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CENTER FOR BIOLOGICS EVALUATION
AND RESEARCH

VACCINES AND RELATED BIOLOGICAL PRODUCTS
ADVISORY COMMITTEE

MEETING

Friday,
January 29, 1999

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The meeting took place in Versailles Rooms I & II, Holiday Inn, Bethesda, MD, at 8:30 a.m., Patricia L. Ferrieri, M.D., Chair, presiding.

OPEN

PRESENT:

PATRICIA L. FERRIERI, M.D., Chair
NANCY CHERRY, Executive Secretary
REBECCA E. COLE, Member (telephonically)
ROBERT S. DAUM, M.D., Member
KATHRYN M. EDWARDS, M.D., Member
MARY K. ESTES, Ph.D., Member
HARRY B. GREENBERG, M.D., Member
CAROLINE B. HALL, M.D., Member
ALICE S. HUANG, Ph.D., Member
KWANG SIK KIM, M.D., Member
STEVE KOHL, M.D., Member
GREGORY A. POLAND, M.D., Member
DIXIE E. SNIDER, Jr., M.D., Member
ROBERT BREIMAN, M.D., Invited Participant
NANCY COX, Ph.D., Invited Participant
THEODORE EICKHOFF, M.D., Invited Participant
CHARLES HOKE, Jr., M.D., Invited Participant
EDWIN KILBOURNE, M.D., Invited Participant
DR. ROLAND LEVANDOWSKI, FDA Participant
KEIJI FUKUDA, M.D., MPH, Presenter
LINDA C. CANAS, Presenter
KUNIAKI NEROME, Ph.D., Presenter
MARIA ZAMBON, MB, BS, MA, Ph.D., Presenter

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PRESENT: (Cont'd)

DAN OFFRINGA, Presenter
GREGORY M. SLUSAW, Ph.D., Presenter
JAQUELINE KATZ, Ph.D., Presenter
JOHN J. TREANOR, M.D., Presenter
JOHN WOOD, Ph.D., Presenter
TAOUFIK MABROUK, Ph.D., DMV, Presenter

ALSO PRESENT:

KATHRYN ZOON, Ph.D.
DR. BETHANIE WILKINSON
CHARLES W. WHITAKER, Ph.D.

PUBLIC COMMENT:

FREDERICK RUBEN, M.D.

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8:38 a.m.

CHAIRPERSON FERRIERI: We need to start our meeting. If everyone could please take a seat. Thank you. I am Patricia Ferrieri from the University of Minnesota, and I am Chair of this committee and have been for a long time actually. This is my last time as chair here, so it is a very special meeting for me.

I would like to start the meeting by having members of the committee and other guests sitting at the table to introduce themselves, name and institution. We will start with Dr. Greenberg.

DR. GREENBERG: Harry Greenberg, Stanford University and the Palo Alto VA Hospital.

DR. DAUM: I am Robert Daum from the University of Chicago in Chicago.

DR. HUANG: Alice Huang from the California Institute of Technology, Pasadena, California.

DR. KOHL: Steve Kohl, University of California, San Francisco.

DR. SNIDER: Dixie Snider, Centers for Disease Control and Prevention, Atlanta.

DR. ESTES: Mary Estes, Baylor College of

1 Medicine, Houston, Texas.

2 DR. KIM: Kwang Sik Kim, Children's

3 Hospital, Los Angeles, California.

4 DR. EDWARDS: Kathy Edwards, Nashville

5 Tennessee, Vanderbilt University.

6 DR. EICKHOFF: Ted Eickhoff, University of

7 Colorado, Denver.

8 MS. CHERRY: Nancy Cherry, FDA.

9 DR. HALL: Caroline Hall, University of

10 Rochester, New York.

11 DR. HOKE: Charles Hoke, Military

12 Infectious Diseases Research Program, Ft. Detrick.

13 DR. POLAND: Greg Poland, Mayo Clinic, the

14 other Rochester.

15 DR. BREIMAN: Rob Breiman, National

16 Vaccine Program Office.

17 DR. KILBOURNE: Edwin Kilbourne, New York

18 Medical College, Valhalla.

19 DR. LEVANDOWSKI: Roland Levandowski,

20 Center for Biologics Evaluation and Research,

21 Bethesda.

22 DR. COX: Nancy Cox, Influenza Branch,

23 CDC, Atlanta.

24 CHAIRPERSON FERRIERI: Thank you very

25 much. I would like to turn it over to Nancy Cherry

1 for any announcements and then we will move on.
2 MS. CHERRY: The first thing I would like
3 to do is welcome all of you to this annual January
4 meeting, and to let you know that my conflict of
5 interest statement is mercifully short this time.
6 This announcement is made a part of the record at this
7 meeting of the Vaccines and Related Biological
8 Products Advisory Committee on January 29, 1999. Drs.
9 Ada Adimora and Dianne Finkelstein are unable to be
10 with us today. Ms. Rebecca Cole is connected to this
11 room by teleconference. And I might add that on your
12 roster of participants you will see the name of Nancy
13 Sander. Ms. Sander had graciously accepted the
14 invitation to stand in for Ms. Cole, but then we were
15 able to hook up Ms. Cole by teleconference. So Ms.
16 Sander will not be here.
17 Pursuant to the authority granted under
18 the committee charter, the Director for the Center of
19 Biologics Evaluation and Research has appointed Drs.
20 Rob Breiman, Theodore Eickhoff, Edwin Kilbourne and
21 Charles Hoke as temporary voting members.
22 Based on the agenda made available, it has
23 been determined that all committee discussions at this
24 meeting for the influenza virus vaccine formulation
25 for the years 1999/2000 and an update on influence A

1 H5N1 viruses present no potential for a conflict of
2 interest. In the event that the discussions involve
3 specific products or firms not on the agenda for which
4 FDA's participants have a financial interest, the
5 participants are aware of the need to exclude
6 themselves from such involvement and their exclusions
7 will be noted for the public record. With respect to
8 all other meeting participants, we ask in the interest
9 of fairness that you address any current or previous
10 financial involvement with any firm whose products you
11 wish to comment on. And that is the end of the
12 statement.

13 CHAIRPERSON FERRIERI: Thank you, Nancy.
14 I am sorry I neglected to announce you, Rebecca Cole.
15 Are you still with us?

16 MS. COLE: I am here.

17 CHAIRPERSON FERRIERI: Thank you. Ms.
18 Cole is our consumer representative. We will start
19 the program with some remarks from Dr. Kathy Zoon from
20 FDA, Director of CBER.

21 DR. ZOON: Good morning. This is a job
22 that gives me great pleasure but also some remorse
23 because today we are seeing some members of our
24 advisory committee for the last time, at least in
25 their capacity at this time. Hopefully many of them

1 will continue to work with CBER as consultants in
2 dealing with future issues.
3 I would like to take a few minutes to
4 thank each of these individuals for their service to
5 our advisory committee, particularly for their sage
6 advice that has been so helpful to CBER on so many
7 issues. As we all know, the regulation of vaccines is
8 filled with many difficult issues. This committee and
9 committees before have risen to the occasion to deal
10 with products as they go through the investigational
11 process, dealing with such complex issues as looking
12 at tumor cell lines as vaccine substrates, looking at
13 some of our more recent discussions that we had at the
14 last advisory committee. They also dealt with many
15 difficult issues regarding adequacy of data; for
16 example, with the rotavirus and the lyme vaccine.
17 They dealt with other issues with respect to safety
18 such as looking at the issues regarding reverse
19 transcriptase in a number of vaccines.
20 I think I would say that the advice of
21 this committee has always been wise, filled with
22 knowledge and science that have helped the agency and
23 will continue to do so, and these four members that I
24 am going to recognize today have played a very special
25 part.

1 First I would like to take the time and
2 ask Greg Poland to come up. Dr. Poland is from the
3 Mayo Clinic and Foundation. I would like to thank him
4 for his service and we have a memo and plaque for you.
5 Thank you very much.
6 I would now like to ask Dr. Caroline Hall
7 from the University of Rochester School of Medicine.
8 Caroline, please come up.
9 And to you, Rebecca, on the phone, who is
10 telecommuting today, I would like to thank you for
11 your service over the past four years. For many of
12 you who may not know, Ms. Cole came to this advisory
13 committee following the work she had done to have
14 warnings added to corticosteroid labeling relative to
15 chicken pox and measles virus vaccines. I think she
16 has done a wonderful job in providing her input on the
17 varicella vaccine as we considered it for licensure.
18 Ms. Cole, thank you. I heard you have your plaque.
19 We will miss you.
20 And last but not least, our Chairperson,
21 Dr. Ferrieri, we want to thank you very much for your
22 service. She has been a member of our advisory
23 committee from 1987 to 1990 and then from 1994 to the
24 present. You are an honorary CBER person, for
25 heaven's sake. And she has been our distinguished

1 chair since 1996. I personally, speaking for myself
2 and CBER, want to thank you. You have led this group
3 very artfully, focused, getting the answers to very
4 difficult questions, and we will miss you. Thank you,
5 Pat.
6 If I could take a minute or two extra,
7 Madam Chair, I just want to say a few comments that we
8 have this year lost a very dear member of our CBER
9 family, who we are desperately trying to get as an
10 SGA. Dr. Hardegree retired this year. For those of
11 you who know Carolyn, and I think everybody in this
12 room does, she will be sorely missed. Carolyn has
13 given 37 years of dedicated service to the Public
14 Health Service, many of which have focused on the
15 vaccines and both the safety and efficacy of vaccines
16 given to the public and especially our children. And
17 I would like to take this moment that we all recognize
18 Carolyn, because I know how strongly you feel about
19 her. So thank you.
20 CBER is actually doing a search for a new
21 director of the Office of Vaccines, but we have
22 selected an acting director. Dr. Bill Egan will be
23 the acting director for the Office of Vaccines. Bill,
24 can I ask you to stand up? And just recently we
25 selected the acting deputy director for Vaccines, Dr.

1 Norman Bayler. Norman, would you please stand?

2 Thank you very much. With that, I will

3 turn it over to the chair. Thank you very much.

4 CHAIRPERSON FERRIERI: Thank you very

5 much, Dr. Zoon. I will turn it back to Ms. Cherry

6 now, who may have other announcements and deal with

7 the Open Public Hearing.

8 MS. CHERRY: I have no other announcements

9 at this time, except to announce an open public

10 hearing. Is there anyone in the audience that would

11 like to make a statement? If not, then we will

12 proceed with the meeting.

13 CHAIRPERSON FERRIERI: Thank you. We will

14 move then into Session 1 on Influenza Virus Vaccine

15 formulation and I will turn it over to Dr.

16 Levandowski. Roland?

17 DR. LEVANDOWSKI: Thank you, Dr Ferrieri.

18 Good morning, everybody. I too would like to thank

19 Dr. Ferrieri for the leadership she has given us on

20 the committee over the past several years. We very

21 much appreciate it and we are going to miss her when

22 she is no longer in the chair. But we hope that we

23 will be working with her in some capacity, as was

24 mentioned -- as a consultant or perhaps come back to

25 our committee one day.

1 I think everybody knows why we are here
2 this morning. And if somebody could turn on the
3 slides over there perhaps and we might need to dim the
4 lights. I will get started. I will apologize in
5 advance. I know that some of my slides are not going
6 to be very visible for people who are way at the back
7 of the room. And I would encourage you all, if you
8 really want to see some of these things, to move as
9 far forward as you can, understanding that there are
10 some obstructions, like other people sitting in the
11 front row.
12 As everybody knows, we are here today to
13 begin the process of selecting the influenza virus
14 strands that we will include in the vaccines that will
15 be prepared for the 1999/2000 season. As you are also
16 probably aware, the match between the antigen and the
17 influenza vaccine and the circulating strains is
18 probably the most important feature in the potential
19 efficacy of inactivated influenza vaccines.
20 The question to be answered by the
21 committee today is shown in this slide. That question
22 is, what strain should be recommended for the
23 antigenic composition of the 1999/2000 inactivated
24 influenza virus vaccine? In order to answer that
25 question, information is needed, and quite a lot of

1 information is needed. We are prepared to supply some
2 of that information this morning to assist in
3 formulating the answer. The data that are needed
4 include most importantly information on the appearance
5 of new influenza viruses. If new viruses are
6 identified, we also need to know how widespread they
7 have become. This helps greatly in judging the
8 urgency and considering changing a component of the
9 vaccine to know how broad the effect is of those new
10 strains that are being identified.
11 If the new strains have appeared and they
12 demonstrate the capability for broad dissemination, it
13 is important to know whether or not the current
14 vaccines are likely to provide some measure of
15 protection. And if it appears likely that current
16 vaccines could be suboptimal, then it is still
17 necessary to know if we have any virus strains that
18 will grow well enough to permit manufacture of vaccine
19 within the current constraints. Actually, I am going
20 to need the overhead projector for just one minute if
21 I could get somebody to put it back up here.
22 The vaccines that are being prepared now
23 must be available by early fall to insure
24 administration of vaccine before the onset of
25 influenza season in winter months. This overhead

1 shows some information about how much influenza
2 vaccine is being produced. The production and the
3 distribution of vaccine seen from a manufacturer's
4 viewpoint is one of having continuous deadlines, and
5 failure at any point can potentially derail the
6 efforts to deliver vaccine, which currently has
7 reached really staggering numbers of doses. I
8 presented information last year to indicate that I
9 thought the production capacity was plateauing at
10 about 80 million doses. But as you can see from this
11 slide, what we are projecting for this current season
12 is that approximately 90 million doses of trivalent
13 and inactivated vaccine have been produced. And it
14 appears from feedback that we have had throughout the
15 year that the demand for influenza vaccine is actually
16 still increasing. So I guess the expectation is at
17 this point that there may be further increases in
18 production capability to keep up with that demand, at
19 least we hope that will be true.
20 The balancing act that is required to have
21 as much information as possible to insure that we get
22 the correct strains in the vaccine also has to be
23 balanced with the need by the manufacturers to get
24 started in manufacturing. So we are at that point in
25 the year where there is some urgency to get the

1 manufacturers going and they need to know at least one
2 of the strains to use in the vaccine today.
3 During the past year, recommendations have
4 been made for the vaccines that are currently in use,
5 and I would just like to mention a few other things
6 that are happening now in the influenza world. In the
7 past year, the World Health Organization has made
8 formal what was previously an informal recommendation
9 for influenza vaccines to be used in the Southern
10 Hemisphere. That development reflects the growing
11 global demand for influenza vaccine, not only in the
12 United States but everywhere the demand is growing,
13 and also a recognition of the utility of influenza
14 vaccines in reducing morbidity and mortality.
15 With the now twice yearly formal review of
16 influenza virus vaccines, WHO has committed to
17 updating its recommendation two times a year to help
18 support the orderly and coordinated global efforts
19 that go on to produce and use influenza vaccines. One
20 effect of this activity is to enhance the continuing
21 efforts to improve and insure safe and effective
22 influenza vaccines. And currently recommendations by
23 the WHO for both hemispheres are the same as what our
24 committee's recommendations were for the past year for
25 vaccines. That includes an A/Sydney/5/97 like H3N2

1 virus, an A/Beijing/262/95 like H1N1 virus, and a
2 B/Beijing/184/93 like virus, which for everybody means
3 a B/Harbin/7/94 like strain.
4 We will begin this morning, as we always
5 do, with information on surveillance. We know that
6 the schedule is quite tight, so I will just remind the
7 speakers that we do want to try to stay on target in
8 terms of timing or for presentations. We would expect
9 that the committee may have questions as we go along
10 with the presentations, and we would certainly want to
11 answer all of the concerns of the committee as we are
12 able to. So Dr. Keiji Fukuda from the Centers For
13 Disease Control and Prevention is going to give us
14 some information on U.S. Surveillance.
15 CHAIRPERSON FERRIERI: Before you start,
16 Dr. Fukuda, I would like to mention that the Chair
17 will only recognize people who have hands up, and then
18 when you are called upon, you will announce your name
19 because everything is transcribed. This may sound
20 rigid, but it is the best for CBER and recording.
21 DR. FUKUDA: Good morning. Thank you, Dr.
22 Ferrieri, and thank you, Dr. Levandowski. Just to
23 remind everybody about the past season. The past
24 season in the United States, the 1997/1998 season, was
25 predominantly an influenza A/Sydney season, and it was

1 a relatively severe season. I think the other thing
2 that I think people remember, at the same time this
3 year, we had just come out of the H5N1 outbreak in
4 Hong Kong, so I will pick up from there.
5 So we were hoping for a quiet summer, but
6 really we had a fairly eventful summer in the United
7 States. And the most eventful thing was that there
8 was really a quite large outbreak of Influenza A in
9 Alaska and the Yukon Territory. Just to put this in
10 context, the population of Alaska is about 652,000
11 people, and it is a quite young population with a
12 median age of 32. It also has a very large tourist
13 season which goes from May to September, during which
14 about 840,000 people come into the state and up to
15 about 70,000 people per week. About one-third of
16 these tourists enter by cruise ship, and last year
17 there were about 28 ships entering the Alaska region
18 with about 1,000 to 2,000 passengers per ship. And
19 quite different from the native population, the median
20 age of these tourists is about 62 years.
21 Now beginning in May, there was a very
22 large outbreak of Influenza A in the state. You can
23 see it began sometime about May. This line here tells
24 you when the cruise ships entered into the area and
25 when they left, and you can see that this outbreak

