of approach being considered?

DR. McINNES: I don't quite understand the question.

DR. ROYAL: Well, since you said that this is a genetically engineered virus, so one would expect that one could introduce mutations in the hemagglutinin and other regions that could be used to generate reference sera to anticipate, so to speak, strains that might arise instead of waiting for various strains to appear on the horizon and sera to be generated at that time.

Is that sort of approach being considered?

DR. McINNES: Along with this program, there is a large effort and CDC is involved in it as well, which is a genomic spaced program to actually characterize a whole series of viruses in the intent that you might select particular cannons for the particular tributes to manufacture vaccines.

The reverse genetics approach that I'm talking about here is specifically for generation of a relevant reference virus which is taking the two relevant genes and inserting them into a backbone, a

worker virus backbone to facilitate the yield.

So, yeah, there is a lot of discussion about how one might generate hypothetical candidates, whether you would utilize genomics technologies, whether you would engineer technologies, whether you would use wild-type, classical reassortant methodologies. It is a great deal of discussion around what might be the appropriate candidates to try.

CHAIRPERSON OVERTURF: I think if there's no further questions we should probably proceed on the agenda with the report from the Department of Defense. Linda Canas.

MS. CANAS: Hello. Good afternoon or morning.

In 1942, the precursor organization to what is now the Armed Force Epidemiological Board began a series of clinical studies using concentrated inactivated Influenza A and B virus vaccine. In 1943, the next year, there was an Influenza A outbreak. In the control group, those people who had not been vaccinated, there was a three to six percent increase

in illness compared to those who had received this trial vaccine.

The incidence of hospitalization was seven percent in unvaccinated individuals and two percent in vaccinated individuals. As Dr. Levandowski indicated, in 1945, the military licensed this vaccine, but in 1947, it was quite ineffective against the circulating virus, and thus we proved that the changing nature of the influenza virus compels us to match the vaccine each year with the virus that we expect to circulate.

In 1976, the Air Force began a surveillance program that was fondly called Project Gargle, which was based with a series of sentinel sites around the world where we had people stationed, and this was also public health, wanting to know what's going on with our active duty members and their families.

And in the process, we were in areas of the world where influenza was emerging and causing disease so that we could share that information with those organizations that would make the vaccine decision.

It is a mandatory vaccination for active duty military.

In the mid-'90s, there was a presidential decision directive establishing the global emerging infection system, GEIS, and their mandate is to be a force against microbial emergence of pathogens and to assure the biosecurity of the United States, and we had influenza already, a program that was well established. So it only made sense to expand this and make it a tri-service program.

So in 1997, it did become tri-service with Air Force, Army and Navy participation, and the influenza part of this program. In the GEIS Program there's two wings. I'm going to be talking today about the part that we do in San Antonio, etiology based, where we have sentinel sites, and whatever they send into our lab in San Antonio we work up for viral pathogens and report anything we find.

The other wing is Naval Health Research Center in San Diego, California, and their sentinel sites are all of the recruit centers of each of the services. They know the demographics. They have an

established population. They keep track of the febrile respiratory illness going on in that population, and based on those demographic numbers, a certain number of samples are collected and submitted weekly to this lab in San Diego. So they can establish rates and know trends and tell when something is getting out of hand.

Now, I always think it's important that the board understand how this program work. We have a variety of layers of cooperation which make it work, make it effective, and also give us opportunities that we can respond to.

As I've indicated, DOD GEIS oversees the program, and laboratorians and epidemiologists from all of the services work together. Each year we do have an annual meeting. Last year it was in late May in San Diego, and 27 individuals from each of the services, again, laboratorians and epidemiologists, representatives of the central hub of GEIS.

We had people from Bangkok lab and the Cairo lab there, and also a representative from CDC, and the purpose is to analyze the program from the

year before, much as we're doing here today, see what we expect to come about, where our opportunities will be, how we can cooperate and work together.

We choose the sentinel sites. The epidemiologists make sure that they have all the information they need to run this program. They put together a PowerPoint presentation that goes out to the Public Health officers of each of these sites that they can use to develop educational programs for their providers so that they understand what the program is about, what it's trying to accomplish and what their role is.

We're very aware that we're adding to their daily work load. So to try and involve them and make them benefit from it as much as possible helps everyone.

During the season, a weekly report is put out on the Web. Anyone who has access to a dot-mil Website can access this report, and this year, actually very recently, it is now being posted on the Epi-X Website.

Over in the laboratory we make sure that

the sites all have the collection materials, they have all the information on what we expect from them, how they should collect the samples and preserve them, and of course, this is all best case scenario.

I need to emphasize that this is a full service virology lab working within a full service reference lab. This is not a stand-alone program, and that helps us because we have the infrastructure of a lab. We have FedEx contracts set up for deliveries from around the world. It's very easy and timely to collect two or three samples that meet a case definition, get the in a FedEx box going out the next day, and arrive in our laboratory in a timely manner.

We do operate this as a clinical program, and for the most part everything is set up the day it arrives in our lab.

When we do get flu isolated, we use conventional methods where tissue culture. We want the isolate. We're not interested in just knowing if flu is there. We want the isolate. So we do use a variety of different tissue cultures, and once we have those, then we become more modern and have various

molecular tests that we can go on to characterize as virus.

And, again, I said it was a clinical program. So when we have it isolated, it goes back to the submitting site as a patient report. The epidemiologist notify the Public Health officer what's going on at their base for two reasons. First, we're giving back to them information that they have provided for us, again, trying to involve them in the program, but also so they can go into the Air Force reportable event surveillance system, AFRESS. Culture confirmed influenza is a reportable event, and this gives them all of the information they need to go in and update those records.

Meanwhile back in the laboratory, after we've identified as Flu A or B, we do go in and subtype selected samples, and some of those go on to be molecularly sequenced, and all of this information is then shared with CDC, and then I get to come here and tell you about it.

This is our map for this year. The red stars indicate those sentinel sites that are new this

year. The blue ones have been around for a while. The green sites are representative from Army in Thailand and the Navy in Lima, Peru, where they've had longstanding research labs, and we've been able to hook on with their protocols. They have IRBs, and do surveillance in the local populations. This has proved to be very helpful.

There's other labs, Kenya, Cairo, and Jakarta, that are also supported by GEIS, but I do not have their information here today.

Reporting from the Air Force, we can say that 80 percent of the active duty Air Force have been vaccinated as of the 14th of February. When the vaccine shortage issue was first announced, the decision was made that the priority status in the active duty military forces would be the same as what had been suggested by CDC with the addition that people who were deployed or deploying would also be in that priority group.

Eventually the recruits got FluMist, and they are all being vaccinated now and FluMist is available for other active duty members. There is now

vaccine for all of the active duty members of either the injectable or the FluMist.

We had a great many questions in the beginning about FluMist. It is an inactivated virus. Were we going to pick this up in the laboratory?

So we had the sequence information for what the seed strain, the A/Wyoming would look like. We did not look for this in every virus we got, but in those that were referred to us as being particularly of concern, we did look at those, and we have not found any that match that.

This slide was submitted by NHRC in San Diego. As I said, they are responsible for surveillance of the recruits. In the military there's a particular problem with adenovirus in the recruit centers.

So NHRC is to adeno what our laboratory is to influenza, but this year right around the time when they were just getting to vaccinating the recruits, they have isolated a total of nine Influenza A viruses. One of those was in a person who had been vaccinated for more than two weeks. All of these have

been subtyped as H3N2.

Now, every season has its own personality. I'm preaching to the choir here, and this graph kind of shows dramatically the last two seasons.

One of the things we do in this program that we're particularly trying to get out that we are a resource for outbreak investigation, both laboratory support and epidemiology support, and this was dramatically exemplified this past summer when we received word from the lab in Thailand that there was evidence of an influenza-like illness in Napal.

So two of the researchers stationed from Thailand that were working in Katmandu traveled to southeastern Napal to a refugee camp. There were actually three. When I made this slide I thought there were two camps, but there were three camps, and between July 1st and July 3rd of this past summer, they collected a total of 64 samples from hospitalized patients.

They only went to the hospitals because of the political situation in Napal. It was not considered safe to be traveling through the

countryside.

Within two weeks, we had those 64 samples in our lab in San Antonio and had isolated 42 Influenza A viruses. All 42 were subtyped to be H3N2, and I want to say here I don't have HI data, nice pretty charts like Nancy Cox does, because we do not have animal studies. We don't have ferrets. We use the WHO reagents, which basically just gives us a breakdown of whether it's H1 or H3. So more extensive studies we send off to CDC and also then we do some molecular work.

And that's what we did here. Twenty-seven samples of the 42 that were positive were randomly selected to see if we could look at a molecular basis for this outbreak, and we studied the hemagglutinin gene, and in the majority of those 27 isolates, there was a four amino acid substitution from the A/Fujian virus. All four of those were either within or near an antibody binding site.

And here we have the signature, K 145N substitution that Dr. Cox mentioned earlier as one of the samples that had drifted, and we do feel that this

shows there had been a significant drift away from the A/Fujian viruses.

This is a graph of our season so far. Judging from the way samples are coming in, we have not peaked. It is an A season predominantly. We had received just under 1,300 samples as of the 9th of February. Thirty-seven percent of those were positive for any respiratory virus. Twenty-six percent were positive for Influenza A.

We have subtyped so far about 40 percent of those, and they have all been H3N2, and a portion of those then have had nucleotide sequencing and all of those have shown the four amino acid substitution that I mentioned earlier.

