, dogs and cats and other animals were dying. And you can see this is pigs kept and dogs running around close-by. Some of these ill animals were tested. Indeed, the virus has infected dogs and cats.

I think in one area, half of the dogs tested were positive. I think the good news is there has been some testing, some systematic testing, done moving away in a concentric fashion from areas with illnesses in pigs and humans. As you move out, the number of infected pigs--I think beyond ten kilometers--you don't see any ill animals. So that is reassuring that at least that is not more widespread.

[Slide.]

There are a number of unanswered questions at this point. I have listed some of them here. Why and how did this outbreak start is not clear at all. The reservoir for this virus, is it a bat? I think preliminary evidence suggests that it may be a bat. Some number of fruit bats, flying foxes, were collected by our Australian colleagues who participated in this investigation.

Preliminary serologic testing suggests that some of these bats may have low-level antibody to nipah and viral isolation and sequencing will close the loop on this one, but that is very possibly the case. Why did it start?

There were some fires reported in parts of Malaysia. Could that have affected roosting of bats and moved them closer to pig farms? Unclear.

Is it safe to return to business as usual? I think I have shown you data that it is spilling into other animals and the fact that movement of pigs outside to suggest that no, and an effort to surveillance and continuing to control this is important.

I think the Malaysians I know are pursuing this very seriously.

[Slide.]

There are a number of other important unanswered questions. One is the question of persistent infection. It brings to mind the hendra experience where one person developed fatal illness a year after infection. And so a long-term follow up of the infected person is important.

The question of latent infection, persistent chronic infection in pigs has not been answered at this point.

[Slide.]

This is the remnant. This is what remains of pig farms in some areas. I put this slide to remind me to emphasize to you that this outbreak has taken a huge toll not just in terms of human lives and morbidity and mortality and deaths of pigs, but also an economic impact on pig

farming and on Malaysia, and to also tell you that there is significant concern throughout the area outside of Malaysia in terms of if the bats turn out to be the reservoir, they are not restricted to one country and efforts to set up surveillance and survey pigs and others will be undertaken.

Finally, I think--and I should have made a slide here of acknowledgements to tell you that the work that I presented is the work of too many people to show here. At the CDC, in our division, I would like to let you know that the pathology is the work of Dr. Sherif Zakil. Dr. Bill Bellini and Paul Rotha did the molecular work and Drs. Pierre Rollin and Tom Ksiazak and others were involved in the viral isolation, in the serologic testing and isolation from the pigs and other animals and humans as well.

We had a large number of people in Malaysia and the team was lead by Dr. Tom Ksiazak who actually spent more than two months there and I know just left Malaysia this weekend, wasn't back in Atlanta when I left yesterday. And also the Malaysian Ministry of Heath, the hospital staff who spent quite a bit of time involved in doing these studies and gathering that information and control efforts.

The veterinary authorities in Malaysia, as well, the University of Malaya. Also Singapore. I mentioned the cases there but we did have a team there go into a case-control study. Preliminarily, it looks like their exposure

to urine and excreta may be associated with cases but, also, the Singapore authorities contributed to this as well as the Australian animal labs. They had a team there and they were experienced with the hendra virus and the bat work was crucial.

I think I will stop there and entertain questions.

DR. AUCHINCLOSS: Thank you very much. To bring us back to work of the committee today, other than the implication that we don't want the nipah virus in our potential donor pigs, connect this outbreak to this discussion. What are the implications, or are there implications, to the infectious risks of a xenotransplantation and FDA policy?

DR. KHABBAZ: I think this is a prime example of an emerging viral infection. Three months ago, we had no idea that this virus existed. It is not primarily a virus of pigs in that we think that the host is a different agent.

In one way, it illustrates the need for vigilance because these agents are there and cross species. So I think it is vigilance. It is sobering to see the impact, the number of illnesses and mortality both in pigs and humans.

In terms of implications of disease outside of the area, I don't think I can comment on that. Until you start looking, you don't know but, clearly, if the bat is the

reservoir, that host species is restricted to that part of the world.

DR. AUCHINCLOSS: Let me put the question to the committee perhaps in a slightly different way. To my way of thinking, when I listen to stories like this, I say, "So why are we interested in xenotransplantation? These kinds of events occur in nature. Xenotransplantation isn't the problem." Is that an implication or is that a way of looking at this that is reasonable, or is that nuts?

DR. CHAPMAN: There are two things I have heard over and over through this whole xeno discussion. One is we have lived with domestic pigs in very close contact for thousands, if not millions, of years and, therefore, we know there are risks and they don't pose much of a risk to us. I think Dr. Khabbaz did a very nice job of outlining the cautionary note in this about new infections.