1 went over a period of about five months and it really
2 didn't come to an end. Basically the ships just left
3 the region and then the outbreak came to an end.
4 Now the cases -- there are about 5,350
5 cases of acute respiratory illness which were counted
6 by a rapidly set-up surveillance system. Most of
7 these cases, 74 percent, were located in tourists and
8 another 24 percent in tourism workers. Only about 2
9 percent of the cases were identified in Alaska
10 residents. 420 clinical specimens were collected
11 during the investigation and 99 respiratory viruses
12 were isolated, of which 66 were Influenza A, and of
13 these, 33 were subtyped and all these were A/Sydney
14 viruses. The remainder viruses were predominantly
15 things like rhinoviruses.
16 Now it is a little bit unclear how often
17 this sort of outbreak occurs, but at least if we look
18 at data from 1997 from ships leaving the Alaska region
19 and then compare it with 1998 data, we can see that
20 there was a much larger number of cases occurring in
21 1998 and the relative risk was about 2.2.
22 So in summary, we had a large outbreak of
23 Influenza A/Sydney virus in Alaska and the Yukon
24 Territory. This outbreak lasted well over 5 months
25 and it involved predominantly over-land and sea

1 travelers. And basically this unusual outbreak was
2 sustained by a continuous influx of people into the
3 region.
4 Now there are several things that are very
5 interesting about this outbreak, but I will just point
6 to two of them that we are working on at CDC and
7 elsewhere. The first question is whether large
8 tourist groups -- whether this kind of outbreak and
9 others which have been reported recently indicate that
10 large tourist groups are at increased risk for
11 exposure to influenza during the off-season or during
12 the summertime, predominantly because you have a mix
13 of international travelers coming together. But a
14 second question is also whether these large tourist
15 groups are an important way that influenza is being
16 spread. I will just note here that the cases occurred
17 in people from over 47 countries and all 50 states,
18 and these people went back to their sites of origin.
19 Before I go on to this slide, I want to
20 point out that this investigation required a huge
21 effort and it was really spearheaded by the Division
22 of Quarantine and also Health Canada with the
23 assistance of the Influenza Branch in the State of
24 Alaska. So you can see that between May and
25 September, we had this large outbreak going on in

1 Alaska. In addition, we were having reports of
2 outbreaks going on in Montana, Florida, Tennessee and
3 California, and these outbreaks were leading us to
4 wonder whether we are going to see unusually heavy and
5 early activity during the fall time. But in fact,
6 activity in the fall time was relatively quiet in
7 terms of outbreak reports to CDC. Although we can now
8 see that in December and January, the number of
9 outbreaks being reported to CDC have picked up.
10 Now in terms of the viruses which have
11 been collected through the WHO laboratory system,
12 again we can see that it has been somewhat of a mixed
13 season. The green bars are Influenza A viruses which
14 have not been subtyped. The red bars represent
15 Influenza A H3N2 viruses and the yellow bars represent
16 Influenza B viruses. So, again, it has been
17 predominantly an Influenza A season, and of the
18 viruses which have been subtyped, predominantly
19 Influenza A H3N2 Sydney viruses. But about 18 percent
20 of the viruses have been Influenza B viruses.
21 In parallel to this increasing number of
22 virus isolations for influenza, we can see that the
23 activity being reported by sentinel physicians or the
24 activity being reported by the state epidemiologists
25 have picked up in the country also. So as of the end

1 of last week, we had 8 states and New York City
2 reporting widespread influenza activity in their
3 states or influenza-like illness activity, and another
4 21 states reporting regional activity in their states.
5 And then again in parallel to this sort of reporting,
6 we see that among the sentinel physicians we have had
7 increased activity reported in the last few weeks. So
8 we are now having about 3 percent influenza-like
9 activity being reported by the Sentinel physicians.
10 Now this map here shows you which states
11 are reporting elevated levels of influenza activity,
12 and down here we can see that in red, these states
13 here, these are the states which are reporting
14 widespread activity. The states in purple are the
15 states which are reporting regional activity. So you
16 can see that activity really is spread out throughout
17 the country. We are seeing activity on the Eastern
18 seaboard and on the Western seaboard, but also in the
19 middle of the country.
20 In terms of mortality -- pneumonia and
21 influenza-related mortality, these measurements have
22 been bouncing around the baseline, but so far we
23 remain below the threshold, the so-called epidemic
24 threshold. So unlike last year, we have not yet seen
25 a sustained elevation in pneumonia and influenza-

1 related mortality. So I think that sums up the
2 activity in the country so far. Are there any
3 questions?
4 CHAIRPERSON FERRIERI: Yes, Dr. Edwards?
5 DR. EDWARDS: Could you comment whether
6 there appeared to be any efficacy of influenza vaccine
7 for the travelers in the outbreak? Whether there
8 appeared to be any protection afforded from vaccine
9 from the year before or was that looked at in the
10 outbreak?
11 DR. FUKUDA: It is being looked at. There
12 are actually a large number of cohorts which were
13 followed or retrospectively followed in that outbreak
14 investigation. Those analyses are still going on. I
15 suspect it will take some number of weeks before those
16 data are analyzed, but it will be looked at.
17 CHAIRPERSON FERRIERI: Yes, Dr. Kohl?
18 DR. KOHL: Kohl, UCSF. We are getting
19 reports in California and Seattle and Portland of many
20 individuals having influenza-like illnesses after
21 previously being immunized with the current vaccine.
22 Are you getting any reports similar to that or do we
23 have any current efficacy data going on right now?
24 DR. FUKUDA: Yes. Every year we get a
25 number of similar reports, and California and some

1 other states have called us with questions about that.
2 There has been a couple of outbreak investigations in
3 which preliminary vaccine effectiveness estimates have
4 been made. There was an outbreak actually in
5 California, and I believe among the staff the
6 effectiveness estimates were somewhere in the range of
7 about 45 to 55 percent. But, again, these are pretty
8 preliminary estimates from small outbreaks. But that
9 is about the only data that we have so far. One thing
10 to note though is that last year CDC was able to
11 obtain data from three HMO's to look retrospectively
12 over the season to see what vaccine effectiveness
13 estimates there were for the entire season, and we
14 will be doing that again for this season. But those
15 data won't be available until the summertime.
16 CHAIRPERSON FERRIERI: Dr. Kilbourne?
17 DR. KILBOURNE: I think the Alaska
18 epidemic is fascinating because it is not an Alaska
19 epidemic. In other words, the venue is probably very
20 unimportant, don't you think, from your data? It is
21 a cruise ship phenomenon and a crowding phenomenon.
22 Do you think Alaska has anything to do with it or it
23 could have been any other port with that level of
24 tourism?
25 DR. FUKUDA: Yes, I think you are right,

1 Ed. I think that one of the things which has
2 characterized the travel industry is that there is a
3 really rapidly increasing number of people who go on
4 these sorts of cruises and there are large
5 conglomerations of people which get together, and I
6 think that this probably could have happened anywhere.
7 I think the really important characteristic is that
8 you just have a lot of people coming together from a
9 lot of parts of the globe at one place. In fact, we
10 are seeing the same kind of activity aboard some ships
11 in other parts of the world right now.
12 CHAIRPERSON FERRIERI: Yes, Dr. Hall?
13 DR. HALL: Dr. Hall, University of
14 Rochester. I am interested in the B isolates that you
15 gotten. In my lab, we have had quite a few B isolates
16 recently, much more than what I expect. And you
17 mentioned that there are 18 percent, I believe, that
18 you know. Are any of those in outbreaks and do you
19 know what strain they are?
20 DR. FUKUDA: Why don't I defer that to
21 Nancy, because I think she will be covering that in
22 detail.
23 DR. HALL: Okay. Thank you.
24 CHAIRPERSON FERRIERI: Any other
25 questions? Dr. Daum?

1 DR. DAUM: Bob Daum, University of
2 Chicago. I would like to hear your comment and
3 perhaps Dr. Levandowski's as well, but I was pretty
4 surprised by the shape of the vaccine uptake curve in
5 that it looked like it was flat for many years and now
6 almost going up exponentially. And I wonder if you or
7 Dr. Levandowski would care to comment on what the
8 factors are that have put the vaccine uptake that high
9 and that big an increase.
10 DR. FUKUDA: Well, Roland may want to
11 comment on this also. I think that that was a vaccine
12 production curve. But I think that one of the big
13 things which has really happened in terms of vaccine
14 uptake is that the vaccination levels have really
15 increased among people 65 years and above. And
16 probably one of the big things which has driven that
17 is that Medicare has started paying for those
18 vaccinations. But in the latest survey data from the
19 BRFSS survey, vaccination levels in the elderly have
20 risen up to about 65.5 percent. This is really a
21 pretty marked increase over the past decade. That is
22 probably what is largely driving that sort of
23 increased production. Roland?
24 DR. LEVANDOWSKI: Yes, I will just comment
25 on that also. I agree, I think that is right. The

1 HCFA demonstration project for making influenza
2 vaccine a Medicare benefit started around 1990, and it
3 is just at that point that the production of vaccine
4 started to go up. I think that has been a very major
5 force behind it. Permitting vaccine manufacturers to
6 increase their production because they know that there
7 will be a demand there to take the vaccine that they
8 are producing. The slide that I showed was for
9 production and not for use of vaccine, so those two
10 curves may be different.
11 CHAIRPERSON FERRIERI: Dr. Breiman? Did
12 you have your hand up also, Dr. Poland?
13 DR. POLAND: Keiji, do you think this was
14 a reporting and surveillance phenomena?
15 DR. FUKUDA: You mean Alaska?
16 DR. POLAND: That this has been happening
17 on cruise ships all along and for some reason we just
18 picked up on it, or is this something new?
19 DR. FUKUDA: Well, I think it is a little
20 bit of both. When you go back to the literature -- in
21 fact, back to some of the earlier pandemics, you can
22 see that in the Alaska region, outbreaks were reported
23 aboard ships. They are not reported very often, but
24 it has been reported in the past. But again, in
25 keeping with Ed's observation, the travel industry

1 really has been changing a lot in that you are having
2 more and more people assemble for these sorts of
3 voyages. And in a sense someone has coined the phrase
4 that these are virtual populations because they come
5 together for a short period of time and then they
6 disperse again. But I think that with the advent of
7 air travel and so on, you can have people coming from
8 all over the world and getting together for a short
9 period of time and then dispersing. So I think that
10 these outbreaks are not unique, but we probably will
11 be hearing about them more and more often.
12 DR. POLAND: Your observation brings up my
13 second question, and that is having never been on one,
14 I kind of assume people come together for this cruise
15 and then they are gone. What sustained this over 5
16 months? Is it the workers?
17 DR. FUKUDA: Well, I think that --
18 DR. POLAND: That is kind of an unusual --
19 DR. FUKUDA: It was probably a little bit
20 of a shifting site of transmission in that the early
21 transmission in the outbreak appeared to be occurring
22 predominantly on land. And then some of the
23 transmission began shifting over to ships as you had
24 outbreaks occurring on ships. And I think that the
25 tourism workers, the people working in staffing hotels

1 and the crews on these ships are one of the links
2 between this large number of people coming in and out,
3 and we think that they are probably an important part
4 of the link, but I think that is not really so clear.
5 CHAIRPERSON FERRIERI: Dr. Hall?
6 DR. HALL: I noticed -- I read somewhere
7 that the age of those on cruise ships has recently --
8 the average age has gone up, and there was obviously
9 a great dichotomy in the age that you noticed on land
10 and whatever. Do you know the vaccination status
11 then? Since many of these must have been at least
12 eligible for the routine immunization.
13 DR. FUKUDA: For the Alaska outbreak,
14 again as part of those cohort studies, I think those
15 data were collected, but I don't know what they were.
16 But that dichotomy in age also brings up a potential
17 artifact for this kind of outbreak in that it may be
18 that the people who are older are a little bit more
19 likely to go to the physicians who were the
20 surveillance physicians and the younger people may
21 have stayed away. So that apparent dichotomy between
22 the population and the travelers may be a little bit
23 less than what it appears. But nonetheless, we do
24 think that it was predominantly located among the
25 tourists.

1 CHAIRPERSON FERRIERI: Dr. Snider and then
2 Dr. Edwards.
3 DR. SNIDER: Dixie Snider, CDC. Just
4 another point of information. Not only has the cruise
5 industry been growing quite rapidly, but the size of
6 the ships has also been increasing. So you have much
7 larger numbers of people on cruise ships these days
8 than used to be the case. So that is another factor,
9 I think, that needs to be considered.
10 CHAIRPERSON FERRIERI: Dr. Edwards?
11 DR. EDWARDS: With the remarkable increase
12 in the number of people over 65 receiving vaccine, is
13 there any data from the CDC that suggests that the
14 morbidity and mortality with influenza related
15 illnesses is indeed going down, or is it staying the
16 same?
17 DR. FUKUDA: This is one of the really
18 tough questions. I think that this has been a really
19 difficult question to answer for a couple of different
20 reasons in that the hospitalizations and the mortality
21 that you see from season to season tends to vary so
22 much for factors unrelated to vaccination. But
23 recently there has been some -- particularly in
24 France, some groups which have looked at how you might
25 calculate how many deaths and hospitalizations are

1 being averted because of vaccination programs. So
2 that is one of the things that we are going to be
3 trying to do this summer to begin calculating those
4 figures for the United States. I mean, this would
5 take me almost a half an hour to go into to just
6 explain why we think that this is a reasonable
7 approach for trying to get at what you are asking. So
8 it is a roundabout way of saying that we are going to
9 try to begin to get at that.

10 CHAIRPERSON FERRIERI: If there are no
11 further questions, then we will move on. Dr.
12 Greenberg?

13 DR. GREENBERG: Just a follow-up on
14 Dixie's point. Is there -- have you found a
15 correlation between either size of cruise ships or
16 duration of cruising? Is there a time period under
17 which this doesn't happen and are you seeing similar
18 things in airplanes?

19 DR. FUKUDA: I don't think that we can
20 really tease out duration and size of ships, because
21 most of these cruise ship vacations last about one
22 week. That is the average time. What you typically
23 see is that the outbreak begins to take off and then
24 people leave the ship. So we couldn't really tell what
25 would happen to a single ship if it were out there for

1 three or four weeks.

2 In terms of airplanes, no, we haven't --

3 again, there have clearly been reports of outbreaks of

4 influenza occurring on airplanes. Notably, there was

5 an airplane which developed engine trouble and was

6 parked on the ground for some number of hours and

7 there was an outbreak which occurred among the

8 passengers, but we haven't had the same kind of

9 reports that we have had from the cruise industry.

10 CHAIRPERSON FERRIERI: Any final questions

11 for Dr. Fukuda? Thank you very much. We will move on

12 then. Dr. Levandowski?

13 DR. LEVANDOWSKI: Okay. We now will hear

14 from Dr. Nancy Cox from CDC on world surveillance

15 strain characterization and molecular analysis of the

16 strains.

17 DR. COX: Good morning. It is a pleasure

18 to be here once again. I think we will start with the

19 first overhead. I will try to condense what is really

20 a tremendous amount of data that has been developed

21 over the past year in an understandable, palatable

22 way. There really is so much information that we have

23 to leave out of what we present today in order to get

24 it packaged so that it really can be understood. So I

25 hope that all of you will ask questions, even

1 interrupt me in the course of presenting a particular
2 overhead, so that we can clarify things as we go
3 along.

4 As Roland has already mentioned, our
5 vaccine strain selection is dependent on our detection
6 of the emergence and spread of variant viruses, and we
7 are looking for variant viruses by testing, using
8 hemagglutination inhibition tests with post-infection
9 sera. For anyone new in the room, these particular
10 methods have been used for many years and have been
11 found to be sensitive for detecting differences in
12 strains which are significant epidemiologically.

13 In the past 10 to 15 years, we have also
14 begun to rely fairly heavily on sequence data of the
15 hemagglutinin gene. We find that these data are very,
16 very useful as an adjunct to the serologic data. They
17 help define precisely what relationships exist between
18 circulating viruses where the HI data may be a little
19 bit less definitive.

20 We are looking for significant influenza
21 activity associated with these variant viruses, and we
22 rely heavily on reports to the World Health
23 Organization or domestic reports of high levels of
24 influenza-like illness during the time that these
25 variant viruses are being isolated. And, of course,

1 we also in recent years have begun to rely more
2 heavily on looking at the post-vaccine immune response
3 to these variant viruses in comparison to the human
4 response to the vaccine strain itself.
5 I would just like to remind you of the
6 time table for vaccine production and use in the
7 United States which Roland alluded to. Here we are at
8 the end of February, and we need to know one or more
9 strains that will be included in the formula. By
10 March, we need to have precise formulation; that is,
11 we need to know exactly what viruses are going into
12 the vaccine.
13 I am going to just briefly summarize
14 global activity for each of the virus groups that we
15 will be dealing with. This particular overhead is on
16 page 9 of your package. We will start with Influenza
17 A H1N1 viruses, and as has been typical in the past,
18 we often tend to start with the group of viruses that
19 to us appears to be the easiest to get our arms
20 around. And that can be for a variety of reasons.
21 One of the reasons we are starting with the H1N1
22 viruses this year is that if you concentrate first on
23 the time period between October 1998 and January 1999,
24 you will see that we really have had relatively little
25 Influenza A H1N1 activity worldwide. We have only had

1 a couple of sporadic isolates in the United States.
2 There has been sporadic activity reported in Europe.
3 There was a single isolate of Bayern-like strain which
4 was isolated in December in South Africa. There have
5 been reports of sporadic H1N1 isolates in Asia and I
6 think Dr. Nerome will speak a bit more about some of
7 those viruses.

8 I we step back and look what happened in
9 the Southern Hemisphere, we saw a similar picture.
10 There was a bit more H1 activity, particularly in New
11 Zealand, where Bayern-like strains circulated fairly
12 widely. However, there really was only sporadic
13 activity over all. There was somewhat more activity
14 last winter during the 1997/1998 season for us, and in
15 Asia in February, there were actually outbreaks caused
16 by H1N1 viruses.