Overall three percent of our viruses were B, and it was a mix. It was a 50-50 mix between B/Yamagata lineage and the B/Victoria, but there was a very definite difference in the season. We got samples in from Hawaii late summer, early fall, and they were split with the Hong Kong and the Shanghai. That made us nervous about what we were going to face this year.

All of our other Hong Kong isolates have come from Peru. One of those was isolated in December, but all of the others had been from the past summer, June, July and August. Everything we've seen so far -- now, we're not talking bit numbers here, 38 total -- have been Shanghai.

Just this past week we already started getting more B viruses. So we haven't had a chance to subtype those yet, but so far everything late in the season has been B/Shanghai.

If we looked at a breakdown of our basis, of our sentinel sites compared to CDC regions, our season started much as everyone else's did with the early results coming in from upstate New York. We're still getting some samples from them, but it has moved much more down coast into New Jersey. All of those As that we have subtyped have been H3N2, and I think there's only one B that we've had in this region prior to last week. It was the B/Shanghai.

We've started getting a lot from Alabama and Mississippi, and of course, we just have specific bases. So it's very dependent on what they send us

from those, but we had a sudden surge of A from these bases and a few Bs that came late.

This is probably the region that has the greatest number of sites, and it does include Lackland Air Force Base with the Air Force recruits. Now, the Air Force recruits are in the sentinel site for NHRC, but ours is a clinical lab, and it's Air Force, and we're in the same city, and we just handle their samples for clinical purposes, and because it is recruits, we've had a great deal of the adenovirus early in the season.

And, in fact, the big mystery right now is where is adeno at this point. We're just not seeing it. We're getting a lot of samples, and we are getting flu. Certainly there's a lot of flu in the area, both A and B at this point.

There's only one site in Illinois, but they're very enthusiastic, and I was concerned last week when we got three Bs from them because I hope they're not getting another wave.

Colorado has the Air Force Academy. We've always been very concerned about those. They are not

in barracks, as the recruits are, but it is a training center, and for public health purposes, we're very interested in detecting early outbreaks, and of course, knowing what they have that's circulating there.

Overseas our early isolates came and still continue to come from Lakenheath Air Force Base in the U.K. They seem to have more flu than the surrounding area. So it's probably enthusiastic Public Health officers making sure that they are doing a good job of surveillance.

Most of what they have is A. It has been very boring I'm told to do their work because they look exactly alike. They are H3N2, again, with the four amino acid substitutions.

They are beginning to pick up a little more B, and the ones here, again, are B/Shanghai.

Italy and Germany are just coming up. We've got quite a few from there, and I should mention in Germany, in addition to our Air Force base there, there's the Army Landstuhl regional Army medical center. They have a very good virology lab of their

own.

And the same is true with Tripler in Hawaii, Tripler Army Medical Center. They isolate the viruses there and then send us the positives. So we're only working up the positives from them.

Of particular interest are those areas that are deployed, and there are many challenges to getting samples out of the deployed areas. Influenza surveillance is not their primary objective, and even if they're concerned about it, they don't have supplies. They have limited storage space. They don't have any dry ice, but they are concerned.

This represents a site in Iraq. I reported last year on a site from Kyrgyzstan. We haven't gotten anything from this year, and we're told they're not seeing any respiratory illness, which of course is the good news.

We have 13 isolates here. Eight of the people had been vaccinated before they arrived in country. The other five were vaccinated when they got in country. We don't have any information on where they got the illness, if it was from the surrounding

community, other individuals. I don't have any denominator status.

We don't understand that this is a particularly big problem, but these are the only ones we have received, and it has worked well. This is where you just do what you can do: collect the samples and ship them however you can. They do come FedEx, but they don't have dry ice. So we've given them gel packs to use, and we're getting very good isolation.

So we go for best case and settle for whatever we can get from some of these more esoteric sites, and of course, the deployed areas we continue to monitor for any of the respiratory pathogens.

I mentioned that in Hawaii we get from Tripler where they isolate their own. We are just beginning to see samples from Asia. They are a primary concern. They're very interested. We get a lot of queries about what's going on. Many of them are very aware of their location in regards to the H5.

But they just now started coming in. We did have reports a couple of weeks ago of an outbreak

in Hokkaido, but it was an exercise. It wasn't an established base of any kind. They had no samples or no collection supplies. We tried to get things to them, but of course, there are limitations, and of course that illness had run its course by the time we got them there.

There was even an effort made to contact the local hospital where the patients had been sent to see if we could get isolates from them, but there was a language problem. So hopefully we'll start seeing more from them.

South America sends us a shipment of between 150 and 200 samples every few months. In fact, we just got a box in this past week, and they're very good. We get a very good percentage of isolation in what they send us.

We have seen a lot of flu A, more flu B from them than we have seen from some of the other sites, as I mentioned, most of it being the B/Victoria lineage.

This slide represents kind of -- there's always an interest. If it's not flu, what is it? So

we put together this slide, actually added on the last year. So it's a fix full seasonal year review. It kind of represents seven different seasons on this slide.

But our case definition is specific for influenza. We have been stressing this year to make sure fever is in the case definition of the samples that are selected. So we look for flu and we get flu.

We do have a variety of other things circulating at the same time, and we have picked up several of the enteroviruses and parainfluenza viruses.

I think there are two unique things to our population that I need to point out, the first being adenovirus. Because of the recruits and what we pick up with NHRC and ours with Lackland, we do have a higher percentage than you would see in the normal civilian population, and just the reverse is true for RSV.

RSV is a very big problem this time of year for babies and immunocompromised, and they don't make up very much of our population. And even if it

is there, the RSV virus is very fragile and is better detected on site with a rapid test. It doesn't survive travel very well. So a program like ours, we get one or two a year at most.

But this is one of our big concerns, and it is certainly a goal of the program because we're back to the same thing. If it's not flu, what is it?

And SARS really brought this to the forefront. When we really needed to know a sample was coming, and we didn't get any samples from SARS, it was just the intellectual discussion in the labs that were getting them, it's difficult to actually identify. So if you're in a SARS endemic area and you have a symptomatic patient and you get a negative, how do you know that it's not SARS? You'd much rather know what you do have.

We're working very hard to get tests. There are tests, but they're not easy and they're not cheap to do in the laboratory for things like chlamydia pneumoniae, mycoplasma, Legionella, pertussis, and of course SARS and the other coronaviruses and things yet to be determined.

We do like to think of ourselves as being in position to handle these kinds of outbreaks. It's a challenge because most of our work certainly with flu, we do large numbers and we emphasize safety all the time, and the laboratory technicians are very aware of safety issues, but I will tell you last February we got a sample in from our lab in Thailand, and the report sheet said, "From soldier sick after culling chickens in avian-endemic area," and that got everybody's attention.

Well, it's nice to have that information because we immediately took that sample into the BL-3 laboratory, and always in the laboratory it's the ones you don't know that you have to be concerned with. So it was a real eye opener to all of us that, oh, yeah, this is true. This can really happen.

We never know what's coming into our lab. We get so caught up in the Flu A and B that sometimes we have to step back and make sure that our procedures are going to be there to cover things that are more pathogenic.

If we summarize our season this year, it

has certainly been predominantly A. All of what we have seen has been H3N2, and all of those have had this four amino acid substitution indicating to us a drift away from the A/Fujian last year and even the A/Wellington from South America.

The B, while it's in our population evenly split between the two, it suggests to us since the latter ones are Shanghai that that is the way it's continuing to go, and we have nothing to add to the H1 story. We know it's out there, but we haven't isolated even a single one.

Thank you.

CHAIRPERSON OVERTURF: Are there cases for Linda Canas? Yes, Dr. Farley.

DR. FARLEY: On the current slide that you have up, I'm just noticing that it seems in 2003 that things changed a bit in the distribution or the prevalence. Did anything change in the methodology. The influenza isolation rates seem to have gone up, and the adeno went down a bit from the previous years.

MS. CANAS: The adeno is difficult to understand because a lot of times they just quit

sending us so many samples. If you look at the '99-2000, everything we got was adeno. The number of samples went from 2,000-some to over 6,000 because they were giving us so many. So adeno went up.

So they quit sending us so many. So adeno, it's hard to tell what that means because we weren't looking at them.

And the other one was Influenza A and what?

DR. FARLEY: Well, the Influenza A isolation rates, they now represent 25 percent in 2003 and 2004.

MS. CANAS: Flu has its own. A lot of times it's the people who are taking the samples. I think in the last two years, I don't have definitive data on this, but I think we put out information on these rapid flu tests about their sensitivity and their specificity, and they should culture the negatives. I think they send us all of their positives, too. I know some places do because I've talked to them. "Oh, but we want you to see what our virus looks like."

So I think the last two years that's been a big part of it, but we've also stressed a lot more the case definition. I reported last year that we did a records review at one of the sites that was overwhelming us, and of those patients that had fever in the diagnosis, 40 percent were positive for a virus. Of those that did not have fever in the diagnosis, eight percent were positive. So that was strong indication to us that this was a way.

This program keeps getting bigger and bigger, and our resources don't, so to try and target those that truly are ill with influenza or respiratory virus. So those two things, I think, have.

DR. COUCH: Just a quickie. You partly answered that, I think. Your case definition for sampling is just acute respiratory illness in the reports for health care?

MS. CANAS: Yes, they do visit, and it is --

DR. COUCH: No fever criteria or syndrome criteria?

MS. CANAS: Fever of 100.5 or greater.

DR. COUCH: They must have fever?

MS. CANAS: Well, that's what we tell them, and we give them sheets and they fill them out, and you say fever and they say 98.7.