The other side of this; yes, this is an explosive new infection. It is an experiment of nature and it occurred in a part of the world that is relatively isolated from here. But I think what it underlines to me is the significance and the importance of the husbandry requirements outlined in our PHS guideline in terms of the importance placed on raising animals, even animals we think we know well, that may serve a sources of xenografts in closed colonies with barriers to prevent just this sort of

introduction of infection from vectors we may not be able to anticipate on the basis of what we know about the past history of the husbandry of the animals.

DR. KHABBAZ: Actually, to follow up on Louisa, I will tell you that during this investigation, it came to our attention a couple of cases in this country and one elsewhere of Malaysians who have traveled there who had an encephalitic illness. There was quite a bit of concern and they turned out not to be nipah virus.

But preliminary information from some of these cases, people had worked in pig farms but did not live there suggested more than two weeks, possibly, of an incubation period. So with travel an with exposure, I think the scenario of this possibly having expanded beyond Malaysia is not too far-fetched.

DR. COFFIN: A perhaps subtle point, but like retroviruses, I would expect paramyxoviruses to be subject to the same kind of an activation by antisugar antibodies as human serum. That seems to be not an absolute barrier to infection in a case like this. It seems not to be a very good barrier at all.

DR. ONIONS: Could I just make a general point? First of all, I want to congratulate everyone involved in this work. It has been an excellent to a really important outbreak. But my comment is these are not the things that

concern me from xeno, apart from the facetious comment that keep fruit bats out of your herd, the real issue is that the viruses that we really need to worry about are certainly new viruses that could, in fact, but don't, necessarily, have a very big impact, perhaps no impact on the pig population and have a long latent period in people, those are the viruses that we need to worry about and, by definition, we don't know what they are.

I would also caution--I agree with Louisa's comments--there are viruses out there we know very little about. I reiterate a story I presented, and I am embarrassed about it because we haven't got any further with it, but there are closed colonies of dogs in which we have found a virus and others have found a virus here in North America which looks like a virus called desoxyvirus. The only other known host of a desoxyvirus is the wart hog and occasionally gets into pigs.

We don't know where this virus came from. We don't know why it is there. But, clearly, in animal populations, if you look very carefully, there are viruses there that we just don't know anything about. I think it is those kinds of cautions that I am concerned about.

These are spectacular but they are not really a risk for xenotransplantation.

DR. PAUL: I think this is an excellent example

just because zoonotic are already demonstrated, but nipah virus infection in Malaysia and hendra virus in Australia, those are only two examples that got a lot of publicity. And yet, if you look at the swine industry in the last year and a half, swine hepatitis E virus, circovirus and there are a number of viruses that we don't know anything about, as Dr. Onions mentioned.

And we also don't have very good diagnostics. We don't know what they do in pigs. They may be totally nonpathogens. They may not pose much of a risk to people. Nevertheless, I think we need to be vigilant and support the studies to better understand what risks they pose.

Again, some of the studies mentioned earlier this morning, with the liver transplantation, are we testing for hepatitis E virus, swine hepatitis E virus?

DR. AUCHINCLOSS: We come back to the FDA implications, however, Louisa's point was this kind of event emphasizes the importance of the breeding in captivity in closed herds kinds of requirements that were in the draft FDA guidelines and those seem to us to be appropriate. Is that the right implication to take from this message?

Thank you very much.

**COMMITTEE DISCUSSION**

I want to return, for ten minutes, before we take a break for some coffee to one unanswered question from the

morning session. I suspect we will come back to the morning session questions yet some more in different ways. When we return from coffee, we will have about an hours' worth of presentations and then end with committee discussion on the afternoon's questions to and from the committee.

The question that I think we have not answered from your morning list was the second of the two which was what if somebody comes up with a positive. Let me phrase that in two different ways. What if somebody comes up with a patient who is positive but the question was put to me, what if somebody in an animal system came up with a positive transfer of a PERV to a cat, for example?

So you could answer either of those two questions. If I recall, the way we answered that question a year ago it seems to me was if you get a positive, call us back and we will talk about it. I am a little bit inclined to come up with the same answer right now, but let me see what the committee as a whole thinks.

DR. WILSON: Can you define what you are going to call "positive?" I guess we should define what we want to call "positive" first.

DR. AUCHINCLOSS: That is a very good point. Could you do it for us? What is positive?

DR. ALLAN: Positive is a sustained PCR-positive signal above microchimerism, RNA, antibody-positive. Any of

those.

DR. ONIONS: You can go in criteria. Again, if we talk about the gamma retroviruses, the majority of animals exposed to exogenous retroviruses recover from infection, so they are not like HIV where, if you are infected, as far as we know, with very few exceptions, you are infected for life. So there is a difference. That is the first thing.