17 So if we move on to the next page in the
18 handout, page 10, we will look at the antigenic
19 properties of these H1N1 viruses that are circulating
20 at fairly low levels. And as you might remember from
21 looking at the data last year, there are really two
22 very distinct groups that one can sort out using post-
23 infection ferret sera. I would like to remind you
24 that we are looking for constant four-fold differences
25 or greater differences in the hemagglutination

1 inhibition titers. I have tried to simply things a
2 bit. We have the homologous titers between the Bayern
3 virus and its ferret antiserum shown in read here and
4 Johannesburg shown in red here and Moscow shown in red
5 here. And you can see that this group of viruses,
6 these three viruses, are similar to each other in
7 their patterns of reactivity.
8 In contrast, we have the Beijing 262 and
9 Harbin 4 strains here, which are really quite
10 different. Antisera to the Bayern, Johannesburg and
11 Moscow strains do not inhibit these viruses very well.
12 And conversely, antisera to the Beijing and Harbin
13 strains do not inhibit these viruses very well.
14 So it is very clear we have two groups of
15 viruses. There are genetic correlates as well, which
16 I will talk about a bit later. And so we will look at
17 some of the viruses that we have tested since we last
18 met. These test antigens 1 through 3 were isolated in
19 the Southern Hemisphere during the summer months,
20 during their influenza season. And they are clearly
21 Bayern and Johannesburg-like. These three strains all
22 isolated from Russia, where some of the European
23 Beijing 262-like strains that we noted last year at
24 this time, and we can see that they are well inhibited
25 -- reasonably well inhibited by antisera to the

1 Beijing 262 and Harbin strains. We also have
2 additional strains from Europe -- two from Hong Kong
3 and two from Japan which fall into the Beijing 262
4 group.
5 Now we are looking at the very latest
6 tests that we have. As I mentioned, we don't have a
7 lot of very recent strains. This is the single strain
8 that we have had in our hands from the United States
9 to analyze. We have one more U.S. strain, which we
10 will be testing next week or the week after. Here is
11 a fairly recent strain from China and one from Russia.
12 Then the rest of these are from Asia. These last two
13 strains were isolated in November of this year, and
14 Dr. Nerome may mention a bit more about them.
15 But let's look first at the Bayern-like
16 strains here, test antigens 1 and 2. They are well
17 inhibited by the reference antisera and are clearly
18 Bayern-like without much change. What we are seeing
19 when we look at the Beijing 262-like strains is just
20 there are some viruses which are less well inhibited.
21 These are 8-fold down. The Ishikawa Japanese strains
22 are 8-fold down with a reference Beijing 262 and
23 Harbin antisera. And I must remind you that Beijing
24 262 is the vaccine -- the H1N1 vaccine component.
25 So we have looked at some of these strains

1 in some detail. Additional strains were examined
2 during the WHO meeting in September to select strains
3 for the Southern Hemisphere vaccine for next year.
4 And I will have a bit more to say about these strains
5 as we go on and as I talk about options for strain
6 selection after lunch.
7 So here is a frequency table. There is
8 really not a lot of data, of course, for the most
9 important recent period, October 1998 to January 1999.
10 We have the two low-reacting Beijing 262-like viruses
11 from Japan, which were sent to us because they were
12 low reactors. We don't know what proportion of the
13 strains in Japan fall into this category, and I hope
14 Dr. Nerome will clarify that for us. And then we have
15 the one Bayern-like strain from the U.S.
16 I think we will just go on to the next
17 overhead. So when we moved to Beijing 262 for last
18 year's vaccine, we were dependent on a couple of
19 different pieces of information. One was that these
20 viruses, which had been circulating only in China for
21 a period of time, now had been detected, not just in
22 Asia but also in Europe, Africa, and the U.S. We knew
23 that this was a travel-related case, but we knew it
24 had been introduced into the United States, and we
25 knew that there were a number of isolates in France,

1 in the Czech Republic and in Russia that detected last
2 winter. And then during a previous period, there had
3 been a number of isolates detected in Senegal and in
4 South Africa. So that was one of the key pieces of
5 information. We can really see that the viruses
6 haven't spread that much more since then.
7 The evolutionary tree or the dendrogram
8 that is in your package is really much more complex
9 than what I have here on the overhead. I have tried
10 to simplify things a bit for the overhead but give you
11 more information in your package for you to peruse
12 later on. Here we have the two distinct antigenic and
13 genetic groups of viruses. Here is our Beijing 262-
14 like group here shown in green with the Beijing 262
15 vaccine strain shown in red. This is the Bayern-like
16 group shown in blue. And I have just asterisked
17 viruses with are egg isolates, and therefore are
18 potential vaccine strains.
19 This strain designated here as Russia 209-
20 98, in other slides it might be mentioned by other
21 individuals with a slightly different designation as
22 Ulan Ude, which is in Russia. So we called it Russia
23 in this particular slide.
24 Now what we have been able to determine is
25 that there are some of the viruses -- you know, with

1 our HI test, we have noticed that some of these
2 viruses are not well inhibited by antisera to the
3 Beijing 262. And these viruses are scattered about a
4 bit. The Malaysia virus was one of those. Hong Kong
5 503, which isn't shown on this slide but which is in
6 your package, was also a low reactor.
7 Now the problem for us has been that we
8 haven't been able to find signature sequence changes
9 corresponding to this low reactivity that we see in HI
10 tests. And furthermore, when we take these low
11 reactors and infect ferrets, we get antisera which are
12 very poorly cross-reacted. They have low homologous
13 titers and they are very poorly cross-reacted. So any
14 of the low reacting viruses, that is those that are
15 less well inhibited by the Beijing 262-95 ferret
16 antisera, have not proven to be good vaccine
17 candidates.
18 We are always trying to keep up with the
19 ball game here, and since we are always tracking a
20 virus that is moving rapidly, we are looking always at
21 potential vaccine candidates. We won't spend but half
22 a second on this, but we do have some egg isolates
23 that are related to Bayern that represent updated
24 strains, and we are looking at the number of
25 differences from the consensus sequence. We do that

1 because we have found in the past that those viruses
2 that are closest in sequence, that have HA's closest
3 in sequence to the consensus sequence, tend to produce
4 very antisera, which are most broadly cross-reactive
5 against the circulating strains. So we are always
6 trying to keep up and make sure that if the picture
7 should change rapidly, we have in our back pockets
8 strains which could be used as vaccine candidates.
9 And so for the Beijing 262 group of viruses, we also
10 have a number of vaccine candidates available.
11 Now I am not going to talk very much at
12 all about the post-vaccination human serologic testing
13 that we do, because that will be summarized later on
14 by Roland. But I would like to remind you that the
15 reason that we -- one of the reasons that we moved to
16 the Beijing 262-95 antigen as the H1N1 component of
17 the vaccine is that not only does it induce a good
18 response to the vaccine strain itself, but it also
19 induces a very nice cross-reactive antibody response
20 to viruses which are on the Bayern lineage. So this
21 was the previous Bayern-like strain in the vaccine,
22 and we see we have an even higher post-vaccination
23 geometric mean titer among human subjects who have
24 received the Beijing 262-like vaccine than they do to
25 the vaccine strain itself. And this has been

1 confirmed in a number of labs using quite a large
2 number of antigens by this time.
3 So I think unless there are any questions
4 about H1N1 viruses, I will move on to the H3N2
5 strains.
6 CHAIRPERSON FERRIERI: Let us move on
7 then.
8 DR. COX: Okay. The H3N2 viruses are
9 always a bit more exciting. Over the past 5 years at
10 least, we have had H3N2 viruses predominating
11 worldwide and causing extensive morbidity and
12 mortality in a number of different countries. If we
13 start with last season, as Keiji reminded you, we had
14 a great deal of H3N2 activity. Certainly in the
15 United States, we had epidemic level activity in
16 January and February caused by Sydney-like strains.
17 There is a similar picture in Canada. Activity was a
18 bit less intense in Europe, but certainly Sydney virus
19 circulated very widely. And in Asia, in countries
20 like Japan, Sydney viruses had quite a large impact.
21 In the Southern Hemisphere during our
22 summer season and their winter season, Sydney-like
23 viruses circulated widely causing epidemics in Central
24 and South America in Australia and in Oceania. What
25 has happened since October of 1998 to the present time

1 is that there have been sporadic isolates and
2 outbreaks in North America and Europe and activity is
3 really on the increase now. We are hearing about more
4 institutional outbreaks as we move on through the
5 month of January toward February. And I would like to
6 mention, as I imagine most of you have heard, that
7 there has been quite intense Influenza A H3N2 activity
8 in China, particularly in the north of China, and we
9 have been very interested in getting viruses from
10 China, and I will be talking about those very shortly.
11 When we look at the antigenic profiles of
12 Influenza A H3N2 viruses, we don't see at the present
13 time such clear differentiation as we do for H1N1 and
14 Influenza B viruses. Here we have our old previous
15 vaccine strain that we are just keeping in to show
16 that the viruses really are evolving and moving on.
17 Here is our Sydney homologous titer of 640. And so we
18 are looking for viruses that have titers of 160 or
19 lower. And then we want to see how those viruses --
20 what sort of a pattern we have for those particular
21 viruses, both in terms of geographic distribution and
22 in terms of their sequence analysis and so on.
23 We received a shipment of viruses from
24 China. The viruses had been isolated between May and
25 September. This shipment was received sometime in the

1 fall, I think, in about October or early November.
2 And among those viruses, we found that there were a
3 number of strains, two of them are here -- Sichuan/418
4 and 346. You will hear more about those as we move
5 through my presentation and the following
6 presentations, which certainly were reduced in titer
7 with the Sydney antisera.
8 Here I have a block of viruses from the
9 U.S. Most of these were isolated in November and
10 December. And you will see that there are occasional
11 strains which are less well-inhibited by the Sydney
12 antiserum. But by and large, the majority of the
13 isolates from the United States are very well
14 inhibited by antisera to the Sydney strain. So we are
15 looking to see what genetic characteristics these
16 particular strains might have that are low reactors.
17 I mentioned that we had this package from
18 China that had a lot of strains from Sichuan that had
19 been isolated during the summer and fall, and among
20 those, there were a number of strains which were less
21 well inhibited by the Sydney antiserum as well as by
22 additional antisera that we had developed for our
23 reference battery because we felt they were
24 interesting strains and important in some way as
25 representing outbreaks that had occurred. And we can

1 see that we have, for the Sichuan/418 a nice
2 homologous titer in this particular test of 1280 and
3 a slightly lower homologous titer of 320 for the
4 Sichuan/346. We also had strains from Japan, which
5 were Sydney-like -- solidly Sydney-like -- in this
6 particular test.
7 I will go through this slide very quickly.
8 We see that there are -- we like to see absolute
9 consistency from HI test to HI test. But as you all
10 know, for serologic assays there is some inherent
11 variation. And here you can see that the Sichuan/346,
12 which will be noted later in the human serologic
13 results, is really quite poorly inhibited by antiserum
14 to the Sydney strain. And this in additional tests
15 has proven to be the case. This strain reacts less
16 well with our battery in general than the Sichuan/418.
17 Now let's just look at these strains from
18 the U.S. Again, we see that there are occasional
19 strains which are less well inhibited, but by and
20 large U.S. isolates are well inhibited by the Sydney
21 antisera. We also have a few strains from Hong Kong
22 which are less well inhibited. Here we have some
23 Korean strains isolated in December which are solidly
24 Sydney-like.
25 And now we move on to the viruses that we

1 have received most recently. And on this particular
2 overhead, you will see at the bottom starting with
3 test antigen number 12 and going on through test
4 antigen 20 a series of viruses which we received on
5 January 20. This was a very large package of viruses
6 from China which included over 130 strains isolated
7 between September and the end of December. And there
8 are some strains among this group which we have
9 analyzed which are lower in titer with the Sydney
10 antiserum. But there are also many strains which are
11 very well inhibited. They are clearly Sydney-like
12 strains. And so perhaps the table has been turned a
13 bit. Northern China was not particularly hard-hit by
14 Sydney-like strains last year, and it would be very
15 unusual to have this sort of picture. But as we know,
16 influenza is unpredictable. And even though we can
17 generalize, there are always exceptions to every rule
18 with influenza. And it appears to us that they had
19 their Sydney epidemic a year after we did in northern
20 China. But nevertheless, we are looking very closely
21 at these strains which are less well inhibited and
22 trying to see if there is a pattern in their
23 reactivity and a pattern in the sequence analysis and
24 so on.
25 These are the most recent strains that we

1 have from the United States. They are all Sydney-
2 like. And we have a series of recent strains from
3 Hong Kong which are also Sydney-like.
4 Now for this frequency table, I am going
5 to concentrate primarily on the two panels at the
6 bottom. First of all, I would like to look at the
7 period between April 1998 and September 1999. And I
8 would like to note that there were some Sydney-like
9 low reactors. On your page 26, the low reactors are
10 actually designated. That was left off this overhead
11 to try to make it a bit prettier. But where I have
12 put little black X's, those numbers here are
13 reflecting the number of low reactors to the Sydney
14 antiserum. They are related to Sydney, but they are
15 low reactors.
16 So we see that we do have a number of
17 viruses that are less well inhibited by the Sydney
18 antiserum. I think it is very important to note that
19 in Australia, approximately 30 percent of the viruses
20 that they have tested are reduced four-fold or greater
21 in titer with Sydney antiserum. And that was a fairly
22 large number of strains. Using a smaller number of
23 strains from Thailand, analyzed by Alan Hampson in
24 Australia, approximately 50 percent of those viruses
25 were less well inhibited by the Sydney antiserum.

1 That is, they were four-fold -- they had four-fold or
2 greater reductions in titer.
3 So as we moved along with the Sydney
4 strains, we have from the very beginning seen the
5 occasional low reactor. And we have been looking for
6 sequence patterns that would give us a clue about what
7 is going on with these low reactors. And what we have
8 been seeing up until very recently is that low
9 reactors were distributed throughout our dendrogram,
10 throughout our HA dendrogram. So there was no real
11 pattern. There wasn't a signature sequence that could
12 be identified with these low reacting viruses.
13 More recently, we have seen that we have
14 got clustering of some of our low reacting viruses.
15 For example, we have our Sichuan low reactors
16 clustering up here at the top of our dendrogram. And
17 then we have this cluster here, which is
18 representative of the most recent strains that we
19 received from China. The CNIC stands for the National
20 Influenza Center in Beijing. So what we are trying to
21 figure out, and we do not have enough sequence data
22 yet, because we had to choose the viruses that we were
23 going to sequence. Because we received the package on
24 January 20, we had to go ahead and select viruses for
25 sequence analysis before we could get the antigenic

1 analysis. And now that we have got a number of these
2 viruses run in HI tests, we are going to go back to do
3 some additional sequencing and see if we can sort out
4 some of the low reacting strains here and how they
5 might relate to some of these other low reacting
6 strains.

7 So we feel that it is unfortunate that we
8 received the package from China as late as we did, but
9 we have a wealth of viruses to look at and we need
10 some additional time to sort out exactly what is going
11 on.

12 But we are trying to keep up with all of
13 our egg isolates, and where there are holes, try to
14 get some additional egg isolates. One of the things
15 that has happened in China is that they have
16 discovered, as the rest of the world had discovered a
17 number of years ago, that many influenza viruses are
18 not well -- are not easily isolated in eggs. And
19 those that are circulating at the current time are
20 great examples of this, particularly the H3N2 strains.
21 So several of the labs in China have moved toward
22 using Madin Darby canine kidney cells and PCK cells as
23 a substrate for isolating viruses. This means we get
24 more viruses from China, but we have fewer strains
25 which are suitable for vaccine production because of

1 the passage history.

2 So we have a number of isolates which have
3 a pure egg passage history. This one was isolated in
4 chick kidney, and of course if viruses have been
5 isolated in chick kidney cells, they are also suitable
6 for vaccine production. And here we see the number of
7 differences from the consensus sequence of the current
8 strains.

9 Again, I am only going to just touch very,
10 very lightly on the human serologic data that we have
11 developed so far. I would just like to move straight
12 away to this column where we are looking at the post-
13 vaccine GMTs. And we have a post-vaccine GMT for
14 Sydney of 143. For this Sichuan/346, we can see a
15 considerable reduction in post-vaccination GMT.

16 In contrast, the Alaskan strain that is
17 shown here is a very typical Sydney-like strain that
18 is recently isolated, and we have a nice robust
19 response to that particular strain. I think that is
20 all I will say because that will be summarized later.
21 If there are any questions about H3N2 strains, I would
22 be happy to answer them. Any burning questions?
23 Otherwise, I will move on to Influenza B activity.

24 Influenza B viruses have continued to
25 circulate world-wide. They have not caused epidemics

1 during the past couple of years. However, they have
2 caused outbreaks. And at the current time, Influenza
3 B strains are predominating in some European
4 countries. As Keiji mentioned, Influenza B viruses
5 constitute about 18 percent of the strains that have
6 been characterized in the United States. There
7 certainly was Influenza B activity during the summer
8 months, and we had some of these strains to look at.
9 I think that is about all I will say about this except
10 to remind you that there are two distinct groups of
11 influenza B viruses circulating in Asia. Both the
12 B/Beijing/184-like and the B/Victoria-like viruses are
13 circulating in Asia. I will mention more about that
14 as we go along.
15 Just to orient you, we will look first at
16 our reference battery at the top. The Beijing 243 and
17 Shangdong 7 strains are Victoria-like. They are
18 recent Victoria-like strains isolated in 1997. And it
19 is very, very easy to distinguish these strains from
20 the Beijing 184 Harbin 7-like strains which are
21 circulating world-wide. These have not been detected
22 outside of Asia, and I will show you a map later on
23 that shows the distribution.
24 On this particular slide, I have viruses
25 that were isolated in Georgia actually in November and

1 December of 1998. These strains were of particular
2 interest because we noted that while the vaccine
3 strain B Harbin 794 has a homologous titer of 640, a
4 number of strains from the United States, and I ticked
5 them here in black, are 8-fold down in their titers as
6 compared to the homogolous titer here. If we look at
7 strains from Europe, we can see that again there are
8 a number of strains that have a four-fold or greater
9 reduction in titer as compared to the Harbin
10 homologous titer.