DR. COUCH: Otherwise your percent of rhinovirus, of enteroviruses and no rhinoviruses would be very --

MS. CANAS: Well, we don't have the right conditions for rhinovirus. So I suspect a lot of our negatives are rhinovirus. Our temperatures are around 36. You need to be 34 to 35 to isolate the rhinovirus.

DR. COUCH: But you are asking for fever.

MS. CANAS: But you're asking for fever, and that's what we're stressing with the FluMist also, when people are reporting after getting FluMist that they're sick. As long as they have the fever and submit it, then we can ascertain whether it is disease or not. Those that don't, the FluMist people feel bad afterwards, but they don't have fever.

CHAIRPERSON OVERTURF: Dr. Monto.

DR. MONTO: Two questions. First of all,

could you update me as to the situation with adenovirus vaccine in the military? I know there was no vaccine for a while. Are you vaccinating now for adenovirus, number one?

And, number two, are you able to get anything about seasonality in other parts of the world from your data? This has become a major issue in trying to identify impact.

MS. CANAS: Right. I'll answer the second one first and then refer to Colonel Phillips for the adenovirus.

I one time made a comment about when South American first started sending samples, that, oh, good, we could get theirs in our off season when they had flu season because they're Southern Hemisphere. That just made sense to me.

And the reply back was, "How do you know that? We're trying to figure it out."

And, in fact, we get flu from them all year, and they say it's because they have so many different sites and their topography in the country where they have some around the coast, some in the

mountains, and it varies more with that, and I can only comment. The rest of them tend to follow the same seasons that we see.

Colonel Phillips.

COL. PHILLIPS: Regarding adenovirus, and you had commented on the epidemiology on the slide there, 1999 was the last year that we had adenovirus vaccine and were able to give it, and have seen steadily cases since then.

We are aggressively pursuing the pursuit of the new adenovirus. Our researchers are working real closely with the folks from Bar Laboratories that has the contract for that.

Phase 3 clinical trials have begun and are underway currently, and we're anticipating FDA licensure of a vaccine by 2007.

CHAIRPERSON OVERTURF: Yes, Dr. Dowdle.

DR. DOWDLE: Thank you.

One of the real advantages, I think, of this system, global system, in terms of influenza surveillance, of course, is sampling sites throughout the world, and it's a real opportunity for sort of

timely sampling in the sense of you know what's going on.

The question I would have is how does the timeliness relate to, for example, other shipments that Nancy would get in from other parts of the world and other WHO centers. Does this system lend itself to completing or being ahead of some of those or would it be lagging behind, depending on how the shipments are arranged?

MS. CANAS: Well, I can only comment on our system, and those that come in from South America and Thailand do have a lag, sometimes as much as five months, but the others are coming in a day or two after collection, and then within a couple of weeks, we try to get anything off to CDC, but I don't have any idea.

DR. COX: I can comment just a bit further on what Linda said and can concur with what she said. In some cases, we do get viruses through the military surveillance sites on a very timely basis, and it's really excellent.

She mentioned but didn't emphasize

probably quite as much as I would like to emphasize that there's a lot of sequence work going on, which feeds directly into the sequence information that we have and, therefore, that goes into the WHO system.

In other cases, we get viruses, for example, from Thailand, directly from their National Influenza Center, in a more timely manner than we do from the military. So it varies depending on site and a variety of circumstances.

What we have found generally is that GEIS is a tremendous value added to the WHO global influenza program, and that we would very much like to see it continue, the GEIS activities to continue.

Along the same lines, we've had the opportunity this past year through efforts of the department to work in closer partnership with some of the military labs and will be providing some financial support for NAMRU-2 and NAMRU-3, to enhance the already existing influenza surveillance infrastructures that are in place there in the hopes of both expanding influenza surveillance into more rural areas in Indonesia and some other countries, and

also having more timely shipments of viruses to our WHO Collaborating Center.

So I think there's very close interaction and real value added is the bottom line.

CHAIRPERSON OVERTURF: Any other comments?

One thing I didn't hear was whether there's any enhancement of this or has been an attempt to enhance the system recently to look for H5, H7, H9 strains, particularly from those areas where they used to come in the past.

What I heard was that you rapidly grow the viruses that you tend to characterize their hemagglutinins. Are there additional epidemiologic data in those sites where these viruses are likely to emerge that are collected that in any way changes your operation at that point?

MS. CANAS: Well, this is what we struggle with. As long as we can keep geography in that mix there, we feel pretty safe that those things that are coming in from endemic areas we can handle in the BL-3 laboratory.

We work again with CDC to know what's

going on in the world. Other than that it's just trying to keep up with what's going on and be prepared.

CHAIRPERSON OVERTURF: Are there other questions or comments? Yes, audience.

DR. GAYDOS: My name is Joel Gaydos. I work with Linda and the other people on the GEIS program.

In response to some of the questions that have come up over the last few minutes starting with Dr. Dowdle, one of the reasons that we meet once a year is to make sure that the programs are complementary, and there are a couple of things that I think are important in addition to what has been brought up.

We have people who are permanently stationed at places like Cairo and in Lima, and we do a lot of bulk processing. But if something is happening, we can speed up the processing. If something happens at the CDC and they think that we should be getting more specimens from Napal or from Peru, then we get the word from them, and then we go

out and collect the specimens.

We also have the opportunity because of having military people stationed there to get into some areas where other people aren't and where specimens would not be easily obtained, like some of the places you have heard about in Napal.

So I think the program has worked very, very well with the CDC. As Linda pointed out, specimens come to her laboratory. She works very closely with Atlanta and also with the Navy group in San Diego.

Not all of our specimens come in that way. Our lab in Cairo for a number of reasons works directly with the CDC. Our lab in Jakarta for a number of reasons works directly with the CDC or with the laboratory in Australia.

So I think it is, in fact, a surveillance system in that we are regularly collecting specimens. We're working together.

We also respond to situations, and we do have the epidemiologic capability to immediately jump on something if it's picked up in the laboratory.

CHAIRPERSON OVERTURF: Thank you.

Are there any other questions or comments?

(No response.)

CHAIRPERSON OVERTURF: We have 15 minutes extra for lunch, and the proceedings of the meeting will begin, again, at one o'clock sharp.

So thank you very much.

(Whereupon, at 11:46 a.m., the meeting was recessed for lunch, to reconvene at 1:00 p.m., the same day.)

AFTERNOON SESSION

(1:08 p.m.)

CHAIRPERSON OVERTURF: I'd like to call the meeting to order please.

Good afternoon. We now open our open hearing, and I'll ask Christine whether there's any members in the audience or otherwise who would like to address the committee.

MS. WALSH: As part of the FDA Advisory Committee meeting procedure, we are required to hold an open public hearing for those members of the public who are not on the agenda and would like to make a statement concerning matters pending before the committee.

Dr. Overturf, would you please read the open public hearing statement?

CHAIRPERSON OVERTURF: Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To insure such transparency at the open public hearing session of the Advisory Committee meeting, FDA believes that it is important to

understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement to advise the committee of any financial relationships that you have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or group's payment of your travel, lodging, or other expenses in connection with your attendance at this meeting.

Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

MS. WALSH: I have not received any requests at this time. Is there anyone in the room who would like to address the committee?

(No response.)

MS. WALSH: I see no response. Dr. Overturf, I turn the meeting back to you.

CHAIRPERSON OVERTURF: So we will proceed with the agenda and talk about vaccine responses. Dr. Roland Levandowski.

(Pause in proceedings.)

DR. LEVANDOWSKI: Okay. While we're working out the technical end of things here, maybe I'll just give a little bit of background information about the serologic information that I'm going to be discussing.

The serological data that I'm going to be presenting actually will be coming from a number of different centers, and I'm going to try to summarize that information as best I can. I think you all are aware that we share serum panels between several different laboratories and test these same serum panels, each within those laboratories.

The whole point of doing this serological exercise really is to try to see whether the responses to current vaccines confirm what we have found already with the antigenic and genetic characterizations that

have been presented this morning already by Nancy Cox.

And whereas you saw for the tables with the ferret sera where there were a few very well characterized ferret sera used to test an enormous number of different antigens, for the serologies we're really kind of reversing that procedure. We're looking at a relatively few antigens that have been selected to be representative of the current circulating strains that seem to be different to us, and we're using a much larger panel of sera.

Now, the sera that we use, those different serum panels that we have available to us are not all identical. They're not all collected the same way. I think that's something that everybody should be aware of also.

Some of these serum panels, you'll see the number of sera on each one of them. Some of these serum panels have been prescreened to select out people who respond well to the vaccine, and that's actually okay. That's good because if there's not a response of any sort, then we won't be able to perform what our primary purpose for this whole exercise is,

which is to try to compare the responses between the different antigens.

We're not really trying to say that any of the vaccines used are immunogenic. We're not trying to say anything about the vaccine itself. Really we're focusing on trying to compare what kind of antibody response we see for the vaccine strain as compared to the test antigen.

And so what I'm going to be emphasizing as I have on some previous occasions are the geometric mean titers, the post immunization geometric mean titers. The tables that I'm going to show you are going to be in the traditional sense. They're going to have percent fourfold increases. They're going to have percent of people who are above an arbitrary cutoff point like one to 32 or one to 40, but I'm really going to be focusing on the geometric mean titers and how that relates the test antigen to the vaccine strain.

You're going to see also as I show you these results that there are going to be differences in the absolute titers between labs, and again, that's

not something I want you to focus on. What I really want you to see are the differences that are shown between the labs.