So if I had a patient that was antibody-positive and there was no other criterion of infection in that patient, I would not be too concerned. There would be evidence accepting the assays. There would be seroconversion of the evidence of exposure to the virus. So that might not be of tremendous concern.

If there was--I don't know who is going to disagree with me--if there is evidence of PCR positivity in peripheral blood cells that was sustained, that is another order of concern. But if the patient was not shedding virus or did not have plasma viremia, whilst that patient was certainly of concern and, certainly, perhaps, at risk of developing some disease, it is less of a concern than the next one which is where that patient has plasma viremia because that patient potentially, it seems to me, is capable of transmitting that virus onwards.

And you want to be very cautious about whether the virus has been secreted in the body fluids. So I think

there is an order of positivity.

DR. MICHAELS: Could I just add a proposal and it could be shot down. If we find a patient that has gone through one of the procedures of porcine xenotransplantation and we feel that there is a true positive that, at that point in time, we would want further samples from that patient. So we found a positive in the peripheral-blood lymphocytes.

We would want to say, "Hold on. Let's go back. Let's get some more samples from this patient, repeat the peripheral blood lymphocytes, look at the plasma, look at serum, look at the saliva, look at other samples which I think some of the groups have put down in their algorithms of how they would approach this.

I think a reasonable approach is should be say to the FDA that we should also, at that point in time, say, let's put a little bit of a hold on until we can analyze those samples at that point in time and maybe look at the close contacts as well. Would that be a reasonable approach?

DR. VANDERPOOL: My questions go beyond some of what we have been talking about in terms of just testing and keeping it in house. I am not sure we need the scare, but it is worth, in the light of this presentation, to raise the possibility that a patient with a xenotransplant organ would

not only become positively infected but be pathogenic and possibly have a serious enough condition that could be passed on to others.

The question would be with respect to an experimental protocol, what would happen, like restricted sexual activity to quarantine, need to be put in any sort of consent form. It seems to me the possibility of that is distant enough for that not to happen, but one of the scary things on this slide is to see all the pigs herded into a pit and shot.

We are not going to do that to humans, but what would we need to do if some egregious infection happened to occur. I think it is at least worth considering without scaring the wits out of ourselves.

DR. ALLAN: I want to get back to the antibody-based assays--

DR. AUCHINCLOSS: Antibody-positive but otherwise negative patients.

DR. ALLAN: Yes. I feel much more strongly in the case when you an antibody-positive individual or an animal that that is a red flag, that is a situation when you have to stop and say, "What is going on?"

I know, in my lab, what we have done, we have taken an HIV-infected cell line and IV injected three monkeys. They don't seroconvert. Those cells are dumping

virus and you are not getting an antibody response. So, many times, the antibody response is in response to an active infection in the recipient cells even if it isn't. In most cases it is, so if you get an antibody response, you have got to stop and wait and see what is going on.

It may be that they have cleared the viral infection. It may be that, but I am not going to assume that.

DR. ONIONS: I don't wish to be misunderstood. First of all, I don't necessarily disagree. I think any of these positives is an indicator that is going to take a very clear look, maybe caution, maybe start. I am not dissenting from anybody. All I was trying to say is that I do think that, again, one has to do things by analogy at the moment and my analogies are not one just going to HIV. They are the gamma retroviruses.

My only comment is that, in fact, if you go and look at 80 percent of street cats, 80 percent of street cats have got antibody to FELV. But they are not, at that moment, at least the majority, not actively infected by that virus.

So, taking that analogy, it is of concern. It means exposure. It means the virus has been expressed in that patient. That is clearly a matter of concern but, to my mind, it is not as great a concern as a patient who has

got persistent PCR positivity, persistent infection in their white blood cells.

That is not as great a concern as the next level up when these patients are viremic. They have got plasma viremia and they are possibly also shedding virus. It just seems to me there is a hierarchy of concern. This is not to say that any of them are not of concern, but it is a hierarchy.

DR. COFFIN: There are two issues of significance. Is that patient who has evidence of having had or having presently persisting virus replication. I should point out, David, the presence of antibodies may mean the virus has gone underground but it almost certainly means it was replicating.

In the case of many oncogenesis models, it could be some time after that that the actual disease appears. But, as far as pathogenesis in that patient, I think probably most of us would agree there is very little you can do but watch them very closely. As far as that goes, that is what you do. You watch them. You take as many samples as you can and get as much information on the natural history and possible progression of disease later on.

The issue of greater significance, of course, is where that patient is capable of transmitting virus. It is to learn to do everything possible to learn whether that