11 The viruses are somewhat better inhibited
12 by the Beijing 184 strain, and we have really learned
13 a lot about the differences, both the genetic and
14 antigenic differences between these two strains, which
15 are pretty similar, but they can be distinguished both
16 antigenically and genetically. And we weren't nearly
17 as clear about this when they were first emerging and
18 were considered more or less antigenically equivalent.

19 Now we have on this slide a number of
20 strains from the U.S. represented here by test
21 antigens 1 through 8. Of course, all of them are in
22 the Beijing 184 Harbin group of viruses. Again, I
23 have ticked those strains that have a four-fold or
24 greater reduction in titer as compared to the
25 homogolous Harbin titer. Again, I would like you to

1 note that the Beijing 184 antiserum covers strains
2 better.
3 We have a number of Harbin Beijing 184
4 strains from Asia that were isolated during the summer
5 and fall up through November. And there are a number
6 of these strains which are also less well inhibited.
7 Here we have two Victoria-like strains from China --
8 one from China and one from Japan that were isolated
9 relatively recently.
10 These -- this test was done very recently,
11 just a few days ago. And the reason that I am showing
12 you this test is that we were actually able to go
13 around the corner to the State Health Department in
14 Georgia and get some original clinical material from
15 some of these isolates where the MDCK isolates had a
16 reduced titer and put the original clinical specimens
17 into eggs. And now we have this particular strain
18 here, B/Georgia/498, which is an egg isolate. And you
19 can see that after re-isolation in eggs, it continues
20 to be reduced in titer with the B Harbin antiserum.
21 And again, I have ticked a number of strains. Here we
22 have a couple of strains from the Caribbean. This
23 fairly recent isolate from a sporadic case in Chile,
24 and we have some additional Asian strains.
25 This Georgia/498 virus has not yet been

1 sequenced. It has just recently been put into
2 ferrets, and we expect that we will have reciprocal HI
3 tests with this particular candidate vaccine strain
4 within the next couple of weeks.
5 So if we look at the frequency table here,
6 we can see that we don't have a tremendous number of
7 Influenza B viruses characterized, but a respectable
8 number -- 76 that were isolated between April and
9 September of 1998, and a total of 50 isolated between
10 October 1998 and January 1999. And we have about 35
11 percent of the viruses isolated in the U.S. which are
12 reduced in titer to the Harbin vaccine antiserum.
13 I should also note that when I was reading
14 Dr. Nerome's overhead that he also sees a number of
15 viruses that are reduced in titer to the Harbin
16 antiserum. And in Europe, I understand, they are
17 seeing a similar picture with viruses being very well
18 inhibited by Beijing 184 antiserum but not so well
19 inhibited by the antiserum to the vaccine strain
20 itself.
21 This map, which shows the geographic
22 distribution of B/Victoria-like viruses really has not
23 changed since September, when we had our WHO
24 consultation on vaccine strain selection for the
25 Southern Hemisphere. We have B/Victoria-like viruses

1 continuing to circulate only in Asia, and we have been
2 looking at the same picture for a number of years. We
3 do not understand why these viruses have not moved out
4 of Asia as there is a growing susceptible population
5 world-wide, particularly among children who are less
6 than 10 years old. We would expect that there would
7 be a very high proportion of these children who would
8 be susceptible to B/Vic-like viruses. But these
9 viruses simply have not spread outside of Asia. I
10 think Thailand was the country that was added to the
11 map since we met last March.
12 So if we look at the evolutionary
13 relationships among Influenza B hemagglutinin genes,
14 we see that it is very, very easy to distinguish the
15 Victoria-like strain shown down here in green from
16 what we used to call the Yamagada lineage. Those of
17 you who have been with us for a long time would
18 recognize the Yamagada virus. So this is the Yamagada
19 lineage, which contains both the Beijing 184 virus
20 shown in purple here and our Harbin vaccine strain and
21 the viruses most closely related to it shown in this
22 turquoise color here.
23 Now what we are trying to sort out and
24 which we still haven't been able to do is to sort out
25 a sequence signature that corresponds to this lower

1 reactivity. But what we have determined is that
2 viruses which are on this particular sublineage, that
3 is, the viruses shown in purple, tend to produce
4 antisera which are more broadly cross-reactive against
5 all of these strains here than viruses which are in
6 this group here. So we are pursuing that line. And
7 of course the Georgia strain, which we haven't
8 sequenced and which is not on here is in this purple
9 group.

10 The viruses that were shown previously in
11 purple and in turquoise are sometimes difficult to
12 distinguish antigenically, but they are very easily
13 distinguishable using restriction analysis or RFLP
14 analysis. so we have been trying to keep up with what
15 is going on in different geographic regions with
16 regard to whether the purple team or the turquoise
17 team are winning, and we haven't really seen a great
18 deal of change. What we know is that the Beijing-like
19 or B -- we will just call them B and B* viruses --
20 have continued to circulate exclusively in North
21 America. So all of the viruses that we have analyzed
22 in using RFLP in the U.S. have belonged in this group,
23 the purple group or the Beijing/184 group. In
24 contrast, in Asia we see both the purple and the
25 turquoise viruses circulating -- the Beijing/184-like

1 and the Harbin-like viruses circulating and continuing
2 to circulate. So there really hasn't been much change
3 there.

4 I think we will skip this one. Now we are
5 looking very carefully at our egg isolates and we see
6 that when we look at Harbin-like, that is, the
7 turquoise group of viruses, when we look at that group
8 of virus and make a consensus sequence for the HAs
9 from those viruses, we see that the viruses have
10 started to move on a bit and the Harbin strain itself
11 has 7 amino acid differences from the consensus. And
12 then we have some egg isolates that have fewer changes
13 from the consensus. Now remember I had mentioned that
14 antisera from the other group, the purple group, have
15 tended to provide better coverage for both groups, and
16 we have a number of candidates here. The Beijing
17 virus itself has 9 amino acid differences from the
18 consensus. And we have this virus, Yamanashi/166/98
19 sent to us by Dr. Nerome, which has only two amino
20 acid differences from the consensus and its antiserum
21 does seem to cover viruses fairly well.

22 Just in case, we are always trying to make
23 sure that we have egg isolates for the Victoria
24 lineage, just in case those viruses do begin to spread
25 and would spread quite rapidly. We have a number of

1 candidates. As you might remember from the past, and
2 as you will be reminded during Roland Levandowski's
3 presentation, there was an experimental vaccine trial
4 done with a virus on the Victoria lineage last year,
5 and there is an additional vaccine trial going on with
6 the Shangdong/7/97 strain in Australia right now. It
7 is just finishing up and we hope to have those sera
8 tested in time for the Geneva meeting. So needless to
9 say, we do have some egg isolates from the Victoria --
10 recent ones from the Victoria lineage that would be
11 appropriate vaccine candidates. But since the viruses
12 do not appear to be spreading, we are just keeping
13 these in our back pockets.

14 When we do our serologic studies in humans
15 for the Influenza B viruses, at CDC we typically use
16 ether-treated antigen. And, of course, we always have
17 to keep in mind when we are looking at these results
18 that we are increasing the sensitivity but decreasing
19 the specificity of the antibody reactions. And what
20 we are seeing is pretty consistent solid cross-
21 reactivity between the antibody induced by the
22 B/Harbin vaccine strain and other strains that we have
23 chosen thus far. We have not yet tested the Georgia
24 strain. But when we use viruses on the Victoria
25 lineage, we see a reduction in the post-vaccination

1 geometric mean titer. A lot more about that will be
2 said later.
3 Now finally I would just like to -- I
4 didn't have time to do a proper overhead, but I did
5 want to acknowledge a number of people in the
6 Influenza Branch. I don't normally do this, but I
7 wanted to do this today. In particular, I would like
8 to acknowledge Alexander Klimov, who heads the Strain
9 Surveillance Section, and in that group I would like
10 to mention Henrietta Hall, Inger Baker, Cuca Perez,
11 Jim Love and a whole variety of visitors from China
12 and Vietnam and other countries who have contributed
13 to the antigenic analysis of the strains that we are
14 getting in. We are actually receiving twice as many
15 strains at CDC as we did three years ago or four years
16 ago, and so it has been a tremendous challenge to
17 figure out how to get these analyzed, how to
18 prioritize these and get them analyzed in a way that
19 serves us best for vaccine strain selection. I would
20 like to acknowledge Keiji Fukuda and his epidemiology
21 section, in particular Lynette Brammer and Lee
22 Schmeltz, who are very integral parts of the U.S.
23 Domestic Surveillance System. And I would like to
24 acknowledge Jacqueline Katz, not so much for what I
25 presented today, but she and her section have worked

1 very, very hard in producing some of the results that
2 you will see this afternoon for the experimental H5
3 vaccine trial. And the, of course, I would like to
4 acknowledge Dr. Kata Subbarao and her molecular
5 genetics section, in particular Huang Jing, Catherine
6 Bender, and Xu Xiyan for getting all the sequencing
7 done in such a timely manner.

8 Okay, now I would like to throw it open
9 for questions.

10 CHAIRPERSON FERRIERI: Thank you very
11 much, Dr. Cox. Dr. Snider?

12 DR. SNIDER: Yes. Dixie Snider, CDC.
13 Nancy, as a context for what you have presented, you
14 alluded to the fact that you are getting more strains
15 in. What is your assessment of the representativeness
16 of the strains as a reflection of world-wide influence
17 or activity, and what has been the trend? Has that
18 representativeness improved over the last couple of
19 years?

20 DR. COX: I would say that the
21 representativeness has improved globally and that if
22 you look at the really big picture, which is the whole
23 WHO global influence network and the work that we are
24 doing in parallel with the work that is being done at
25 Millhill in Melbourne and in Tokyo, that we really

1 have a much better handle on what is going on. But I
2 think you have to pull it all together. We are
3 getting a lot more viruses from South America and a
4 few more from Central America. We are getting a lot
5 more viruses from Asia. There are still areas of the
6 globe that are not well covered, and obviously we are
7 doing our best to try to enhance surveillance and work
8 with our partners in the military and elsewhere to try
9 to plug some of those gaps. But I would say that we
10 have -- generally speaking, we have a much better
11 handle on what his actually circulating out there at
12 the present time than we did 10 years ago.

13 CHAIRPERSON FERRIERI: Dr. Kim?

14 DR. KIM: Dr. Kim, Los Angeles. I have
15 some questions on HI, the titer. It appears that --
16 you indicated that titers of let's say 640 is better
17 than 160 and 320 is better than 80. Again, I am not
18 a virologist, but are there data indicating that
19 better means biologically relevant? That means 640 in
20 this is biologically better that 160? That is my
21 first question.

22 DR. COX: Okay. We have a lot of
23 experience looking at hemagglutination inhibition
24 tests using post-infection ferret sera, and this is
25 the common method used world-wide to distinguish

1 antigenic variance. We know that there is sort of an
2 inherent two-fold variation in HI tests. And of
3 course an HI test done on one day may have higher
4 titers generally than an HI test done on another day.
5 We have found through many years of experience that if
6 we consistently see that there are reductions of four-
7 fold or greater in titer of a test antigen compared to
8 what we see for the homologous antigen antiserum
9 interaction, that this is significant and we need to
10 pay attention to it and use our sequence analysis and
11 pull that in and try to see if there are genetic
12 correlates. And the way that we really try to put
13 that all together is to look for patterns, both
14 geographic patterns and sequence patterns.
15 DR. KIM: All right. The second related
16 question is that it appears that looking at the
17 numbers, your post GMT titer appeared to correlate
18 with the somewhat pre-GMT titer. I wonder whether
19 that is sort of your impression post-GMT titers?
20 DR. COX: Yes. And I am sure that Roland
21 and others may comment about that. But, yes, that is
22 true to some extent. That if you have higher pre-
23 vaccination geometric mean titers, which often means
24 -- it can mean a couple of different things, but it
25 often means that you have a primed population and you

1 often have a higher post-vaccination geometric mean
2 titer for that particular strain.
3 DR. KIM: And then looking to the
4 speculation of why let's say some individuals may have
5 higher pre-GMT titers compared to others, does that
6 perhaps relate to any preexisting -- different kinds
7 of influencing activity? For example, if someone has
8 a vaccine and then has antibodies being induced and
9 then has exposure or has the influence of H3N2,
10 perhaps that individual's antibody may decrease and
11 then resulting in lower pre-GMT titer and therefore
12 that person might not be better antibody inducers in
13 the following season?
14 DR. COX: I am not quite sure I --
15 DR. KIM: What I am trying to get into is
16 that perhaps endogenous decay of antibody may depend
17 on the exposure to a homologous or related antigen
18 that perhaps may determine the titers of a pre-GMT.
19 This is entirely speculation. I just want to see --
20 DR. COX: Yes. I think that the
21 population's exposure both to vaccine and to
22 previously circulating strains certainly will have an
23 impact on the pre-vaccination geometric mean titers.
24 CHAIRPERSON FERRIERI: Are there other
25 questions? Yes, please.

1 DR. HOKE: Charles Hoke, Fort Detrick. As
2 you attempt to correlate the sequence changes with the
3 changes perhaps in HAI, you express the information in
4 terms of numbers of mutations and so forth. And I
5 know that you are sequencing through an area that is
6 important, but are all mutations equal?
7 DR. COX: No, certainly not. And we have
8 a limited amount of time today, so I am trying to
9 present what I think will be the most useful data at
10 this point in time. But we actually look in much
11 greater detail at the changes that occur and
12 oftentimes -- I mean, you have probably seen graphic
13 representations of the three dimensional structure of
14 the HA and where the changes are in the HA, whether
15 they are in antigenic sites or not. And whether or
16 not they are conservative changes -- conservative
17 amino acid changes or whether they are more dramatic
18 changes which we would expect to have a greater impact
19 on antigenicity.
20 Very often when we see a significant
21 antigenic variant, there are a number of signature
22 changes as we have come to call them that we see in
23 association with the antigenic change. It is very
24 hard to dissect out which of those signature changes
25 -- often there are three or more signature changes --

1 and it is very often hard to dissect out which of
2 those changes are most important for the antigenic
3 changes that we see. But nevertheless, we do look at
4 those in relation to the three-dimensional structure,
5 which antigenic site they are in, and whether or not
6 they are conservative or more dramatic changes.
7 CHAIRPERSON FERRIERI: Dr. Levandowski?
8 DR. LEVANDOWSKI: I have one comment that
9 I would like to make. I think Nancy mentioned it, but
10 I just wanted to comment on the B status, the
11 Influenza B virus vaccine component. The fact that we
12 are using B/Harbin/7/94 in the vaccine relates to the
13 fact that the B/Beijing/184/93, which was actually the
14 strain that was named, does not grow well enough to be
15 useful for manufacturing. But I think I would just
16 like to emphasize that. I think Nancy touched on
17 that, but I think everybody needs to be clear on why
18 that strain was substituted at the time that it was.
19 CHAIRPERSON FERRIERI: Thank you.
20 DR. COX: I didn't mention that and I
21 should have, Roland. Thank you for bringing that up.
22 The B/Beijing/184 grew very, very poorly. I think all
23 the manufacturers had similar experiences with it and
24 it just was not suitable for production.
25 CHAIRPERSON FERRIERI: We have time for

1 one brief question and brief answer. Dr. Huang?

2 DR. HUANG: You focused on the low

3 reactants and even pulled them out in your data

4 presentation. I am not sure that I understand why you

5 did that.

6 DR. COX: We seek -- I focused on those

7 viruses that are reacting lower because we are looking

8 for the next variant. And the only way we are going to

9 do that is by focusing on those low reactors and

10 comparing their sequence -- their genetic sequence to

11 the sequences of the viruses that are typical. I

12 perhaps didn't give enough emphasis to that. So we --

13 DR. HUANG: But don't you always have a

14 background of those?

15 DR. COX: That is right, you do. But

16 you've got to be looking at those, or you will never

17 know what the new strain is until it is on you. So

18 you focus on the low reactors. We have learned this

19 from all of our past experience. You've got to look

20 for those low reactors. You have got to know where

21 the sequence changes are, what the signature is, what

22 the antigenic and the genetic signatures are so that

23 you can pick up the next one and the next one and see

24 where they are, so that you can get a pattern. You

25 don't want to just pull a low reactor out of one of

1 your tests or a couple of them and say, okay, this is
2 probably our next epidemic strain. And you don't want
3 to wait until it is upon you, because then it is far
4 too late. So you are constantly sifting through your
5 data in that way, comparing the low reactors to the
6 typical viruses.

7 CHAIRPERSON FERRIERI: Thank you, Nancy,
8 for a very comprehensive presentation. We will move
9 on to Ms. Linda Canas, who will present on additional
10 surveillance.