And what I can tell you generally when we look at this, although there are differences in the absolute values from each lab, when you do a rank order of the way the different antigens fall out, the test antigens, they come out to be not exactly the same but pretty close to the same in terms of which is recognized as the highest response and second and third and fourth, and so on.

So these are the serum panels that have been provided shown on this slide and the next one, and you'll see here that the vaccine strains used in all of the centers were pretty similar. I just point out some slight differences.

All of the centers using A/New Caledonia and A/Wyoming as the H1N1 and H3N2 strains, but there are differences in the B strain. The vaccine used for preparation of these sera from Australia contain the older B/Victoria-like vaccine antigen, B/Brisbane/32, but the other centers have used more recent

B/Yamagata-like vaccine strains, B/Jiangsu/10/2003 or B/Shanghai/361 and that should be 2002.

We also have a couple of panels of sera from children. Nancy Cox mentioned that they had tested two. There's only one that I'll be discussing, one panel from the CDC that I'll be discussing with the following information.

We had even a third panel of sera from children, and I'll be presenting some information from those. So these are not exactly the same two panels that Nancy mentioned, but they are two panels of sera from children that we have to look at.

So jumping right into it, these are the different antigens that are used for the H1 serologies, and all of them are H1N1 viruses. As Nancy mentioned, there really haven't been any H1N2 viruses circulating, but we have a good representation of strains from around the world, from Asia, from North America, from Europe, from Oceania.

And in terms of responses, I'll be showing you some tables that are not all the tables that were available or all of the data, but some that are just

representative of results that came out from the different centers.

So here is a table of results for three different serum panels from the United States, from Australia and Japan. These are sera that were done in the lab here at Center for Biologics, and these are the numbers of serum pairs that we had from each of these sites. The antigens that were tested are shown here, and in blue on every one of the slides I'll have the vaccine antigen, and in red I'll point out the ones where there's a 50 percent or greater reduction in the post immunization titers as compared to the vaccine strain.

So from this you see that we tested not only New Caledonia, the vaccine strain, but also A/Florida/4/2004, A/Bangkok/1544/2004, and A/Okinawa/42/2004.

And for the most part the post immunization responses of people who were immunized with A/New Caledonia were very similar for the other strains that were tested. The one exception here is that in this particular serum panel with A/Florida,

there was a more than 50 percent reduction against the A/Florida strain as compared to the vaccine.

So those were adults, and now elderly, from the tests that were done at National Institute for Biological Standard and Control, or NIBSC. They're ordered somewhat differently here and different numbers and so on, but again, A/New Caledonia was the vaccine strain. A/Netherlands/128/2004, A/New Caledonia/9/2004, and A/Okinawa/42/2004 were the test antigens.

And by saying that, I'd also point out that we didn't test all of the same antigens in every lab, but there is overlap between antigens that were tested in the different labs. They're not all exactly the same. So we have a somewhat broadening of the numbers that are tested and some similar or some identical strains that are tested in the different labs to give us some idea of where we're standing in terms of the overall results in comparison between labs.

But here, again, you see in the post immunization geometric mean titers for the most part

the new strains were well inhibited by antisera that were raised against the vaccine strain. In this case, the one exception, it was for this A/Okinawa/42/2004 H1N1.

So those were adults and elderly. For the pediatric population, the two different groups of children were children who were six to 23 months of age. I don't know exactly what the mean age is, but they were all less than two years old, and another group of children who had a mean age of 21 months with a range of eight to 38 months.

And, again, the antigens that were tested are shown here. For the somewhat older children, we did not really see much of a difference between the vaccine strain and the test antigens, although it's a little bit low for this Florida strain, which is similar to what I showed on the first slide that didn't quite reach this 50 percent reduction

And here these younger children for these two antigens, A/Florida/4/2004 and A/Netherlands/128/2004, there were more than 50 percent reductions, and I'm just going to go right on

to a table where we've tried to summarize all of these results looking at the percents that were greater or the reductions in geometric titer that were more than 50 percent.

Why are we emphasizing this? This is a way to try to get some idea of how severe or how the magnitude of the difference. It's a somewhat arbitrary way to do things, but it does give us a way to try to handle the data which are somewhat disparate between the different labs and for the different antigens.

And if you look at this, there are a number of different antigens that were tested. Many of them were tested in more than one lab, and I'm not including some of the information for strains that were just tested in one lab, although I have a little bit here.

But what you see generally is that for all of these H1N1 strains, for the most part on average there was not really a 50 percent reduction either by the total numbers or by the mean averaging the percent reduction for all of the studies all together.

Actually they're quite low.

So the difference here wasn't very much, and you can see from the range also that for the most part, very few of these antigens when they were tested were there 50 percent or more reductions.

So now moving on to the H3 strains, A/Wyoming, of course, was the vaccine strain, and there were a whole range of representative current viruses. All of these, I think, can be categorized as California/7/2004, although I'm not entirely sure about the Singapore strain. That might not be truly considered California/7-like, but it's a more recent strain than Wyoming, and if anything, it would be somewhat like Wellington or out farther than Wellington on the genetic dendrogram.

But, again, we have a range of viruses that were tested, and here I'm showing some data from the CDC lab for adults, from the United States, Europe, and Australia. The antigens that I'm showing here, Wyoming is the vaccine strain, and here there were A/California/7/2004, A/Singapore/36/2004, and A/Tennessee/6/2004. Those strains were tested.

And I would call your attention to the fact that for every one of these serum panels and with all three of those California-like antigens, the reductions in post immunization geometric mean titer are really quite obvious. They're very large reductions. It's much more than 50 percent in each one of these cases.

And as you'll see on the next slide, that holds true for the elderly here as well. These are sera that were run at NIBSC for several serum panels. They tested California/7/2004, Singapore/37/2004, Oslo/807/2004, and Shantou/1219/2004. And here, again, you see it stands out very much that there's a big difference between the post immunization responses for the vaccine strain and for these newer California-like antigens, and it's a lot more than a 50 percent reduction.

And, finally, for the H3, here are some data for the pediatric population again, and again, just not to belabor this too much you see that even in these young children or particularly in these young children, you see that there's a big difference

between the post immunization titers for the California-like strains and the Wyoming vaccine strain.

For a summary of all of this, in this table I just call your attention to the totals over here. You'll see that almost every one of the serologic tests that was done show that there was a 50 percent or greater reduction in geometric mean titer as they were tested, and the magnitude of this difference was really quite amazing and substantial. All of these are much, much more than 50 percent, and in many instances the percent reduction -- none of the tests were under 50 percent and most of them were almost 100 percent.

So now moving on to Influenza B, this is a little bit more complex. As I mentioned in the vaccine, the Australian vaccine has B/Brisbane/32 as the vaccine strain. The vaccine that we're interested in here in the United States would have contained B/Jiangsu/10/2003. So I'm really not going to be focusing on results that came from the vaccine that was used in Australia in this case.

There were both Victoria-like Influenza B viruses that were tested here. I've listed them as Hong Kong/330-like. That was the last vaccine strain that we used here in the United States.

And there are also a group of viruses that are more like Yamagata/1688 or our current vaccine is Shanghai/361-like. So there's a range of different antigens to look at here.

And, again, this table from data from the CDC in adults for a number of the different serum panels. In this particular instance B/Jilin/20, which is equivalent to B/Jiangsu/10, was used as the test antigen, but that's equivalent to the vaccine.

And I guess I should point out that in this case the antigen was ether treated. Not all of the labs do this, but many of the labs do ether treat the antigen before doing the serologic testing.

There are a number of antigens that were new viruses that were included in the test, including B/Colorado/4/2004, B/Florida/2004, and B/Fujian/2004, and again, all of those are in the same HA lineage as the vaccine, and you can see all of these in each of

these instances were very well inhibited by the current vaccine.

The only thing that was not in this particular test was B/Hawaii/13/2004, which is representative of the other HA lineage that's not in the vaccine right now, and there were more than 50 percent reductions here, and this is very consistent with what we have been seeing in the past in the same situation. This is really not new. It's what we have identified in previous years when we've been comparing the responses of vaccine containing one HA lineages to viruses in the other HA lineage.

These are data from the Australian center, an elderly population, and here they were testing -- that should be B/Jiangsu/10. Sorry -- but they were using B/Jiangsu/10 as the equivalent of the vaccine strain, and then tested B/Brisbane/4/2004, B/Victoria/501/2004, and B/Hawaii/13/2004.

And here the results are not quite as clear-cut as in the previous slide. Here in this instance, compared to the vaccine response, we would say that the response for all of these test antigens,

including the one that's not from the same HA lineage, the B/Hawaii/13, looked pretty similar.

But in this other panel from Europe, one of the strains that's in the same HA lineage as B/Jiangsu actually gave responses that seemed to be low in this particular center's tests, as did the Hawaii which we would have expected because it's in the other HA lineage.

And then the last in this particular panel of sera here. Again, there was not really a difference seen even between the Hawaii strain which was in the other HA lineage and the vaccine strain.

So these are results from children, these two different groups, and here it's a little bit more difficult to interpret because the vaccine responses to the vaccines themselves were on the low side. The sera tested at CDC using B/Colorado/4, B/Florida/7, B/Fujian/430, Hawaii/13, and the other similar strain to Hawaii, Phitsanulok -- I think I'm getting that right or close -- even though these results are low, it looks like for all of the strains that are equivalent to the vaccine there's a similar response,

whereas it was a lower response for the non-vaccine antigens.

For these children, these slightly older children, however, although we had some response to the vaccine, we really weren't able to detect response to any of the antigens, including those that are similar to what's in the vaccine.