11 DR. LEVANDOWSKI: Dr. Ferrieri, could I
12 just --

13 CHAIRPERSON FERRIERI: Yes?

14 DR. LEVANDOWSKI: Could I just comment on
15 this presentation and the ones that are coming up?

16 CHAIRPERSON FERRIERI: Yes.

17 DR. LEVANDOWSKI: Many of you know that
18 there is a long history of military involvement with
19 influenza vaccines, and we probably wouldn't be
20 sitting here today if the military had not wanted to
21 have inactivated influenza vaccines licensed back in
22 the 1940's. For many, many years, we have been very
23 fortunate to have information collected through
24 extensive networks within the military in the United
25 States, and a lot of our most important efficacy data

1 comes from studies that have been done. We were very
2 pleased and happy that we were able to get Linda
3 Canas, who is the chief of diagnostic virology at
4 Brooks Air Force Base to come here today to give us
5 some information on the military's current status in
6 terms of surveillance. And I think you will hear that
7 it is a fairly global activity as well. And that will
8 be followed up by some of our international visitors.
9 So thank you for that.
10 CHAIRPERSON FERRIERI: Thank you.
11 MS. CANAS: Good morning. The Department
12 of Defense has long recognized that the trivalent
13 vaccine is currently the most effective measure of
14 preventing influenza illness, and in fact it is a
15 requirement that every active duty individual be
16 vaccinated annually. It is in our institutional
17 interest as well as the global interest that this
18 vaccine be right. And we are very happy to present
19 our data for your consideration.
20 The program has been in existence since
21 1976 and was started by the Air Force to determine
22 what influenza was doing in the local communities and
23 the impact that it would have on those bases as well
24 as to establish control measures. We do collect
25 throat swabs today, but in the early days, they

1 actually did have the patients gargle.
2 Today, it is a tri-service operation. It
3 is headed by the global emerging infection system
4 located here in Washington and the Army, Navy and Air
5 Force do participate along with some very creative
6 partnerships with other federal and international
7 organizations. This program is evolving as we speak.
8 It is probably one of the strengths that we can be as
9 flexible as we have been, and there is a good deal of
10 excitement about what we are doing.
11 To give you a very brief overview of how
12 the program works, the people in the global emerging
13 infectious system office, the epidemiologists at the
14 local level, and laboratories for each of the three
15 services decide on the sentinel sites. I will be
16 presenting the Air Force data because we have the
17 international component.
18 These sites are chosen on the basis of
19 their location as well as the mission. Are they a
20 training site? Is this a port of entry into the
21 country? Do we have troops that are going out to
22 other areas of the world of interest? Or perhaps that
23 base itself is a location we are interested in. In
24 addition, we have been able to collaborate with other
25 areas to establish surveillance in more non-

1 traditional areas. These have represented clinics
2 that have been established and have a fairly long-
3 running history with other areas of the service, and
4 we have been able to establish surveillance in
5 conjunction with other efforts. We now have a pretty
6 good established program in Nepal, Thailand, Argentina
7 and Peru. The Navy has a lab in Cairo, and they are
8 attempting to establish surveillance sites in Egypt
9 and Syria. We are currently not collaborating with
10 them and sharing resources and information, but we
11 hope that this can be another component of our program
12 in the future.
13 We also have some very real possibilities
14 for expanding our surveillance, some of them quite
15 creative. One of them that is very real and probably
16 will come about shortly is being able to put liquid
17 nitrogen tanks on board air craft carriers so that
18 samples can be collected from shipboard personnel and
19 at clinics at the various sites where those ships may
20 stop. We also look for increased surveillance in
21 South America and more in Asia.
22 This is just a quick view of what our map
23 looks like today. We send out collection supplies to
24 each of the sentinel sites along with information on
25 how to store and ship these specimens and ask that

1 they collect according to a case definition. This
2 case definition is consistent with influenza-like
3 illness. We ask that the specimens be collected from
4 the active duty population. They will serve as a
5 sentinel for vaccine efficacy. The dependent
6 population are less likely to be vaccinated and they
7 will be likely to give us an idea of what is going on
8 in their local community.
9 The specimens are then shipped to our
10 laboratory in San Antonio, Texas. If there is one
11 component of this whole program that should be at the
12 top of the list of importance, it is probably the
13 shipping issue. Getting timely specimens is important
14 to the submitting facility to know what is going on,
15 but also for the viability of the virus. And I would
16 mention that our laboratory is a reference laboratory
17 -- a full service reference laboratory with customers
18 around the world. And in that regard, we have
19 established Federal Express contracts. So we receive
20 2,000 to 3,000 specimens per day in our lab for any
21 tests. Our project gargle specimens are easily added
22 in to those shipments. And even those non-Air Force
23 sites that are using us are usually in a position to
24 be able to get the samples to our sites. We have been
25 able to hook onto some other arrangements. But this

1 basic contract to get the specimens to us quickly has
2 greatly increased the programs capabilities.
3 In our laboratory, we have our laboratory
4 procedure for how we are going to isolate those
5 viruses. When we do get a flu virus, we have just
6 this year begun doing our own subtyping. We do share
7 what we get with the CDC to make sure that you all
8 have the information that we have and so it is
9 available for your vaccine decisions. It is a full
10 service lab and we are going to report any virus that
11 we happen to isolate. Just as a point of interest, we
12 do expect to get a specimen in an average of about
13 four days. Influenza grows rather quickly and we have
14 been able to get most of those results out within the
15 first test, which will come off at 48 hours. We don't
16 work on the weekends, so it comes out a little bit
17 longer. I would say that by the time a negative is
18 reported out, we have done at least two screening
19 tests and have held the culture for at least 10 days.
20 In addition to our laboratory, the result
21 is put into our computer where the submitting facility
22 receives it as a patient report for those records. We
23 also e-mail the results to the Public Health Office,
24 so they have real-time data on what is going on in
25 their area. This can be used then for public health

1 decisions or command decisions that may need to be
2 made. This has also been an important addition to the
3 program. The submitting facility is now getting
4 something back. When we were just giving patient
5 reports, it was very difficult for the public health
6 people to get that information. So with the minimal
7 effort of putting it into e-mail, they have some
8 information to make their program worthwhile, and
9 their physicians are much more likely to collect also.
10 We are attempting to establish the
11 epidemiology. This is a real strength. We have this
12 information. We know vaccination status and travel
13 histories. It is just a matter of getting it all
14 together. That is an ongoing process, but one that we
15 expect to be able to work on and one that we
16 collaborate closely again with the CDC. And here is
17 an idea of where our isolates have come from in the
18 last year.
19 We collect and report anything we get
20 throughout the year, but it has officially run October
21 through May. As we add more of South America, I
22 expect that will change. I notice the numbers got cut
23 off on this graph. Just as a point of reference -- if
24 I find it -- the January peak, we received 540
25 specimens and we isolated 160 Flu A's. That was last

1 year, but it is just to give you an idea. As you are
2 well aware, every year has its own personality of
3 influenza. And last year was pretty much exclusively
4 Flu A for us. We had five B's in the entire year from
5 all of our sites. We are seeing a few more this year,
6 but it still is exclusively influenza A.
7 Now looking at our recent data, if I
8 divide up our sites into areas, when we look at Asia,
9 last year we had 53 specimens that were typed in this
10 area, and all of them came back as A/Sydney H3N2. I
11 would say that this year has been fairly light until
12 last week. This data is current until last Friday.
13 From Monday until Wednesday before I left, we had
14 received 85 specimens and had reported out an
15 additional 34 influenzas. So I will add those as we
16 go along. And 31 of those specimens came from Asia,
17 and 8 of those influenza isolates came from this area.
18 In December, we saw an outbreak in Korea. We had 15
19 of our isolates from this area came from Korea. All
20 of those were in active duty individuals. We don't
21 have confirmed vaccination status on those, but we do
22 know that our vaccination status overall in this
23 population is 90 percent. So we have sequenced -- or
24 excuse me, not sequenced, but we have typed 15 of
25 those and they have all been the A/Sydney.

1 The ones that we have received this week were
2 from Japan. We haven't had quite as many lately from
3 Korea, but we are now starting to see them in Japan.
4
5 We have three sites in Europe. England
6 has Lakenheath Air Force Base, Ramstein, Germany, and
7 Incirlik, Turkey. I would also comment that the Army
8 has a lab at Lonstull, Germany, where they run a
9 similar program although on a smaller scale. Bosnia is
10 supposed to be conducting surveillance and sending
11 through them. The data I have seen has been small, but
12 their isolates have also been the H3 Sydney. We are
13 continuing to receive more from them. We have this
14 week reported 5 more A's and one B, the one B being in
15 Germany.
16 Most of our specimens do come from the
17 United States. We have 8 sentinel sites, but these
18 public health officers move around and know about the
19 program. Flu, of course, is becoming a much more
20 important topic, even in the lay press. So we get
21 specimens from a lot more sites than just our sentinel
22 sites who are required to send to us. We are really
23 seeing an increase in activity. We are picking up --
24 in California, we are starting to pick up more, and we
25 have had two B's in Texas so far. That is the only

1 B's we have seen in our United States population.

2 And to comment on our work with the

3 Nepalese and South America. This has been a very

4 exciting part of our program, mainly because it works.

5 It takes a tremendous amount of cooperation to collect

6 the samples in these remote communities and get them

7 to a place -- in Nepal, they go to Bangkok -- where

8 they are repacked in dry ice and then shipped to us.

9 We have had wonderful success from Nepal. We have had

10 a total over two years of four shipments, and we have

11 had good isolation. This year out of two shipments,

12 we had 91 samples. We had a 44 percent isolation of

13 viruses in those shipments. 34 percent of those were

14 influenzas. Of the 28 influenza A's that we received,

15 21 have thus far been identified as being the H3N2

16 A/Sydney. We have had 3 B's altogether.

17 And this gives some idea -- we just

18 finished -- Wednesday, as I was leaving, we just

19 finished the latest shipment. So not all of those are

20 represented here, but we have had a very broad range

21 of the time scale. The latest we had was in November.

22 Another important point is how flexible it is. Dr.

23 Cox mentioned all the discussion about activity in

24 China. So our contact in Thailand put out the word to

25 the providers to be alert and got a call that they had

1 an Embassy official who had just returned from
2 Thailand and was very ill and they were able to
3 actually get that specimen in the shipment. We
4 isolated parainfluenza 3, but they had the result back
5 in 7 days.

6 In our results in South America, we had a
7 little more problematic beginning in the shipment. It
8 was a learning experience. It got held up in Customs
9 once. There wasn't enough dry ice once. So we had a
10 couple of shipments where we got nothing. But this
11 last shipment that we received in October, we had
12 quite a nice selection of viruses and age groups. But
13 again what we have seen -- we have limited
14 identifications on these yet, but it seems that they
15 are still being the Sydney that we are seeing.

16 As any program moves on, it continues to
17 develop, and we are growing too. Our latest component
18 is that we are going to add the molecular and be able
19 to do our own sequencing. Dr. Lohman has just joined
20 our lab in October. I am not the only one standing
21 outside his door with priorities, but he is here today
22 and I think that shows our corporate commitment and
23 his personal enthusiasm for what we have to do. He is
24 coordinating closely with CDC so that we are doing the
25 same thing. We can share our resources and not

1 duplicate our efforts but maximize the information
2 that we have to present to you. We are very pleased
3 with our program. It is actually a lot of fun. My
4 techs get overworked, but they are very committed to
5 it. It is important to them to know that you are
6 interested in their work and it helps them to work a
7 little harder. And we thank you. Would there be any
8 questions?
9 CHAIRPERSON FERRIERI: Yes, Dr. Breiman,
10 a brief question.
11 DR. BREIMAN: Rob Breiman. This sounds
12 like a wonderful adjunct to the global surveillance
13 information. I wonder -- you mentioned that shipping
14 has been or can be a problem or it could be the weak
15 link. Do you have an idea -- have you been able to
16 look at viability to see what proportion of specimens
17 have had some effect to them during transport so that
18 you had loss of viability and inability to grow the
19 organisms?
20 MS. CANAS: We actually have very good
21 results. The ones from Nepal that we just received
22 were actually collected in April and May. So the
23 average was five months that they had stored it. So
24 the trick is to be stored properly. It doesn't matter
25 how long, as long as it is -- and those were held in

1 liquid nitrogen and then shipped to us on dry ice.
2 South America stores at -70, and they shipped on dry
3 ice, and the average that they held them was over two
4 months. So that has not been the problem. It is
5 maintaining that cold link in transit.

6 CHAIRPERSON FERRIERI: One brief last
7 question. Dr. Hall?

8 DR. HALL: I just wondered, is viral
9 isolation the only means of diagnosis you are using or
10 are you using the antigen detection tests in addition?

11 MS. CANAS: Currently we are only using
12 the culture because we want to have the strain.

13 DR. HALL: Right.

14 MS. CANAS: This is certainly an area that
15 would help tremendously in these remote areas if we
16 could use the antigen test. They would get something
17 out of it more quickly. Expense comes in there. We
18 also then hope to work on that as far as our molecular
19 goes also. The other problem with those direct
20 antigen tests, at least stateside, is they do them and
21 they know what is going on and they forget to give us
22 the culture.

23 CHAIRPERSON FERRIERI: Thank you, very
24 much. I wonder, Dr. Levandowski, if you would please
25 introduce our next two presenters, our international

1 reports.

2 DR. LEVANDOWSKI: Okay. Thank you. We
3 are fortunate to have some guests to fill in some of
4 the information that Nancy Cox was alluding to earlier
5 in her presentation. First on our list is Dr. Kuniaki
6 Nerome from the NIH in Japan. He, as you know, works
7 at one of the WHO influenza centers, the most recently
8 established one in Japan, and he too will give us some
9 information on the status of surveillance.

10 DR. NEROME: Good morning, ladies and
11 gentlemen. It gives me great pleasure to give a
12 presentation about the situation of flu activity in
13 Japan. I am also grateful to the staff of the CDC and
14 the FDA for providing this chance to attend this
15 meeting.

16 I left all color slides in the airplane
17 between Atlanta and Washington. I think this is
18 another type of risk due to jet lag. But I overcome
19 this risk by the aid of the staff of the FDA. Thank
20 you very much.

21 Our flu activity in Japan starts with
22 first isolation of the H3N2 antibodies in the southern
23 part of Japan. Antigenic analysis indicates that
24 this --

25 CHAIRPERSON FERRIERI: Can you turn it up?

1 DR. NEROME: Antigenic analysis of these
2 past isolates indicated a close identity to A/Sydney
3 vaccine. Second are reports of isolation of H3N2
4 viruses from the southern part of Japan, Okinawa. And
5 now H3N2 virus has spread all over the country. First
6 of all, I'd like to show the differences of the
7 epidemic strain by two colors. The darkened bar
8 indicates the isolation of the A viruses, H3N2
9 viruses. This dark bar indicates the number of
10 isolations of H3N2 viruses. This white bar indicates
11 the number of isolations of Influenza B viruses. So
12 H3N2 and B viruses cocirculate in Japan.
13 So we have used five indices to understand
14 epidemic occurrence in and the magnitude of the
15 outbreak. This information was collected in grammar
16 school and junior high and high school. This affords
17 a good indicator for understanding the immunizations
18 in Japan. This is the virus isolation in primary
19 school.
20 CHAIRPERSON FERRIERI: Excuse me, Dr.
21 Nerome, would you please speak louder? I am afraid
22 the acoustics are not good enough for people to pick
23 up in the back of the room. So louder, please. And
24 maybe you can help over here our assistant.
25 DR. NEROME: You can see here whole

1 indices to understand epidemic occurrence and the
2 magnitude of the outbreak. This information was
3 collected in schools such as primary school, junior
4 high and high school through absentees, class closures
5 and school closures. These big peaks through the
6 indices portray in dark last season. But this season,
7 only a very small peak can be seen here. This slide
8 shows activity in Japan, very small.
9 The Japanese government decided to start
10 a nationwide serological surveillance of all age
11 groups before the start of the flu season. You can
12 see here antibody distribution of A/Beijing/262 H1N1
13 viruses. This is borderline to prevent epidemic at
14 this time. All age groups, the antibody titer of this
15 age group rested on this borderline. This is very
16 important information to predict the next epidemic
17 strain.
18 This table shows the antigenic analysis of
19 H1N1 viruses. Could you look at this slide here? So
20 the Okinawa isolates show directly to a higher titer
21 to Beijing 262, the antigen similar to the vaccine
22 strain. The remaining three viruses are isolated in
23 the western part of Japan, Ishikawa 42, Ishikawa 43,
24 correlate directly to a low titer to the vaccine
25 strain like this.

1 These slides show the evolutionary pathway
2 of H1N1 influenza viruses. You can see most of the
3 isolates are isolated from this scene are located in
4 new branch across here and the second branch located
5 here. These two -- this one is related to vaccine
6 strain to Beijing.
7 This slide shows the antibody distribution
8 to two Influenza B viruses. Above here is the
9 antibody distribution to all age groups. You can see
10 here that most of the age groups contain antibodies to
11 higher than 40 --? So this data suggests to us that
12 this Harbin-like strain cannot cause intense strain in
13 the coming season. Please remember this antibody
14 distribution. And the other figure indicates antibody
15 distribution to B/Beijing/243/97. This is a Victoria-
16 like variant. This is a Harbin-like variant. These
17 two viruses were quite different originally.
18 The Japanese isolates reacted to the low
19 titer to reactive strain Beijing/243 as you can see
20 here. And one European strain isolated in France,
21 B/Pasteur/266, reacted to low titer to reactive
22 strain. The antigenic analysis of the B influenza
23 viruses, one French strain reacted to low titer to the
24 vaccine Harbin/07/94 strain.
25 This here is very interesting for us. So

1 last season, Japan recommended Harbin-like variant of
2 the vaccine B viruses, but I was beginning to wonder
3 if this Beijing-like strain may appear or not. But as
4 a result, two types of B variant cocirculated in
5 Japan, one variant, a Harbin-like vaccine strain,
6 and the second variant a Beijing-like strain. These
7 viruses cannot -- a vaccine cannot contain this
8 variant. By a lower antibody distribution. So
9 B/Beijing-like variant, Victoria-like variant, could
10 circulate a major strain like this here.
11 This pie chart indicates the proportion of
12 the isolates of B viruses. So 62.8 percent isolates
13 to Victoria-like variant, B/Beijing/243, and the
14 remaining 37.2 percent is Harbin strain. So the
15 B/Beijing-like variant or the Victoria-like variant is
16 predominant in Japan as the major strain. This is
17 very important to understand the choice of the vaccine
18 strain for next season.
19 This is the geographical distribution of
20 the two B variants. The red indicates the number of
21 isolates of Harbin-like viruses. And blue indicates
22 the area where B/Beijing-like viruses are isolated.
23 And light blue is mixed. You can see here that in
24 most of the areas, the two variants are cocirculating.
25 This is very interesting data for us.

1 This is an evolutionary tree of B
2 influenza viruses. Most of the recent influenza B
3 viruses have evolved into two evolutionary lineages.
4 This is the first lineage, the Yamagata-like variant
5 and this is the Victoria-like variant. So most of the
6 recent isolates are located in this top branch cluster
7 here. And second, the Victoria-like viruses in Japan,
8 Shizouka, Shiga, Chiba, Osaka and Chinese Shangdong
9 viruses belong to the top branch cluster of Victoria-
10 like genus.
11 In the last slide, the figures indicate
12 the antibody distribution to the H3N2 viruses. This
13 top figure indicates antibody distribution to all age
14 groups to A/Sydney/5/97 strain. All age groups,
15 particularly the lower age groups, contain a higher HI
16 antibody titer than had been considered. So this
17 Sydney-like strain may not cause a major strain in
18 this season. This bottom figure indicates the
19 antibody distribution to the Yokohama-like variant.
20 Roughly, these two antibody distribution of the two
21 viruses are similar to each other.
22 So all isolates from Japan antigenically
23 are very similar to A/Sydney/5/97 strain. This is the
24 substrain Samara and Scichan, and the Scichan isolate
25 reacted to a low titer to A/Sydney vaccination, around

1 10.

2 Two Sichuan strains were sent from CDC and
3 we captured them again. Dr. Nancy Cox already pointed
4 out that, these two strains are directly related to
5 the vaccine strain in Chile in 1997. This is a
6 proportion of the isolate in the 1996/1997 season, the
7 Wuhan strain was the prevalent strain. In the last
8 season, this Sydney-like strain increased in
9 proportion like this. And this season, all isolates
10 were actually 5/97 strain.

11 This is an evolutionary tree, a Sydney-
12 like variant, an Okinawa or Japanese strain to top
13 branch. This is related to vaccine Sydney-like virus
14 here.

15 In conclusion, first in 1998/99 influenza
16 activity in Japan was characterized by epidemic
17 influenza A/Sydney/5/97-like activity.

18 Second, cocirculating influenza B viruses
19 were antigenically and phylogenetically separated into
20 two lineages. The first lineage, B/Yamagata-like
21 viruses (B/Harbin/07/974-like viruses). Second, the
22 Victoria-like viruses (B/Beijing/243/97-like viruses).
23 Third, a few A H1N1 viruses from sporadic cases
24 drifted from A/Beijing/262/95 viruses similar to
25 A/Harbin/04/97 viruses.

1 Serological studies conducted several
2 months before the start of the 1998/99 season indicate
3 the following. Antibody levels to A/Sydney/5/97-like
4 viruses were highest in the lower age group, primary
5 school, junior high school and high school students.
6 Mean antibody titers to A(H1N1) viruses in groups
7 ranging from young adults to the elderly were low and
8 below protective levels.
9 So even though antibody titers were low to
10 A/Beijing/262/95 H1N1 viruses, a few drifted variants
11 were isolated in this season. Antibody titers to
12 B/Harbin/07/96 were elevated in persons over 49 years
13 of age. Antibody titers to B/Victoria/2/87-like
14 viruses were very low in all age groups except in
15 persons 20 to 29 years of age.
16 With regard to the vaccine strain
17 recommendations, a few points should be considered.
18 A/Sydney/5/97-like virus variants may cause little
19 activity in the coming season. New antigenic variants
20 should be considered to replace the A/Sydney/5/97
21 virus component. In Japan, B/Beijing/243/97 viruses,
22 variants of the B/Victoria/2/87-like viruses, appeared
23 to cause major activity.
24 For the B component, should we consider
25 B/Harbin/07/96-like strains? Second,

1 B/Beijing/243/97-like strains? Or third, strains
2 which efficiently cover the above two strains? And
3 how should the few A/H1N1 variants, drifted from
4 A/Beijing/262/95, be evaluated?
5 Regarding new potential pandemic influenza
6 viruses, Avian influenza A/H5N1 viruses disappeared at
7 the end of 1997. In Southern China, avian A/H9N2
8 viruses have been isolated from poultry and pigs.
9 It was Sunday night or Monday night --
10 anyway, it was midnight. I received some information
11 from the southern part of China. A large number of
12 H9N2 viruses were isolated in humans from some lady
13 who travelled in China. I was beginning to wonder if
14 this new H9N2 virus may cause the next pandemic strain
15 and tried to confirm it with the Chinese Government,
16 and then with the party who has authority over the
17 southern part of China. Finally I confirmed that this
18 information is very wrong. So the large number of
19 H9N2 viruses were isolated from Southern China, but
20 all of them were avian viruses, not human. So that is
21 wrong information. So we have to carefully handle this
22 kind of information. So it turns out that we are
23 still waiting at the starter gate and looking for a
24 tail of the pandemic strain. Thank you for your
25 attention.

1 CHAIRPERSON FERRIERI: Thank you, Dr.
2 Nerome. I am afraid we have to move on or we won't be
3 on time for the open public session later in the day
4 and some of you want to apparently leave early. So we
5 have a maximum of 15 minutes to present by Dr. Zambon
6 of the Public Health Laboratory Service in the United
7 Kingdom.

8 DR. ZAMBON: Good morning, ladies and
9 gentlemen. It is a pleasure to be here. In England,
10 just as in the United States and in Japan and in many
11 countries of the world, we use many different ways of
12 monitoring clinical influenza activity. The one which
13 we place the most reliance on through experience
14 really in terms of its usefulness is a consultation
15 index derived from continuous morbidity registration
16 by sentinel physicians scattered throughout the
17 country. This allows us to derive a population-based
18 rate for influenza-like illness in the community, and
19 we term this the RCGP index.

20 We know over the last 10 years or so that
21 we can recognize activity above a baseline of about 50
22 consultations per 100,000 in association with
23 circulating influenza viruses. And we describe
24 activity above baseline usually for somewhere between
25 6 and 12 weeks every year. An activity between 50 and

1 200, we describe as normal seasonal activity and we
2 would expect to see this in most years. In unusual
3 years, such as in 1989, when there was a circulation
4 of a novel influenza H3N2 variant, we saw consultation
5 rates of well over 400 per 100,000. And in maybe 3
6 years out of 10, we see consultation rates of between
7 200 and 400, which we describe as higher than normal
8 seasonal activity.
9 So if we come to look at this season, the
10 1998/99 season, we have had a consultation rate of
11 somewhere of approximately 270 per 100,000. So very
12 similar in terms of impact to the season of 1996/97,
13 when we had circulation of a new influenza H3N2
14 variant, the Wuhan variant. Shown in color here along
15 the index are the actual virus isolates which we have
16 had, and this season so far in England has been
17 predominantly influenza A H3N2.
18 This is confirmed from the total
19 laboratory isolates, where we have seen laboratory
20 reports from all hospital laboratories, predominantly
21 Influenza A activity with very little Influenza B
22 activity. This line here should actually be labeled
23 1996/97. The reason for the comparison is that the
24 death registrations that we have had are very similar
25 in terms of total impact to the season of 1996/97.

1 And as I should also point out, our peak consultation
2 was in week 1 of the year, and we have seen a downturn
3 in the consultation index, which indicates that we are
4 tailing off in terms of flu activity.
5 We have looked -- one of the problems of
6 making slides for this meeting is that they are very
7 rapidly outdated. Here we had, when I prepared this
8 slide towards the end of last week, we had actually
9 looked at some 300 viruses, and we have now looked at
10 well over 400, the vast majority of which have been
11 H3N2 viruses with only a handful of Influenza B
12 isolates. The viruses themselves have actually been
13 obtained from all regions of the country, almost in
14 equal measure between northern/central and southern
15 parts of England with some Scottish, Northern Ireland
16 and Welsh isolates.
17 As I have mentioned, one of the things
18 that we have in England is a community-based
19 surveillance system. And we have linked that
20 community-based surveillance system with continuous
21 morbidity registration to sampling for influenza in
22 particular. So that individuals presenting with flu-
23 like illness to certain sentinel physicians are
24 automatically swabbed, and those swabs sent centrally.
25 So that allows us a very rapid, timely handle on

1 influenza isolates coming directly from the community
2 as opposed to from the hospitalized population. And
3 what we see there is the onset of influenza activity
4 towards the end of the year and peaking from the
5 community point of view around the turn of the year.
6 And the hospital isolates are just now starting to
7 come in from other laboratories, although we have the
8 same picture in the community as compared with the
9 hospital in that we have predominantly H3 activity in
10 both sides with very little Influenza B.
11 In terms of the age distribution of the
12 isolates which we have looked at, in the community
13 isolates and in the hospital isolates, we do see some
14 differences in the age distribution, not surprisingly
15 in that the hospital isolates reflect the populations
16 which become hospitalized with influenza, in
17 particular the very young. I haven't disaggregated
18 this data in the under 5's, but the vast majority of
19 these are actually from the under 1's as opposed to
20 the 1 to 5's and in the elderly. Whereas in the
21 community, the majority of isolates that we get are
22 from the working age population. Unusually for us, we
23 have seen rather more isolates from the elderly than
24 we would normally expect, and although we don't have
25 the complete disaggregated consultation data, this

1 also reflects the preliminary evidence that for some
2 reason -- we don't have a good explanation for it --
3 Influenza A or Influenza this year has been
4 particularly hard on the elderly population in the
5 United Kingdom.
6 I have mentioned that we have seen
7 Influenza H3 and Influenza B virus. We have not seen
8 any influenza H1N1. But for the sake of completeness,
9 I have included the HI data on the very last H1N1
10 virus that we had in the last season which was
11 isolated in February/March of 1998. And the main
12 point to notice there is that it was very similar in
13 properties to the Bayern-like viruses, and indeed very
14 similar to other influenza viruses circulating
15 throughout the United Kingdom during the last season.
16 If we turn now to the influenza H3N2
17 isolates. As I have mentioned, we have had
18 approximately 400 or so isolates which we have looked
19 at. The vast majority of these isolates have shown
20 good reactivity with Sydney 5 antiserum. Very
21 recently, though, in January, we have had five
22 isolates from a variety of different sources in the
23 United Kingdom which we can see have a four-fold or
24 more reduced reactivity to Sydney 5. We do not yet
25 have molecular information about these viruses. As

1 you can see, the dates of the specimen are actually
2 very recent indeed. So it will be sometime next week
3 before we have molecular data pertaining to those. So
4 the molecular data, if you will, that I am about to
5 present on the H3N2 really pertains to the first
6 strains that we have isolated.
7 I should draw your attention to this one,
8 A/656, which still continues to show good reactivity
9 to Sydney 5 in this HI test, but which was actually
10 obtained from a person who had been vaccinated. And
11 of some interest is the fact that it had rather lower
12 reactivity to older, earlier strains.
13 In terms of the molecular analysis of
14 viruses, we don't really have anything much which we
15 can comment on in terms of important differences or
16 features that we could identify as being perhaps
17 evolution of new lines of influenza. But of some
18 importance, perhaps, in the virus recovered from the
19 vaccinated individual, we do see an isolated leucine
20 to asparagine change here which potentially creates a
21 novel glycosylation site and then some changes here in
22 position 192, which is antigenic site B, which may
23 possibly account for that escape from vaccination,
24 although that may well be a rather speculative
25 conclusion at this time.

1 This slide should actually read H3N2 here.
2 So of the data that we have so far, the phylogenetic
3 analysis of the sequence data indicates that the
4 England strains looked and mapped very closely to
5 Sydney 5, and I would point out that these are viruses
6 which continue to show good antigenic reactivity to
7 the Sydney 5 antiserum. They are not the viruses
8 which have the rather lower reactivity which we have
9 seen and cannot attribute just yet.
10 We have had a sporadic number of Influenza
11 B's, a total of about 23 or 24 from a variety of
12 sources, both hospitals and communities. And as has
13 already been remarked upon by Dr. Nancy Cox, some of
14 the characteristics of the viruses are overt. They
15 react rather better with the Beijing/184 antiserum
16 than they do with the Harbin. In our England strains,
17 we do see a four-fold reduction in reactivity to
18 vaccine strains or antiserum rates to vaccine strain.
19 However, it is also fair to say that when we have
20 analyzed these strains, at least the ones that we have
21 sequenced, we have seen a number of sequence
22 differences, as you would expect from the vaccine
23 strain itself. But we are not necessarily able to
24 attribute, if you will, importance to the particular
25 amino assay changes that we have seen, and I would

1 guess that in order to do that, this type of data
2 needs to be pooled with data that is available that is
3 available world-wide to give a picture of what is
4 actually happening with Influenza B, and in particular
5 the importance of the reduced reactivity that we seen
6 in hemagglutination inhibition.
7 To conclude then, our Influenza B's look
8 very similar in terms of phylogeny to the more recent
9 Influenza B's that we have had from the last season
10 and are showing a little bit of movement from the
11 Beijing 184, perhaps slightly closer to the Harbin in
12 terms of phylogeny.
13 So in conclusion then in England we have
14 had a moderately severe influenza season in
15 association primarily with influenza H3N2 A/Sydney-
16 like virus. We have seen one or two variants from
17 Sydney/05 virus, which have low reactivity, although
18 we don't yet have molecular correlates of that. We
19 also have Influenza B viruses associated with sporadic
20 cases which have a slightly reduced reactivity to
21 antiserum raised in the vaccine strains, and I would
22 suggest that more data needs to be accumulated on the
23 importance of that observation. Thank you.
24 CHAIRPERSON FERRIERI: Thank you, Dr.
25 Zambol. We have time for -- we don't have time, but

1 we will take it anyway. Does anyone have a question
2 for Dr. Zambol? Dr. Breiman?
3 DR. BREIMAN: Rob Breiman. When you look
4 at the homologous responses to your A/Sydney, your
5 scale is quite a bit different than the one that CDC
6 showed in the sense that it is 2,500, and I think
7 their homologous was 640. Is there that much lab to
8 lab variation that is expected? And I guess the
9 corollary is then when you look and try to extrapolate
10 to these new viruses, particularly the new Scottish
11 and England isolates, would you then -- instead of
12 looking at them as 320, if one wanted to compare with
13 the results that Nancy gave, would they be more likely
14 to be like 80 in that vaccine?
15 DR. ZAMBON: Okay. Well perhaps if I
16 could answer the question broadly. There is often
17 variation in HI tests, laboratory to laboratory. But
18 if you look at the numerical values, that is perhaps,
19 if you like, more emphasized if you concentrate solely
20 on the numerical side of things. If, on the other
21 hand, you look at, if you will, the overall meaning of
22 those results, what we found historically, I think, is
23 that our data correlate usually pretty well with the
24 CDC data in terms of meanings of tests.
25 The second point to make is that we are a

1 national influenza center and that our representative
2 portion of our isolates always goes to one of the
3 world influenza centers. So that our data can be
4 confirmed in HI testing. And I think in some ways
5 that answers the second part of your question, which
6 is relating to the numerical values that you see. I
7 think they are less important than the scale of
8 differences that you find.
9 CHAIRPERSON FERRIERI: Thank you. I think
10 we will take a break now and then resume at
11 approximately 11:30. Thank you.
12 (Whereupon, at 11:18 a.m., off the record
13 until 11:37 a.m.)
14 CHAIRPERSON FERRIERI: We will need to get
15 to our seats right away. Will all committee members
16 please come to the table? We will start with vaccine
17 responses, and Dr. Levandowski is going to lead off.
18 This is a very critical part of our meeting this
19 morning before we adjourn for lunch, so that we try to
20 remember information presented, options for strains,
21 and so on. Roland, please start.
22 DR. LEVANDOWSKI: Okay. If I could get
23 somebody to turn on the slides for me, please? And I
24 again need the lights down. I will apologize if you
25 are not able to see some of these things in the back

1 row. Some of these actually are better seen by
2 Gestalt anyway, so it may be all right.
3 I am going to be talking about the 1998
4 vaccine studies that were done to get information
5 about serologic responses of people who have been
6 immunized with the current vaccines. For the
7 convenience of the committee, the handout includes the
8 slides and the overheads that are being used to
9 present this information, so you can follow along on
10 those. What I am going to try to do is summarize what
11 is really very voluminous information from many
12 collaborating centers to provide information for this
13 purpose, for the function of making strain selections.
14 Now this slide -- and maybe that is not in
15 focus. I can't really tell from here whether it is or
16 not. So maybe someone can help me with that. This
17 slide shows the serum panels that were used for the
18 serologic studies, and they include four separate
19 serum panels from adults and elderly in Australia,
20 Europe, and the United States. The vaccines used for
21 the immunizations are shown here. And I will call
22 your attention to the vaccine used in Australia, which
23 includes as the H1N1 component 8 Johannesburg 82/96.
24 Data for the H1N1 viruses for the Australian sera are
25 not going to be presented because of that because it

1 is not representative of the vaccines that are being
2 used here.
3 The laboratories participating in
4 performing the serologic testing to be discussed
5 include the WHO Influenza Center in Melbourne,
6 Australia, the National Institute of Biological
7 Standardization and Control in London, the Centers for
8 Disease Control and Prevention in Atlanta, the Centers
9 for Biologics Evaluation and Research in Bethesda, and
10 the University of Rochester in New York.
11 The first four labs, I should point out,
12 share the first three sets of sera that are shown in
13 the table here. And that includes about approximately
14 172 serum pairs. And then the University of Rochester
15 supplies an additional 200 serum pairs for these kinds
16 of studies.
17 Now this slide shows the H1N1 antigens,
18 and I am sure you won't be able to see these in the
19 back, but these are the antigens that were used for
20 the serologic testing. And I don't think it is really
21 critical to see all the names up here, because I will
22 discuss some of them more specifically. But you can
23 see that there are quite a few different antigens that
24 have been used for testing. Not every antigen was
25 used by all the laboratories in order that some of

1 these strains could be explored more discretely, but
2 there is a core of the different strains that are used
3 for testing in each of the laboratories, and that is
4 used as a gauge for confirming when there is a signal,
5 since there are, as has been pointed out in some of
6 the previous discussions, some technical differences
7 between laboratories doing hemagglutination inhibition
8 tests.