So trying to put this into a summary slide, looking at the 50 percent reduction between the different sera, I'd say overall it still sort of holds up that the strains that are in the HA lineage, not currently in the vaccine, Hawaii/13 and Phitsanulok/2053, that there are some reductions, but you know, they're not really high magnitude all the time. It's not really consistent between all the centers and all of the tests that there's a 50 percent or greater reduction, and in only about half of them, if I've done the math there right -- and I might have made a mistake, but it looks okay as I do it quickly in my head -- although it did reach like 50 percent for mean, there was a huge range here for the results.

For the viruses that are in the same HA

lineage as the vaccine strain, here again there's some data that suggests that there may be some reductions for some of these viruses like Brisbane/4, and on the other hand, other data suggest that for new, more recently circulating strains similar to the B/Shanghai/361 that there's not so much reduction.

But, again, you see there's a wide range here in the results for these different serum panels, and the highest percent reduction actually would come from the children. So if I excluded those, you would see for adults and elderly that it looks like even less a difference between those more recently circulating strains that are like the vaccine strain, B/Shanghai/361.

So in summary, I guess what I would say overall is the studies were done with sera that were collected after immunization from a number of centers with a number of different vaccine products, and at this point it looks like the representative H1N1 viruses are very well inhibited by the current vaccine.

On the other hand, it looks very clear

that the A/California/7/2004-like viruses are poorly inhibited compared to the current vaccine.

For the B strains, the B/Shanghai/361/2002-like viruses seem to be reasonably well inhibited, but those that are in the other HA lineage as represented by B/Hong Kong/330 are less well inhibited than the vaccine strains.

And I will stop there and see if there are any questions.

CHAIRPERSON OVERTURF: Are there questions for Dr. Levandowski? Dr. McInnes.

DR. McINNES: Roland, the infant data one presumes that some of these children are receiving their first dose of flu vaccine and some are maybe second, maybe, I guess, second. So what was the timing around the sera collection post dose, and for those who are receiving it for the first time, was this collected post two-dose?

DR. LEVANDOWSKI: Okay. I should have made that clear. Both of those serum panels were from children who had received two doses of vaccine. It was post the second dose of vaccine. I don't have

information. I can't tell you at this point whether any of those children had been immunized previously or infected.

Some of the older children in the second serum panel more than likely were either immunized or infected. The younger children though you'd have to -- since these were studies done some time in the summer to early fall, late summer to early fall, this would have been happening in between flu seasons, but I can't really totally tell you how all of their exposure history fits in because I don't have those data.

And I think, you know, still these are really very young children, and it gives you an idea of what you would expect in a population of very young children.

CHAIRPERSON OVERTURF: Yes, Dr. Karron.

DR. KARRON: Two questions. the first is I actually first want to commend you on having these pediatric panels of sera. I think a couple of years ago we were discussing how important that was, and I think it's very useful to have them.

I guess my first question is now that we have two types of vaccines in this country -- we have an activated and we have live attenuated -- would it be useful to have data from individuals immunized with live attenuated vaccine to have that as a comparator, obviously not in the children under five, but you could have school age children and adults 18 to 50. I think, particularly when these issues of antigenic drift come up, that might be a useful comparator to have.

I'll let you say something, and then I have a second question.

DR. LEVANDOWSKI: I think it would be interesting, but I just would like to point out that the whole point of this, again, is to compare. It's not so much to say whether the vaccine is immunogenic or not. I don't think with the strategy that we're using here, I don't think we can really make any comment on that. So really we're trying to focus on getting a comparison between the different antigens, the vaccine antigens versus the newly circulating strains.

Having said that, I think it would be very interesting to have access to more sera all together from different populations, and it might make sense to explore that and see if that gives us any additional data compared to what we get from inactivated vaccines. I don't see any reason it wouldn't be useful information really.

DR. KARRON: And I guess the follow-up question really relates to something that Dr. Couch was talking about earlier, but you know, I guess my question is really we see very poor responses in very young children to the B strain. Is that consistent with what we've seen before, data that we have before?

And if so, I mean, I'm almost wondering whether it matters which B strain we choose because responses are poor. You know, it's hard to look at reductions when your geometric mean titer is ten.

DR. LEVANDOWSKI: Right. Well, those numbers are small, and I don't know how much of that is technical artifact and how much of that is significant in terms of what protection would be. Most people are used to thinking about titers of one

to 40 or greater being representative of protection, but actually the data are fairly sparse in terms of indicating that there's a correlation between the titer and efficacy.

We do know the higher the titer of antibodies the more likely you are to be protected, but I don't think we know for sure that, you know, even at lower titers there might not be some level of protection. So I would hesitate to comment on what the meaning of those titers that we saw there are.

But we have for children, we have seen titers that have been very low. Usually it's for the H3 strains that we've seen that in previous years, but they vary quite widely, and when I said there may be some technical artifact, I guess we occasionally see differences that may be related to some aspect of the virus itself in terms of their avidity for binding to red cells, other things that are going on in the system.

But I'm rambling around here and not trying to answer your question directly because I don't think there is a direct answer to it.

DR. KARRON: But I guess my question is that I'd have to go back and look at these data, but the titers to be were not uniformly low across populations. You know, if you thought it was a technical issue, then presumably whether you tested adults or elderly or children, you would have uniformly lower titers.

My question is given how low the titers are to the B strain in these very young children, is that inconsistent with what we've seen before or is that consistent with what we've seen before in TIB in very young kids?

DR. LEVANDOWSKI: Well, I did answer that part directly. I think we've seen low titers for different antigens, not necessarily for B, but we have seen low titers for different antigens, not necessarily for B, but we've seen low titers with different antigens in very young children, and I don't think we always would predict what that would be. I think we've seen differences between children and adults for, you know, the same antigen and we also have seen if you look at a series of antigens like H3,

for example. It seems that some of them are just more inherently immunogenic than others.

CHAIRPERSON OVERTURF: Dr. Couch.

DR. COUCH: Just for a moment, a lot of what we're looking at here is very test dependent, as Roland pointed out, and the split product is used for the B which, in order to give it the kind of sensitivity that will permit these spread out comparisons, and what he is giving us is really relative, not absolute, and when we're using the A strains, we're using it there in an entirely different HI test.

But in general, Influenza B responsive is when you do one type of assay, you see, which carries, as we know, the same kind of sensitivity. Neutralization tests are generally speaking not quite as good as they are to A, but that's all fairly relative. So it's difficult to compare B responses to A responses when we're looking at them here because it's so person and so test dependent. But he's going with relative findings for strain selection, you know, this percent of GMT, and then the same is true for the

equal to or greater than 40, you know. What is the validation of a one-to-40 with a split product versus a one-to-40 with a whole virus antigen when you're doing Influenza A, and certainly a major difference is if you do B with whole versus the split product.

So it's all sort of a test-test nuance in that. So one of the reasons that I think that really caught my eye that you didn't comment on, Roland, was page 37 in the CDC surveillance where the pediatric population age is five to eight, 274 children by Kathy Neuzil, and only ten percent of them had a rise to be Hong Kong. There's a major disparity in that age group, both of which has inactivated and live recommended for.

You really would have liked to have seen the live to see how it does in that comparison in that regard, but as we go back to Roland's point, that doesn't influence our selection here. That's the kind of thing that the ACIP would want to be looking at when they talk about relative criteria for recommendations, but that data would have really been interesting to add to that one.

I only had one other just minor question, and I couldn't pick it up here, but in the individuals it looked like the elderly in the U.S. and Japan didn't respond as well as the elderly in Australia and Europe. That's got to be partly population dependent in testing. What do you --

DR. LEVANDOWSKI: No, no, no, no, please. I said at the very beginning that you should be aware that some of these serum panels have been prescreened to select out higher responders to begin with. Not all of these serum panels have been so prescreened.

DR. COUCH: I missed that.

DR. LEVANDOWSKI: So I think you have to be very careful. There are differences in how the sera were handled before they got to the individual labs to be tested. So I wouldn't draw any conclusions from that. I'd try to focus on the relative responses between the antigens.

CHAIRPERSON OVERTURF: Dr. Monto.

DR. MONTO: Just a further comment about B responses in young children. They don't even respond well to HI ether treated antigens post

infection because we've had, when we were back doing our community studies in Tecumseh years ago, we could show that children under five were heavily infected when there was a B outbreak, but if you look just in terms of rises in antibody titer, a fourfold rise in titer, it looked like they were spared.

So there basically is a problem in just immune responses and young children measured by HI, we don't know how that translates to other things.

DR. COUCH: Just a quickie for general information. That same thing is true for the neutralization test, and I think our test is fairly sensitive. The same finding.

CHAIRPERSON OVERTURF: Yes, Dr. Dowdle.

DR. DOWDLE: Like Bob, when I saw that chart, the Australia sera were looking so good that I was ready to move to Australia, but --

(Laughter.)

DR. DOWDLE: So thank you very much for your explanation, Roland.

But this is just to say that I think that the evolution in sort of the use of human sera to

interpret the strain differences has been really quite helpful and is moving forward quite a bit.

I wonder if there's further discussion on how this can be standardized even more because particularly I think there were core viruses, but could there be more core viruses, it might be used. In addition, the labs could use whatever other strains that they want, but I'm sure those discussions must be taking place.