9 The serologic studies that I am going to
10 describe have been performed in two separate campaigns
11 that coincide with the WHO recommendations for the
12 Southern Hemisphere in September of 1998, and the
13 current evaluation of influenza viruses that is going
14 on now. The antigens shown include representative
15 strains for both of the H1N1 lineages that are
16 circulating, including the A/Beijing/262, which is our
17 current vaccine strain, and the A/Bayern/7/95-like
18 strains, which was our previous vaccine component.

19 Now I would like to turn the slides off
20 for a minute and use some overheads here. And, Dan,
21 if you can put up the overhead from page 3. That is
22 the same page number that is in the hand-out, so it
23 may be more convenient for you to look at them that
24 way. This overhead shows the results that were
25 obtained in September of 1998 from two of the

1 laboratories using a panel of sera from adults in
2 Europe. And by the way, I am going to just pick and
3 choose here to try to make some points from the
4 serologic testing that was done. The table includes
5 data on geometric mean titers, percent greater than or
6 equal to 32 of 40, and percent four-fold rises. The
7 data that are shown here are from NIBSC at the top and
8 from the WHO Influenza Center in Melbourne at the
9 bottom. The vaccine strain for these tests was the A-
10 Beijing/262/95 strain. The vaccine used was clearly
11 immunogenic and it produced brisk homologous antibody
12 responses. And in this particular instance,
13 A/Shanghai is a Beijing/262-like strain, and A-
14 Johannesburg, A-Prague and A-Aukland are Bayern/7/95-
15 like strains. In both cases, the A-Beijing 262
16 vaccine produced antibodies that cross-reacted very
17 well with the Beijing/262-like strain and also with
18 the Bayern 7-like strains.
19 If I can get the next overhead for page
20 four. This overhead shows the results obtained in
21 January of 1999 from two of the laboratories using a
22 panel of sera from elderly in Europe. These data are
23 from CDC at the top and CBER at the bottom. The
24 vaccine strain, again, was A/Beijing/262/95. A/Hong
25 Kong/4847 is a Beijing/262-like strain, and

1 A/Johannesburg and A/Michigan/24 are both
2 Bayern/7/95-like strains. Although the absolute
3 antibody titers are somewhat lower than in the
4 previous overhead, and this is again because of
5 technical differences and also partly because these
6 sera are from elderly patients, the vaccine did elicit
7 response to the homologous and the heterologous
8 antigens. And although the response to the Hong Kong
9 strain was reduced, the difference here was less than
10 a two-fold difference in geometric mean titer.
11 The next overhead for page 5. This
12 overhead shows the results obtained in January of 1999
13 using a panel of sera from adults in the United
14 States. These data are from the CDC at the top and
15 from NIBSC at the bottom. The data from the CDC
16 indicate a greater than two-fold difference between
17 the vaccine strain and the Hong Kong strain, but quite
18 good titers for A/Michigan. Conversely, the data from
19 NIBSC indicate a greater than two-fold reduction in
20 the geometric mean titer for A/Michigan, which again
21 is a Bayern-like strain, and A/Ulan Ude, which Nancy
22 mentioned as being from Russia, which is a
23 Beijing/262-like strain. There was no difference seen
24 here for the other Bayern-like strain, A/Madrid, as
25 shown in the NIBSC information.

1 Then I am going to skip the next overhead
2 there and go back to the slides. If you can turn the
3 overhead off. This slide shows the H3N2 antigens that
4 were used for serologic testing, and all of the
5 antigens chosen represent strains that are related to
6 the A/Sydney/5/97 current vaccine strain. So I don't
7 really want to dwell on those, but just again to
8 reiterate that there are a number of strains that are
9 tested.
10 Go back to the overheads, the overhead for
11 page 8. This overhead will show results that were
12 obtained in September of 1998 using a panel of sera
13 from adults in the United States. The data shown here
14 are from the CDC at the top and from NIBSC at the
15 bottom. The data are similar in both instances and
16 show again that the vaccines used are immunogenic.
17 They also demonstrate that the current vaccine strain
18 produces antibodies that cross-react reasonably well
19 with the other new H3N2 viruses such as A/Chile/37/92,
20 A/Shanghai/72 and Johannesburg/29.
21 The overhead for page 9 shows the results
22 obtained in September of 1998 using a panel of sera
23 from elderly in Europe. The data are from the CDC at
24 the top and from CBER at the bottom. And although the
25 absolute titers are lower in the elderly than seen

1 with the adult serum panel just before, the vaccine
2 responses to the new H3N2 strains appears similar to
3 the vaccine except for the Johannesburg/3/98 virus,
4 which shows a result that is more than a two-fold
5 reduction in the CBER test.
6 The overhead for page 10 shows results
7 obtained in January of 1999 for sera that were from
8 elderly in Australia. These data are from NIBSC at
9 the top and CBER at the bottom. And they demonstrate
10 that the current vaccine strain produces antibodies
11 that cross-react reasonably well with other new H3N2
12 viruses such as the A/Genoa/5/98, the A/Genoa/8/98,
13 the Sichuan/346 and the Sichuan/418.
14 The overhead for page 11. This overhead
15 shows results obtained in January of 1999 for adults
16 in Europe. The data are from NIBSC at the top and
17 from the WHO Center in Melbourne at the bottom. The
18 data here demonstrate that the current vaccine
19 produces antibodies that cross-react well with the
20 other strains such as Genoa/5 and Genoa/8 and also
21 Sichuan/418. However, the results here suggest a two-
22 fold reduction for the Sichuan/346 strain.
23 You can take the overheads off again,
24 please. This slide shows the antigens used for the B
25 component of the serologic testing. These antigens

1 include representative strains for both of the B
2 lineages that are circulating and include strains that
3 are related to the B/Harbin/7/94 current vaccine
4 strain and also similar to the B/Victoria/287-like
5 strains.
6 Back to the overheads, page 13. This
7 overhead shows results that were obtained in September
8 of 1998 using a panel of sera from adults in
9 Australia. The data are from the WHO Flu Center in
10 Melbourne at the top and CBER at the bottom, and the
11 data again demonstrate that the current vaccine strain
12 produces antibodies that cross-react reasonably well
13 with the newer B/Harbin-like strain, which here is the
14 B/Romania/318/98, which I think I remember was in the
15 cluster that Nancy indicated was more like the
16 Beijing/184/93. But the antibody titers are extremely
17 low against the newer B/Victoria-like strains such as
18 B/Beijing/243/97 and B/Shangdong/7/97. And actually,
19 this is very consistent with the experience that we
20 have been noting over the past several years for the
21 B/Victoria-like strains.
22 We can skip page 14 and go to page 15.
23 This overhead will show the results that were obtained
24 in January of 1999 using a panel of sera from the
25 United States with data from CDC at the top and CBER

1 at the bottom. They demonstrate that the current
2 vaccine strain again produces antibodies that cross
3 reasonably well with other new Harbin-like strains
4 such as B/Foshan/396 and B/Delaware/2/98. And again,
5 the antibody titers are very low for the B/Victoria-
6 like strain, the B/Shangdong/7/97.
7 Although the reduction is not as great as
8 two-fold, the results for the B/Singapore/35 strain
9 are on the low side for a B/Harbin-like virus. I
10 don't know if I have got both slides and overheads for
11 this. Would you turn the overhead off and I will see
12 if the slide projects here. Actually, I think, let's
13 put the overhead up. I think that will probably show
14 up better. This should be H1N1 viruses.
15 So to try to summarize all of the
16 information, of which I have given you some flavor
17 from the bits that have been presented so far, we have
18 taken the information and converted it so that we can
19 show you the frequency with which we found new test
20 antigens giving a 50 percent or greater reduction in
21 geometric mean titer compared to the current vaccine
22 strain. We use 50 percent because that is a fairly
23 dramatic difference for a geometric mean titer. A
24 two-fold reduction in geometric mean titers is
25 actually fairly marked. The data included in this

1 table are only for those antigens where more than one
2 lab is tested. So I am not showing you -- there is
3 more information in the specific handouts from each of
4 the places, but I have tried to summarize where we
5 have some level of confirmation from other
6 laboratories.

7 For the first four labs that are indicated
8 here, the serum panels were shared. And again, for
9 the H1N1, the Australian serum panels were excluded
10 because the vaccine antigen was not A/Beijing/262/95.
11 The top two antigens on this panel are the
12 Beijing/262-like strains and the lower three are the
13 Bayern/7-like strains.

14 Paying attention for the moment just to
15 the total results here -- the column that says total
16 -- the data for Shanghai/2, A/Johannesburg and
17 A/Moscow uniformly indicate that there is no reduction
18 in the geometric mean titer. However, the results for
19 the Hong Kong/4847 and the Michigan/24 are somewhat
20 mixed, with some laboratories showing some reduction
21 and some not, and even differences within the
22 laboratory for the particular virus. However, on the
23 average, the reductions for all of these strains are
24 less than 50 percent, which is shown in that last
25 column, trying to take the average of all the

1 serologic panels that were examined and showing you a
2 range of the differences that were found.
3 The next overhead. This should be a
4 summary for the H3N2 viruses. There is a typo at the
5 bottom that says something about Beijing/262, so
6 please ignore that. All of these strains are Sydney-
7 like and generally the A/Chile/37/92 and the
8 Sichuan/418 viruses appear to be well inhibited by
9 sera from person who were immunized with the current
10 vaccines. However, the Johannesburg/3 virus and the
11 Sichuan/346, which represent Sydney-like variants, did
12 not appear to be well inhibited, and there is more
13 uniformity in finding reduced antibody titers compared
14 to the vaccine strain for the different laboratories
15 that are testing. On average, the responses to both
16 of these viruses are reduced by more than 50 percent
17 in the labs testing, and I think that gives some
18 indication of the degree of divergence.
19 The next one, please? This slide shows
20 summary data for the Influenza B viruses. And most of
21 these panels are done with ether-treated antigen.
22 Where that information was available, if we didn't
23 have that information, I have included information for
24 non-ether-treated antigen. And in some instances,
25 there is not really a difference in the relative

1 proportion of the antibodies, but sometimes there is
2 as Nancy was pointing out. There are differences in
3 specificity and sensitivity for those different ways
4 of looking at the Influenza B serologies. So this
5 data shows -- this slide shows the summary data for
6 the B viruses, and the top two strains are B/Victoria-
7 like, and the bottom three strains are like the
8 vaccine strain, which is B/Harbin. And generally the
9 B/Harbin-like viruses appear to be well inhibited by
10 the sera from persons who are immunized with the
11 current vaccines, although there is a suggestion that
12 some antigenic drift is occurring. And I would point
13 out that the data for B/Delaware here, where there
14 seemed to be more than a 50 percent reduction, it
15 actually is somewhat remarkable with non-ether-treated
16 antigen to see differences. So I think that is
17 something that we need to take into account.
18 As would be expected, the B/Victoria
19 viruses do not appear to be well inhibited and there
20 is more uniformity in finding the reduced antibody
21 titers for these strains as compared to the vaccine.
22 And on average, the responses to both of the
23 B/Victoria-like strains is a bigger difference than 50
24 percent in all of the laboratories.
25 We can turn that off and turn the lights

1 back on. So in summary, the vaccines used for the
2 clinical studies appeared to be immunogenic in the
3 populations that were tested. For all three of the
4 vaccine component strains, there is some evidence of
5 antigenic drift, which is possibly most notable for
6 the H3N2 virus strains representing drift variance of
7 the current vaccine strain. And as we have known for
8 several years now, the B/Victoria lineage is
9 persisting and the current vaccines may be very
10 limited in their protection against those strains.
11 So I will stop there. I hope we are
12 getting back on time. And if there are questions or
13 comments, I will take them.
14 CHAIRPERSON FERRIERI: Thanks, Roland.
15 Yes, Dr. Estes?
16 DR. ESTES: Mary Estes. Would you clarify
17 for me the importance of the ether treatment? I don't
18 remember hearing about this before. Apparently it is
19 only used for the B viruses, and is this something
20 that is new this year or just put that into
21 perspective?
22 DR. LEVANDOWSKI: No, it is not new. It
23 is something that has been done for many, many years.
24 There are a number of studies that suggest that
25 serologic responses for Influenza B viruses are more

1 comparable to what would be found for -- 2 hemagglutination inhibition assays for B viruses with 3 ether-treated antigen are more comparable to 4 neutralizing antibody. The correlation is much 5 better. And I don't think that has been entirely 6 explained on a chemical basis, but it is something 7 that has been noted in many different studies. My 8 personal feeling is that I would find the ether- 9 treated antigen hemagglutination inhibition titers to 10 be a better representation of what protective effect 11 there might be from the vaccine. But I don't think 12 that we should ignore the titers that we get 13 otherwise. What usually happens is that it is a 14 flatter looking curve. It is more difficult to show 15 a difference at all if there is one there with the 16 non-ether-treated antigen. And of course what we are 17 trying to do here with both the ferret sera and the 18 human sera is to see can we detect differences between 19 -- immunologic differences between the current vaccine 20 strain and the newer strains. So our comparison is 21 not really to show that these vaccines are being 22 protective, but really to have that comparative 23 difference between the strains. And we think maybe we 24 equalize things with ether treatment for B.

25 CHAIRPERSON FERRIERI: Dr. Daum and then

1 Dr. Greenberg.

2 DR. DAUM: Just a quick question, I think.

3 Bob Daum, Chicago. What does the ether do?

4 DR. LEVANDOWSKI: Ether disrupts the virus

5 by dissolving the lipid envelope. Influenza viruses

6 are lipid envelope viruses and it is one way to do it.

7 It is actually used in manufacturing the vaccines to

8 make the subunit vaccine. One manufacturer uses ether

9 as their solvent, whereas others may use detergents,

10 but it is the same concept.

11 DR. DAUM: Why only B?

12 DR. LEVANDOWSKI: There are some studies

13 with Influenza A as well, but I don't believe -- and

14 somebody could correct me if I am wrong -- but they

15 don't seem to show as much difference as Influenza B.

16 We talk about these two viruses. They both are

17 obviously orthomyxo viruses, but they are structurally

18 somewhat different and physiologically or

19 pathophysiologically, they may be somewhat different.

20 I don't think I really want to get too deeply into

21 that, but there are differences between Influenza A

22 and Influenza B, and we call them that because of the

23 disease that they produce, which is the febrile

24 respiratory illness, but they probably are somewhat

25 different.

1 CHAIRPERSON FERRIERI: Harry?

2 DR. GREENBERG: Harry Greenberg, Stanford.
3 The lack of cross-reactivity between Harbin and
4 Victoria on the B side, that has been the same for
5 several years, correct?

6 DR. LEVANDOWSKI: Yes.

7 DR. GREENBERG: Is there anything
8 different now, this year, for B than there was last
9 year for B? I mean last year the same thing, that
10 Victoria was different but limited basically to China.

11 DR. LEVANDOWSKI: Right. Well, in terms
12 of the serologic results, I would say I guess that
13 there is some evidence of antigenic drift going on in
14 the B/Harbin-like strains that I think we are
15 detecting in these serologic studies as well. In past
16 years, when we have done comparisons between the
17 vaccine strain and the non-vaccine strains, often the
18 antibody titers have been even higher against the non-
19 vaccine strains. So I think we are seeing some
20 reductions here, and I think that corresponds with
21 what was being described earlier for characterization
22 of the strains. There are antigenic differences that
23 can be distinguished with the ferret antisera.

24 DR. GREENBERG: But that is related to
25 other Harbin-like strains, not to the Victoria-like

1 strains, right?

2 DR. LEVANDOWSKI: Right. Well, those are
3 two different issues. There are two lineages of
4 Influenza B that are cocirculating. But amongst the
5 B/Harbin strains, which are predominant everywhere, it
6 appears that there are some antigenic changes.

7 DR. GREENBERG: I was asking on the other
8 side, vis-a-vis the Victoria things are pretty much
9 status quo?

10 DR. LEVANDOWSKI: I don't think we can
11 gauge that from the serologic studies because we are
12 not immunizing -- at least -- and I am not going to
13 discuss any of the studies that Nancy was eluding to.
14 But I don't know if John Wood or Nancy Cox might have
15 something to add about the experimental vaccine
16 studies that have been done to try to -- if that in
17 some way indicates anything about what is happening
18 with the B/Victoria-like strains. I mean obviously
19 they have antigenic changes going on also that are
20 clearly detected by the ferret antisera, but I don't
21 think I can answer the question you are asking about
22 serologic responses in people.