DR. LEVANDOWSKI: You're right. There are discussions going on all the time about how to try to do this better. It's very difficult because of the logistics of transport of all of these materials, and although I did not get into that as part of the presentation, there are differences in the antigens, too, because in order to do the testing, it's done with a pool of virus that is grown usually in the laboratory where this is being done, and so there may be some subtle differences from passage levels in the different laboratories that have an impact on that.

But the logistic part of it is very tricky, and I'm not emphasizing that, but of course,

these California viruses have only really been recognized as a separate group of interests since about mid-January. So to get these shipped around to the different labs and to be able to do the testing on those has been, you know, a challenge, quite honestly.

But in the ideal world, if we had more time and it wasn't flu, then maybe we could get it right.

CHAIRPERSON OVERTURF: Dr. Cox.

DR. COX: If I could just add a couple of more comments, we really have put a lot of effort into improving the testing and the consistency of choice of antigens and so on. Every year there's some different factor that's a problem, and this year, in particular, we had a rather slow start to the influenza season not only in North America, but also in Europe, and this year, as in other recent years, we have had two preparatory WHO conference calls, part of which are dedicated to talking about what strains we should include in the human serologies and where the serum panels are in terms of their availability for shipment and so on.

So there is really a lot of effort that goes into this. It's just a matter of, as Roland said, getting the viruses out there and then seeing what viruses grow best and how many you can actually put in test given the limited amount of antiserum that you actually have.

CHAIRPERSON OVERTURF: Dr. Eickhoff.

DR. EICKHOFF: Just a general comment. I think this year particularly illustrates the importance of having human serum panels available for study because as Nancy pointed out to me earlier in response to one of my questions, the difference between A/Fujian strain and the A/California strain wasn't really that clear in the ferret antisera data, but in the human sera data it's apparent that there's a real problem.

CHAIRPERSON OVERTURF: If there's no more comments, we can go ahead and progress to the availability of strain reagents. Dr. Ye.

DR. YE: Thanks.

In his presentation, Dr. Levandowski described the immune response to the current influenza

virus vaccine, and I'm going to present this status of candidate virus vaccine that is used for production as a viral vaccine and the potency reagent that had to be used for standardize the antigen of vaccine product.

Influenza vaccine contains three antigens: H1N1, H3N2, and B virus. Current vaccine for H1N1 is New Caledonia/20/99. IVR/116 is a reassortant between New Caledonia/20/99 and A/Puerto Rico/8 or PR/8 high growth viruses in the lab.

This reassortant grows quite well in eggs. Currently we do not have antigenically divergent strains available for this particular subtype.

Wyoming is the current vaccine reassortant. NYMC-X-147, NYMC-X-149, RVR-134 are the reassortants between Wyoming and PR/8. All of them grow pretty well in eggs.

The candidate strain for H3N2 is a little bit complex. The first one is the A/Wellington/01/2004. There are three reassortants. RESVIR-20, IVR-139, NYMC-X-155 are the reassortants between Wellington/01/2004 and PR/8. All of them grow reasonably well in eggs.

And as Roland mentioned, Wellington/01/2004 is a strain recommended for the Southern Hemisphere in the 2005 season.

The next one is A/California/7/2004-like strain. Currently we do not have high growth reassortant for this particular strain. Although IVR-140 is a resortant between Singapore/37/04 with PR/8, this particular reassortant does not grow very well in eggs, neither grow well in eggs nor ideal strain represent A/California/7/2004.

However, we have at least six circulating viruses that represent A/California/07/2004. All of them are egg isolates. One of these strains, A/New York/355/2004, are the most among the six -- grow reasonably well in eggs, and this strain may be ideal strain to be used for generating high growth reassortant between this particular strain and PR/8, and the rest of them do not grow very well in eggs.

I'm putting up this slide to give you the sense of the possibility we can generate high growth virus for this particular subtype. There are at least the six laboratories work wholeheartedly to generate

high growth reassortant for A/California/7/2004. There are three in the United States, one from CBER, CDC, New York Medical College, and three from overseas. They are NIBSC from New York (sic), CSL from Australia, National Institute, Infected Disease, from Japan, and hopefully, by mid-March we can have this high growth reassortant for A/California/704.

The reason I'm saying that is I mentioned one strain grows reasonably well. Then the possibility we have with high growth reassortant for this particular strain would be likely.

The current Influenza B strain -- current vaccine strain is B/Shanghai/331/2004-like. There are three strains: B/Shanghai/361/04 itself, B/Jilin/20/2003 and B/Jiangsu/10/2003. All of them grow moderately, at a moderate rate in eggs.

And currently we do not have a new antigenically divergent strain available for this B strain.

Now we move on to the potency reagents. We have antisera and antigen for standardization of the vaccine for both H1N1, which is New

Caledonia/20/99 and Wyoming/03/2003. If the new strain is going to be chosen and the particular reagents needed to be prepared so that they wouldn't be available before May.

We also have antisera and antigen for both HA lineages, one for Shanghai/361/04, which is a Jiangsu/10/2003, and also we have antisera and antigen for Yamagata lineages, which are B/Hong Kong/330/2201 and Hong Kong/1434/2002 and B/Shandong/7/97.

Again, if the new strain are choosing the reagents, it would be available in May in the earliest.

Thank you.

CHAIRPERSON OVERTURF: Are there questions? Yes.

DR. BUCHER: I just wanted to mention that we had submitted a high yield B reassortant for Jiangsu, NYMC -- I'm from New York Medical College -- NYMC-BX-7. So it doesn't -- in our hands it grows about twice as well as the B/Jiangsu. So just to let people know that that's available. We did submit it to the CDC. It was also sent to your lab.

CHAIRPERSON OVERTURF: Maybe you can clarify something for a bacteriologist. What is it that you do to make the -- is it all trial and error or are there genetic or molecular elements known for egg growth?

DR. BUCHER: No, it isn't that random. We do use, although we do take advantage of a lot of possibilities here -- Ed Kilbourne had the main lab, and we're continuing on with his assistant as well and his reagents, and we've generated improved selection reagents.

So he developed the system for As, and that's what people have been using, using an old, well adapted egg strain as the donor, which is A/PR/8/34. So it's been in an egg since 1934.

For the Bs now, we -- and that's how we made X-147 and X-149, which were mentioned, and now we're working on the high yield A/New York/55.

For the B strains, we selected B/Lee/40, which has been in egg since 1940, and as I said, it doesn't give us the tremendous enhancement that we see for the As, but the Bs generally -- they generally are

growing better than the new isolates of the A strains, but we did see an increase in yield.

So we know that it has the eight gene segments. Of course, we have to have the two from the current strain, the strain that's circulating currently. So we have to have the hemagglutinin and neuraminidase from the B/Jiangsu.

We know we have the M gene from B/Lee/40, and we're in the process of analyzing the rest of the genes.

CHAIRPERSON OVERTURF: Thanks.

Are there any other questions or comments?

(No response.)

CHAIRPERSON OVERTURF: Thank you.

We now have time for the comments from the manufacturers, and that's going to be presented by Albert Thomas of Sanofi Pasteur.

MR. THOMAS: Good afternoon. My name is Albert Thomas. I'm the Director of Viral Manufacturing for Sanofi Pasteur, the vaccine maker previously known as Aventis Pasteur.

I'd first like to thank the committee for

the opportunity to present today, and I'd like to begin by talking about some of the critical factors that are involved with influenza vaccine supply and how the strain selection process can impact each of those factors.

First of all is the growth potential of the seed virus. Obviously there are many factors that can impact the number of doses of influenza vaccine that can be produced, such as the overall capacity that is available to each manufacturer, the average yield of all three of the monovalent strains currently in that formulation, but many times it is the yield of basically the lowest performer, the least productive monovalent strain that will ultimately impact or determine the number of doses that can be produced in that given season.

You may be successful in producing 40 million doses of your H1N1 monovalent component, 40 million doses of your B component, but if you can only produce 20 million doses of your H3N2 component, you will ultimately only be able to distribute 20 million doses of trivalent influenza vaccine.

Probably the most critical constraint is time. Given that the timing for influenza vaccine manufacturing is limited at the beginning by the timing of strain selection and is then limited at the end by the necessity to be able to manufacture, release, distribute, and administer the vaccine prior to the onset of the influenza season, your actual time is very limited, in which case you can actually manufacture the monovalent components and then formulate the trivalent vaccine, fill, package, release, and ultimately distribute.

Also, please keep in mind that the working seeds typically require about four weeks for development and release prior to them being able to be utilized in large scale manufacturing, and usually that four weeks is based upon the time that we initially receive the seed candidate.

The potency test reagents are also a factor that must be taken into account. Given that each monovalent component, the potency of that component or the amount of hemagglutinin must first be determined prior to formulation of the trivalent

vaccine, and that's done via single radial immunodiffusion, which requires a strain specific reference antigen and antiserum.

Those two components, the potency test reagents must first be manufactured and calibrated or standardized for each new strain prior to the formulation. That can be anywhere from, say, an eight-week process to about a 12-week process, depending on when the seed candidate is first involved or first available.

The time line that I've got listed here has several assumptions built into it. I'd like to first describe those. This is based upon assuming that there is one strain change from one year to the current year, and the new strain here is listed as B or is listed as the blue component.

The way this is broken down is really first the upper half here is related to the production of the individual monovalent components. The lower half here is involving the formulation of the bulk trivalent vaccine, the ultimate filling, packaging, and then distribution of that vaccine.

Again, as I mentioned before, the time constraints are we are required or limited by the time that we need to begin distributing the vaccine, again, typically the August to the, say, early November timing. This past season was a bit of an exception. Obviously we've been able to extend the time, in which case we can distribute the vaccine.

This basically ends or defines when you need to begin distributing, and again, the actual beginning of the strain selection really determines when you can begin production.

Now, something you might notice here is is I've got this arrow here listed as essentially the timing that the strains are announced, basically the middle of February. If you're not familiar with influenza manufacturing, you may see that manufacturing is already underway.

Some manufacturers may choose to manufacture one of the strains at risk, and that risk is the fact that that strain may not actually be included in the vaccine the coming year. The reason why manufacturers may choose to do that is, again,

because the time here is very limited, in which case you can actually produce those monovalent strains and be able to distribute that vaccine in time.

So, again, here, you know, assuming a model of one strain change for the year, assuming that manufacturers would have already had some of that production underway, manufacturers would typically be looking to begin production at that second strain very soon after the strain announcement. Basically the concern with the fact that they want to build too much of one strain without ultimately knowing the yield of all three strains that will be in the vaccine.

So typically following the strain selection, you know, assuming a working seat is available, manufacture of the second monovalent strain would begin. Once a working seed would be available for the strain, manufacture of that would also commence.

That depends now upon what is the timing or the availability of the reassortant. This time line here would assume approximately a mid-March availability, which again for this year may be

aggressive, but at least it's something to start with.

Again, approximately the four weeks necessary for each manufacturer to produce the working seed to use in large scale production from that reassortant, and then the beginning production of that third strain.

Now, what then happens in parallel with that is the production and standardization of the potency reagents, again, the reference antigen and the antiserum. Until these reagents are available, the final or true yield to this third strain is not known. Once the reagents are available, manufacturers now have a very good idea of what their yields of the three strains are. So they then begin the strain balancing process here.

Again, each of the three strains are produced independently, and depending on what the yield is of each strain, manufacturers will emphasize one or the other with the ultimate goal at this time to have an equal number of doses produced of each of the three monovalent strain components.

And, again, once the potency reagents are

available, the formulation of the trivalent vaccine can begin, which then would be followed by the filling, packaging, and ultimate distribution of the vaccine.

Current manufacturing status, as I alluded to in the previous time line, for some manufacturers the H1N1, New Caledonia, the 20/1999 manufacturing is underway, and again, that was initiated at risk, again, of that strain potentially not being selected to be in the vaccine formulation for the 2005 and 2006.

Again, the reason why manufacturers decide to produce that risk is, again, because the manufacturing time point here is very limited. We have a limited time. That's really our most critical constraint that we have to work with, and by manufacturing at risk with some knowledge of the surveillance data that's available allows us the opportunity to produce some of that product prior to that very critical time frame.

The H3N2 and B strain, obviously we're awaiting the strain selection process and are

currently evaluating any new potential seed candidates that we're receiving, whether they're the A/California type of other type strains.

Basically, in conclusion, it's really cooperation. I think it's very clearly necessary for the successful influenza vaccine production and supply, and that really comes from all parties involved in the overall process. I would say the timely selection of the appropriate antigens, both consideration of the antigenic match, as well as the potential growth potential for that strain; the availability of the height of the seed viruses, especially the high growth reassortants.

Again, because time is so limited, the best yielding strain you have to produce each day will maximize the number of doses that can actually be distributed. And something that worked, I believe, very well last year and I think is a good representation of the availability or the greater availability of egg isolates is the opportunity for manufacturers to evaluate the growth characteristics of strains that are antigenically similar but may have

very different growth characteristics and large scale production.

Again, the example last year, evaluation and selection of the B/Shanghai/361/2002 light strain. Manufacturers were given several strains to pick from, and actually it was the Jiangsu strain here which was received last but had by far the best growth potential, and that's what was utilized in production.

Again, just showing the fact that antigenically similar strains can have very different growth characteristics, especially in large-scale production, and the availability to have additional egg isolates is a key factor for a lot of the manufacturers to pick potentially what would be significantly a greater yielding strain which ultimately will yield many more doses for the marketplace.

And, again, the availability of the potency test reagents and the time frame or the time required to produce those must be taken into account in the overall campaign.

CHAIRPERSON OVERTURF: Thank you.

Are there questions for Mr. Thomas? Yes.

DR. SCHWARTZ: I'd like to ask you a question about your last point about the B/Shanghai-like strains. Two questions. One is: did all of the manufacturers come to the same conclusion or will one strain or a different strain grow better with one particular manufacturer's processor, a different manufacturer's process?

Secondly, I'm wondering if when those three different strains were supplied to industry whether there was an indication that the Jiangsu would be the best growing of those three strains or whether it was something that you had to test out in your manufacturing system before that recognition came about.

MR. THOMAS: Sure. Could I possibly defer the first question to someone on the committee? Again, I'm not -- typically each manufacturer is not involved with what the other manufacturers are selecting. I believe we all chose that same strain, but I'm not sure if possibly anyone from CBER would like to talk about that. Yeah.

CHAIRPERSON OVERTURF: Roland.

DR. LEVANDOWSKI: We've had two different strains based on preferences for manufacturers in past years, and I don't remember what year it was, but it was in the late 1990s we had -- actually it was the Hong Kong/330/2001 and the Hong Kong/1434/2001. We had different manufacturers producing different strains, and that went even further because in Europe the strain that was being used was the B/Shanghai/7/97 strain. So all three of those strains that Zhiping mentioned in his talk were used by different manufacturers based on what they saw as what was best for them.

It's a little bit stressful for the system to have that happening, but it can be accommodated.

MR. THOMAS: And then answering the second part of the question, I believe that's pretty much each manufacturer will probably come to that decision independently. I mean, there is data usually given to us when the seed candidate arrives, but eventually based on what each of our -- whatever process each manufacturer would follow to develop and evaluate that

seed candidate, if it would be a good candidate for their process.

DR. SCHWARTZ: So that you actually tested all three of those before selecting that particular strain?

MR. THOMAS: Oh, yes, yeah, tested them in multiple passages. Sort of it's when the race begins. When you start receiving the seed candidates you begin very aggressively looking at each of these and try to quickly narrow the list down for those that you would continue to evaluate.

CHAIRPERSON OVERTURF: Kathleen.

MS. COELINGH: It's Kathleen Coelingh from Medimmune.

And just so people know, the live attenuated has the B/Jilin strain as the B component last year, and because we use a reassortant, the early growth of the wild-type strains is not necessarily a good indicator of how our reassortant will grown, but at the same time, I would agree with what Albert said. It's important to get these wild-type strains in early because we make our own reassortant. So we're sort of

on the same time line with CDC and the FDA and Doris Bucher and so forth.

So it's the same. It's true we need to get these strains in early, but for different reasons.

CHAIRPERSON OVERTURF: Dr. Couch, did you -- oh, I'm sorry. Go ahead.

DR. HJORTH: Richard Hjorth from Sanofi Pasteur.

I'd just like to also say about yields that I don't believe CDC or CBER are that focused on assays that can really pick up differences between yields, such as the manufacturers are. I think Nancy has mentioned that before. You don't focus on early assays, especially, before the potency reagents are available.

So I think the manufacturers are probably in the best position to evaluate the yields coming out in the allantoic fluid, not that we necessarily run them all the way through our process, but we look at the allantoic fluid in a more quantitative way.

CHAIRPERSON OVERTURF: Dr. Couch, you had a comment?

DR. COUCH: Yeah, I had a question that's along that same line, and if you would confirm or perhaps deny the impression that many of us have that while we know you can balance a number of eggs you put in one strain or another, in terms of the actual yield of a single strain, is it true that the B is most commonly your limiting antigen?

MR. THOMAS: Historically, yes, the B strain has been limiting.

DR. COUCH: And that need for a high yield that Dr. Bucher referred to that's being worked on right now still carries a high priority that it has carried for a number of years now.

CHAIRPERSON OVERTURF: Comment from the floor?

DR. SUN: This is Wellington Sun from Walter Reed.

One question I had based on your time line, it's very stressful, and you said that manufacturers manufacture the first strain at risk before the selection. So what is the impact if the selection is the wrong one, and how often has that

happened?

MR. THOMAS: Well, probably the first impact is probably a different person standing up here next year --

(Laughter.)

MR. THOMAS: -- based on the selection.

The second is I would say now that's manufacturing time that you just lost. So obviously you try to make the best educated decision to pick that strain as to which one you think is the greatest probability of being in the vaccine for the following year.

But, again, because your overall time is fixed and you're working within the time constraint of strain selection to really when do I need to stop production to meet the last available time to distribute vaccine. That time is fixed. So you try to find any way to potentially build up a bit of a hedge if you have a potential low yielder. Right now the yield, say, of the new strain is unknown. So by beginning that production, that gives you a bit of a cover factor knowing that you could potentially build

up enough doses of the other strains in time.

But, again, obviously it is a decision made at a business risk of that product, potentially not being able to use in the vaccine.

CHAIRPERSON OVERTURF: Yes.

DR. FARLEY: If I remember correctly, the California strain is at an earlier phase of development from the previous presentation. Can you comment on concerns from a manufacturing standpoint about getting a California-like strain up and ready and going in the time frame this year?

MR. THOMAS: I think that's probably similar to -- I'll give you the analogy of last year -- the B strain. Again, we had two strain changes last year, both the H3N2 and the B strain, and it was actually the -- I believe the B/Jiangsu seed candidate we did not receive, I think, until some time in March, which means that we had very little time to pass that, to develop a seed candidate ready for large scale production.

Now, the issue is now currently with the A/California type, assuming that is what is selected

for the vaccine. It's really how successful this process is going to be and how quickly a reassortant with reasonable growth potential can be completed, released and given to the manufacturers in time. You know, it's kind of obviously that's a strain that we're just seeing very quickly show up, and we're just beginning to receive candidates for that. It's really how quickly can that reassortant be put together and a working seed developed, and what will ultimately be the yield of that.

Probably no different than in previous years in which case, you know, a strain shows up near the middle or end of the season and it appears to be the right match for the next year's vaccine and how quickly can manufacturers put that into production.

CHAIRPERSON OVERTURF: Yes, Dr. Schwartz.

DR. SCHWARTZ: And just to follow up on that, in the previous presentation, it was mentioned that there were six different laboratories working on the reassortants for the A/California strains. Is it possible to tell us are they working on different strains or is it all the New York/55 or are there

different California-like strains that are being investigated by these laboratories?

DR. YE: They are working on all six strains. So their own preference is to see which strain grows well in their hand. It's early in the development of the candidate strain.

CHAIRPERSON OVERTURF: Dr. Cox.

DR. COX: Just as a matter of information I'd like to mention that at CDC we are not using the traditional or the classical reassortment techniques, bur rather we have decided that in this particular instance we're going to actually use reverse genetics to attempt to make six, two reassortants for California and for one other California-like strain and we're choosing based on the knowledge we have about those particular viruses.

I think a number of other labs will be using the New York/55 because it does appear to grow better. So there will be some consistency in that, but you know, there will be a lot of effort and some diversity, I'm sure, as Zhiping has said in the candidates that eventually come out.

CHAIRPERSON OVERTURF: Dr. Markovitz.

DR. MARKOVITZ: Nancy, I'm happy to hear you're going to use reverse genetics, but I have a question. I thought with reverse genetics you have to go through a cell line before you go into eggs, and then that brings up all of the problems of certification of the cell line. Is that incorrect?

DR. COX: That is not incorrect, but we will be using certified cells, certified virile cells in laboratory conditions as close to GLP condition as it's possible to maintain. So we'll be making sure we have introduction of no TSCs and so on, and we're documenting everything so that we believe that the strains that will be made at CDC will be suitable than to go into eggs for production should the necessity arise.

DR. MARKOVITZ: Yeah, that sounds like a very good plan. I'm just curious in terms of who decides that a cell line is okay? I'm not speaking against that in any way. I think that's great, but I'm curious. Who gives you the green light to do that?

DR. COX: These are cells that have been certified through a long process of testing by one of the vaccine manufacturers, and they have provided those cells to us. FDA has the dossier on these cells, and so it's all very well contained and controlled.

DR. MARKOVITZ: So it's an FDA decision that the cell lines are okay?

DR. COUCH: But that approval is FDA, is it not?

DR. BAYLOR: This is Norman Baylor, FDA.

It's not an approval, but when we get information and, say, at a drug master file and what have you, we can review the characterization of the cell line, and then we can give that a green light. We don't approve, quote, unquote, cell lines, but we can evaluate whether they've been adequately characterized to our satisfaction to make products.

DR. COUCH: Just a clarification. I think what we're understanding is that you don't approve a cell line. You approve a vaccine.

DR. BAYLOR: Correct.

DR. COUCH: If it's made in a substrate of a particular type, that's a part of the review process.

DR. BAYLOR: Correct.

DR. COUCH: And that's what would be going on with this one also. So it's still an FDA review because the vaccine has to be approved if it had that substrate in its preparation.

DR. MARKOVITZ: Right, but I'm wondering who at FDA. I mean who finally says, "Yes, it's okay to do this"? I'm a little perplexed. It sounds very good. I'm not in any way against this. I think it's a great idea, but I just want to know who finally says yes.

DR. BAYLOR: CBER's office. if it's a vaccine, CBER's Office of Vaccines.

CHAIRPERSON OVERTURF: That's fairly nonspecific.

(Laughter.)

CHAIRPERSON OVERTURF: Robert. Yes, Dr. LaRussa.

DR. LaRUSSA: Just a clarification. Let's

assume a worst case scenario, that you weren't able to make high growth reassortments from the A/California-like strains. Are you saying that the reassortment that's available for A/Singapore 37/04 would not be appropriate and would not work as a vaccine strain? Because that's the one you do have a reassortment.

DR. COX: Roland warned me that I would need to go first on this.

I don't know if you recall, but when I showed on one of my H3HI tables, what we found when we put the Singapore/37 strain into ferrets was that the ferret serum generated did not cover the current strains quite as well. We went back and very carefully went through the genetic data to see if we could find a reason for why this might be true, and we found that there were two potentially significant genetic changes right in the same neighborhood in the HA molecule, and although this neighborhood, this area of the HA molecule hasn't typically been associated with antigenic changes or major antigenic changes in viruses, there is right adjacent to an isoleucine to methionine change, and changes to methionine are

fairly infrequent I would say overall.

We also see loss of a potential glycosylation site, which certainly could affect anogenicity. So these are changes relative to the majority of the California-like viruses.

So while the Singapore strain shares the change at amino acid/145, which we believe is critical, it also contains these two other changes which are not part of the consensus and may cause a slightly different antibody response in the recipient.

So we would view the Singapore/37 strain as less than idea.

DR. LaRUSSA: So that's a very gracious way of saying no.

(Laughter.)

CHAIRPERSON OVERTURF: Yes, Dr. Karron.

DR. KARRON: Just as a follow-up question to that, of all the other A/California-like strains, are any of those like the Singapore or none of the other California-like strains have those changes?

DR. COX: Correct. None of them do.

CHAIRPERSON OVERTURF: Yes, from the

floor.

DR. BUCHER: I just wanted to raise the issue of our high yield A/Wellington strain, which was NYMC-X-155, which I had understood from the sequencing was a step ahead of the A/Wellington and I would presume toward the A/California type.

We had discussed this at the previous meeting, and there was a mention of that going into ferrets. So I wondered if that had been looked at any further. That is the highest yielding reassortant I think we've ever seen. So I wonder if that could be under consideration, as is the A/Singapore as fail safe.

DR. COX: If you can give me two minutes to look at our data again so that I'm sure that I give you the correct answer, maybe we could go on to another question and then I'll have the answer to that.

CHAIRPERSON OVERTURF: A question from the floor?

AUDIENCE MEMBER: Assuming all reassortants grew poorly, can a wild-type virus be

used in a vaccine?

CHAIRPERSON OVERTURF: I'm sorry. Could you repeat that? It was not quite clear through the mic.

AUDIENCE MEMBER: Oh, yeah, that's regarding discussion on the H3N2 virus. So if the reassortants obtained not growing as well as the wild-type virus or even poorer, then can the wild-type virus originally isolated be used in the vaccines?

CHAIRPERSON OVERTURF: Yes, Roland.

DR. LEVANDOWSKI: The simple answer is yes. The wild-type virus can always be used in the vaccine, and that's what we use typically for Influenza B, and as you recall, even for Influenza A, the A/Taiwan/186 strain was a wild-type virus which was not a reassortant.

So, yes, we would make use of whatever seemed to be appropriate. If the question is related to the California-like strains, it doesn't seem very likely to me that that's going to be the case because the California-like strains as Zhiping showed are generally low to moderate growth, if any, so that it's

unlikely that those would be suitable for large scale manufacturing, at least not within this decade.

DR. BUCHER: Since I'm not a manufacturer, in our hands the New York/55 is growing quite well. We've been passing wild-type along with our reassortant passes, and it's gaining. So it may not be so hopeless to use that as wild-type.

DR. LEVANDOWSKI: Thanks for that comment, but I wasn't saying it's hopeless. I'm just saying that generally the wild-type strains do not grow better than the reassortants. That's the whole point of doing the reassorting in the first place.

CHAIRPERSON OVERTURF: Dr. Cox.

DR. COX: Yes. So now I'm prepared to answer Doris' question with 100 percent accuracy. We had taken your new Wellington virus, your new Wellington reassortant, and we put it into ferrets and did the cross-test, and most unfortunately -- well, the antiserum that we generated had a very, very high homologous titer, but when you look at the relative titers to the current strains, it covers many of the current strains no better than the Wellington wild-

type antiserum did.

CHAIRPERSON OVERTURF: Dr. Schwartz.

DR. SCHWARTZ: I just have one other question for you, Nancy, and that is that we talked this morning about some potential limitations to the commercial use of a vaccine that's made using reverse genetic reference strain, and so I'm just wondering if you can just describe the decision that you and your laboratory made to use reverse genetics for this California-like strain rather than to use traditional reassorting methodologies.

DR. COX: Well, it was really a very -- it's a forward looking strategy really. We do feel that it's very important for federal laboratories to be practicing reverse genetics on a regular basis for generation of vaccine strains, even knowing that there are some potential intellectual property issues associated with them.

We made this decision at a time when the North Dakota virus, which was one of our candidates, was growing extremely poorly, and we were getting reports back that California was also not performing

well.

So we decided that we would go ahead and really work toward perfecting the system and moving viruses through making reverse genetics modified strains as quickly as possible just in case we were unable to generate one using classical reassortment.

It looks now like some of the additional egg isolates that have come on line since then are much more promising. So it may not be necessary, but nevertheless it's good practice in many senses of the word, and I think we'll be doing this in the future as well.

DR. LEVANDOWSKI: Yes, sir.

DR. COUCH: A very minor question. I would have guessed at least two and possibly three other sites that are also at least trying reverse genetics. Would you agree with that?

DR. COX: I don't believe so. I believe NIBSC is --

DR. COUCH: Leaving it alone?

DR. COX: Going to use classical reassortment.