23 CHAIRPERSON FERRIERI: Yes, Dr. Edwards.
24 And then this will be the last question.

25 DR. EDWARDS: In the past you have had

1 some pediatric serology. Is it felt that the
2 pediatric responses so clearly mimic the adult
3 responses that they don't provide additional
4 information, or do you have that information and it is
5 not here?
6 DR. LEVANDOWSKI: We would dearly love to
7 have that information, but it is not very easy to find
8 someone who can immunize children and provide the sera
9 in a timely manner so that we can use that. We would
10 very much like to have access to those kind of sera.
11 As you know, we had a contract at FDA to support that
12 for a number of years, and we are no longer able to do
13 that. And we have been searching to find others who
14 could help out in that regard. We do get some support
15 from Vanderbilt and from the University of Rochester
16 in providing some of these sera, but we just have not
17 been extremely successful in finding a good, stable
18 place to make it possible. Particularly for the very
19 young children. It is a little easier for older
20 children. But for the very young children who give a
21 response that is not influenced by any previous
22 exposure, we have a very hard time finding those.
23 CHAIRPERSON FERRIERI: Thanks, Roland.
24 Our next speaker is Dr. Offringa from FDA, who will
25 speak on the availability of strains and reagents. My

1 voice is altered due to a respiratory infection this
2 week that was flu-like, but having been vaccinated and
3 being adamantly attached to the protectiveness of flu
4 vaccine, I do not believe that I have an influenza
5 illness. I prefer to think it was adenovirus -- maybe.
6 RSV or paraflu, we have lots of affinities here.
7 DR. OFFRINGA: Good morning. I am going
8 to give a brief overview of the status of candidate
9 vaccine strains and potency reagents. I will begin
10 with the Influenza B vaccine strains.
11 The current B vaccine strain is
12 B/Harbin/794. As you have heard earlier, the name
13 strain is B/Beijing/184/93. Currently, reassortants
14 are not made for B strains, and Harbin is used for
15 vaccine production because it is antigenically similar
16 to the Beijing strain but has better growth
17 characteristics. As you can see, it is a moderate to
18 high yield growth strain. There are two candidate
19 strains listed, B/Shangdong/7/97, which is in the
20 B/Victoria lineage, and B/Foshan/396/98, which is a
21 B/Harbin variant. The B/Shangdong/7/97 strain has
22 been sent to manufacturers and has a moderate growth
23 character. We are preparing to send the B/Foshan
24 strain early next week, and as Dr. Cox mentioned
25 earlier, there are also some other B variants which

1 may be better candidates, such as B/Romania/318/98 and
2 B/Bucharest/311/98. These strains will be included in
3 the shipments as well.
4 If I could move on to the H1N1's. The
5 current vaccine strain is A/Beijing/262/95. The
6 reassortant being used for vaccine production is X127,
7 which has a high yield growth character. At this
8 time, we have no candidate strains identified for
9 H1N1. As Dr. Cox mentioned earlier, there have been
10 a few Beijing low reactors isolated, but there is no
11 general trend in any particular antigenic direction.
12 Influenza H3N2, the current vaccine strain
13 is A/Sydney/5/97. There are two reassortants being
14 used for this, IVR-108 and RESVIR-13. Both of these
15 reassortants have a median to high yield growth
16 character. I have two candidate strains listed,
17 A/Sichuan/346/98 and A/Sichuan/418/98. Both of these
18 are Sydney-like variants and have been sent out to the
19 manufacturers. Dr. Kilbourne's laboratory and our lab
20 are both currently working on reassortants for these
21 viruses, but at this time none have yet been isolated.
22 The wild type strains have a moderate growth
23 character.
24 For the potency reagents -- for the
25 current vaccine strains, reference antigens and

1 antisera used for the potency testing are available
2 for all strains, B/Harbin/794, A/Beijing/262/95, and
3 A/Sydney/5/97. Based on previous years experience, if
4 a new strain is chosen or new strains are chosen,
5 reagents would be available in May at the earliest.
6 Are there any questions?
7 CHAIRPERSON FERRIERI: Committee members?
8 Now is your chance.
9 DR. OFFRINGA: Thank you.
10 CHAIRPERSON FERRIERI: Thanks very much.
11 We will move on then to Dr. Slusaw from the
12 Pharmaceutical Research and Manufacturing Association,
13 and he will present on behalf of the manufacturers.
14 Apparently the current pharmaceutical firms that will
15 be making vaccine are Wyeth, Pasteur Merrieux
16 Connaught, Evans in the UK, and Parke Dale, related to
17 Parke Davis. Pardon me? The other Rochester. Okay.
18 DR. SLUSAW: Thank you. Dr. Levandowski
19 had asked me to give a brief discussion of some of the
20 logistics and other timing issues that are of concern
21 to the manufacturers. Kind of an explanation of why
22 the manufacturers tend to look perplexed at various
23 times during the strain selection process. So I
24 thought I would recap some of that information for
25 you.

1 Although there are a lot of critical
2 pieces of the puzzle that have to fall into place in
3 order to successfully manufacture the number of
4 vaccine doses that we have seen have been distributed
5 and released over the last few years, probably the
6 three most important pieces are first of all insuring
7 that a supply of embryonated eggs is available to
8 produce the vaccine. And this is a process that
9 really begins far in advance, actually a year in
10 advance of the current vaccine manufacturing cycle.
11 And the critical part of the egg supply is not only
12 making sure they are there, but once the supply is
13 started and that flow of eggs is turned on, it is
14 impossible to turn off. And right now, the
15 manufacturers in the U.S. probably have about half a
16 million eggs per day available that need to be made
17 into some sort of monovalent concentrates.
18 Strain selection is also very critical,
19 the activity that we are doing here today. And there
20 are two components to that, both the timing of the
21 strain selection as well as having suitable viruses
22 with high growth characteristics, particularly the
23 availability of high growth reassortants of the A
24 strains, and having vaccine strains that we can purify
25 and inactivate in our manufacturing processes.

1 And then the third important piece of the
2 puzzle is the availability of the potency test
3 reagents for the SRID test. For new strains that are
4 selected each year, until these reagents are produced
5 and standardized, we really don't have a good handle
6 on how much monovalent we are manufacturing, as well
7 as we can't formulate trivalent vaccine until these
8 reagents are made.
9 I would just like to run over a little
10 overview of the time table that Drs. Levandowski and
11 Cox eluded to in their presentations. As I mentioned,
12 the birds to ensure the egg supply have to be ordered
13 about a year in advance. Each year in October or
14 November, those birds are actually moved into the
15 houses and soon begin producing eggs. Also in this
16 time frame, we begin receiving candidate seed viruses
17 from the FDA and from CDC, potential viruses that may
18 be considered for inclusion in the next year's vaccine
19 formulate. And happening kind of in parallel with
20 this is the work on high growth reassortants of some
21 of these viruses that look like they may be serious
22 candidates for the formula.
23 Manufacturing the first monovalents
24 generally begins -- I think all the manufacturers
25 currently are starting in January and may actually be

1 starting before the official strain selection, taking
2 our best guess based on the scientific and
3 surveillance data that is available at the time and
4 beginning production with one of the strains from the
5 previous vaccine year. Of course with some of the
6 high growth viruses we have these last few years, we
7 can't continue to produce a single strain for a very
8 long time without risking overproducing the number of
9 doses of that strain. So we need to switch to the
10 second and third strains in fairly short order. And
11 typically we would like to have, if possible, the
12 second virus strain in February and then the third
13 virus strain for the vaccine formula sometime in
14 March. After some discussion actually in years where
15 it is suitable to make the decision, we would like to
16 see perhaps a provisional recommendation for a second
17 strain pending the decisions that come out of the WHO
18 strain selection meeting in February.
19 Monovalent concentrate production
20 continues for about a 7 or 8-month period from January
21 through August or so, and for new vaccine strains that
22 have been included, potency test reagent production
23 and standardization of those reagents occurs in
24 parallel and is usually completed by about May or
25 June. We generally target to manufacture the first

1 bulk vaccine about the first week of June, and that
2 material is filled and released and the license is
3 typically issued about the first week of July, and the
4 manufacturers begin distribution of the final product
5 at that time. And the final product is distributed in
6 the July through about October time frame, typically
7 anything available later than that, the uptake by our
8 customers and distributors will be very low and much
9 of that material is returned. So we have a fairly
10 narrow time window where all the material that we
11 manufacture has to be sent to our customers.
12 So just to recap from the previous
13 overhead some of the more critical timing issues that
14 concern the logistics and issues that the
15 manufacturers have to consider. There is about a year
16 lead time for the egg supply. We have good systems in
17 place for that and it is generally not a concern. We
18 know we need to make vaccine each year. The problem
19 is turning them off if we have to wait for strain
20 selection. About 8 to 10 weeks are generally required
21 to make and analyze a high growth reassortant virus.
22 And I think as Drs. Kilbourne and Levandowski will
23 attest, that is with a good strain. Certainly it can
24 take much longer than that depending on any technical
25 problems that may be encountered.

1 Once we have a suitable seed virus in the
2 manufacturer's hands, it takes at least about a month
3 and sometimes longer depending on the passage level
4 and so on to produce a working seed that is suitable
5 for use in production. About 10 to 12 weeks are
6 required from beginning to end to manufacture and
7 standardize potency test reagents. And from start to
8 finish, it is about three to four months,
9 approximately 15 weeks, from the inoculation of a
10 monovalent concentrate through the production of a
11 final container of a trivalent vaccine. So one of the
12 issues I would like to emphasize is that although from
13 a scientific standpoint it is often useful to be able
14 to wait for additional data on strain selection, any
15 candidate strains that may be identified say a month
16 from now also have these lead times for reassortant
17 production and working seed production that must be
18 added onto them. So if a candidate strain selection is
19 made very late, there may be several months of
20 activity that is needed to be done by the
21 manufacturers before those strains can actually be
22 brought into production.
23 CHAIRPERSON FERRIERI: Thank you very
24 much. Questions for Dr. Slusaw? Well, we will be
25 finishing up the morning session then with options for

1 strain selection, Dr. Nancy Cox from CDC.
2 DR. COX: Okay. So I will try very hard
3 to summarize what we have been hearing today and lay
4 out the options in some kind of a logical framework.
5 I just wanted to remind you that global data were
6 reviewed extensively in September, and the WHO
7 recommendations at that time for the Southern
8 Hemisphere's 1999 season, which will be coming very
9 soon, are for an A/Sydney-like H3N2 component, a
10 Beijing/262 H1N1-like component, and
11 A/Beijing/184/93-like virus, which of course is the
12 B/Harbin/7/94 in all the countries using vaccine.
13 I think we need to review the need for a
14 trivalent vaccine. We do have Influenza A H1N1
15 viruses continuing to circulate worldwide. There are
16 two distinct antigenic and genetic groups, as you have
17 seen. Although they have been isolated rarely during
18 the past four months, if we look back over the past
19 couple of years, we see that there have been outbreaks
20 caused by H1N1 viruses in different areas of the
21 world.
22 Influenza H3N2 viruses have continued to
23 circulate worldwide in large numbers and to cause
24 significant morbidity and mortality. They have
25 predominated during the past year if you look

1 globally, and of course the Sydney variant is the
2 culprit we are talking about.
3 Influenza B viruses also continue to
4 circulate worldwide. There are two distinct antigenic
5 and genetic groups, as you have heard, and they have
6 been isolated fairly frequently in North America and
7 Europe.
8 For H1N1 viruses, sporadic isolates have
9 been reported in Asia, Japan, Thailand and China; in
10 Europe, Finland, Italy and Spain; in the United
11 States; and a single strain in South Africa. Overall,
12 activity has been low, as I just said.
13 There has been antigenic variation noted
14 among the Asian A/Beijing/262/95-like strains, yet we
15 have no clear genetic correlates for these antigenic
16 changes. And when we have taken these strains that
17 have the lower reactivity to the Beijing/262 antiserum
18 and put those strains into ferrets, we found that the
19 antiserum produced really did not either clearly
20 identify these viruses as a group or cross react well.
21 The current H1N1 component induces broadly
22 cross-reactive antibodies versus the Bayern-like
23 strains, and this particular vaccine component was
24 updated last year.
25 So our options -- I will put all the

1 options up. Of course, I have sort of put them in the
2 order of -- well, in some sort of order. You can
3 judge for yourselves. But we could retain a
4 Beijing/262/95 strain in the vaccine. We know that it
5 induces broadly cross-reactive antibodies. It has
6 known properties.
7 We could update to a more recent low
8 reacting virus on the Beijing/262 lineage, but we
9 really don't have a good candidate. And there have
10 been so few isolates, we would be stabbing in the dark
11 to some extent.
12 There is another alternative, which I
13 think is not a very good one, but of course I should
14 list it. We could update the H1N1 component and go
15 back to the other lineage, that is the Bayern lineage.
16 But we already know that viruses from that lineage
17 have not induced broadly cross-reacting antibody to
18 the Beijing/262 lineage strains.
19 Okay. So now I will summarize what we
20 have seen today regarding Influenza A H3N2 viruses.
21 The Sydney strains have circulated worldwide, both
22 during the 1997/98 influenza seasons, the 1998 season
23 in the Southern Hemisphere, and this season that we
24 are just moving through. There have been widespread
25 outbreaks and epidemics during the 1998/99 season in

1 Asia, particularly in China. Hong Kong has also had
2 activity. Japan and Korea have also reported H3N2
3 activity. A number of countries -- a large number of
4 countries in Europe have isolated H3N2 viruses in
5 association with influenza-like activity and Canada
6 and the U.S. have had predominantly H3N2.
7 So there has been extensive H3N2 activity
8 overall. There is some antigenic heterogeneity
9 observed, although the majority of the strains clearly
10 are still Sydney-like. We have the A/Sichuan/436/98
11 strain, which is the best characterized of the low
12 reactors, and we have seen that in human serologic
13 tests that the post-vaccination GMTs to this strain
14 are reduced by about 50 percent in some tests in some
15 laboratories.
16 Now we have a number -- actually a fair
17 number or quite a large number of additional strains
18 to analyze from China, and we have noted variation
19 among those. I just mentioned the reduced post-
20 vaccine response to the Sichuan strains, and we did a
21 rough count before we received 7 more packages of
22 virus yesterday, and we had 200 H3N2 strains that are
23 moving through the system. We did update the vaccine
24 component last year from Nanching to Sydney.
25 So we could retain the A/Sydney vaccine

1 component. Most current strains are Sydney-like,
2 although we are seeing evidence that they are changing
3 a bit. The Sydney high growth reassortant has known
4 characteristics, and that is always an advantage. Or
5 we can update the Sydney component. And the reasons
6 that we might think that this would be necessary to do
7 is that this variant has circulated widely for two
8 years. It seems unlikely based on past patterns that
9 we have observed with H3N2 viruses that we would have
10 a third year of circulation of the same strain,
11 particularly given the intensity of circulation.
12 The H3N2 viruses certainly are associated
13 with more severe disease, including hospitalization
14 and death. And we have detected this particular
15 variant. We have, as I mentioned before, many more
16 viruses to test from Asia, the U.S. and Europe, and I
17 think it would be worth our while to do some
18 additional serologic analyses with the human serum
19 using some of these recent strains from China.
20 We will move on to Influenza B viruses.
21 Of course, we have two lineages, the B/Victoria and
22 the B/Yamagata groups that are co-circulating -- B/Vic
23 in Asia only. Outbreaks and sporadic activity have
24 been reported this season in Asia. In Europe and the
25 countries that I have asterisked are those in which B

1 has really been the predominant strain. In the United
2 States, approximately 18 percent of our isolates are
3 type B, and there was a B virus from Chile.
4 Influenza B activity overall has been
5 fairly moderate worldwide. We have noted that over
6 half of the U.S. viruses that we have characterized
7 that were isolated during this season are four-fold or
8 greater reduced in titer with the B-Harbin serum. And
9 similar results have been seen in Europe.
10 Now the current B component introduces
11 antibody that cross reacts with many non B/Vic-like
12 viruses in human serology when we are using ether-
13 treated antigen. This vaccine component was last
14 updated in 1995. So we haven't changed our B
15 component for a number of years. And the last time we
16 changed the B component, we were seeing a similar
17 pattern with our ferret antiserum to the vaccine
18 strain, B Panama, not covering the recently isolated
19 strains in Europe and North America. So there is sort
20 of a parallel from the last time that we updated the
21 B component that I can clearly see.
22 So what are our options? We can retain
23 the B/Harbin component. It does appear to cover most
24 strains tested in our human serologies. This strain
25 has known characteristics. It grows well for the

1 manufacturers and so on.

2 Or we could update the B/Harbin component
3 to another virus, a more recent strain on the same
4 lineage, and we would do this because of the reduced
5 ability of the Harbin ferret serum to inhibit the
6 current strains. We have a number of additional
7 strains to test, and in particular I think it would be
8 useful to assess this B/Georgia virus and the other
9 strains that Dan mentioned.

10 The other possibility that I must put on
11 the table is that we could update the B/Harbin
12 component to a virus on the B/Vic lineage. We have
13 discussed this several times in the past, but as you
14 have seen, the B/Victoria viruses remain more or less
15 confined to circulation in Asia and you've seen what
16 the WHO recommendations were for the Southern
17 Hemisphere. But we always need to keep in mind that
18 there is a growing susceptible population to viruses
19 on this particular lineage. Thank you.

20 CHAIRPERSON FERRIERI: Thank you, Nancy.
21 Are there questions for Dr. Cox? Yes, Dr. Breiman?

22 DR. BREIMAN: Nancy, could I ask a few
23 questions that might clarify things for me? The data
24 for me at least are a little dense and getting through
25 it is, for me, a little challenging. I just want to

1 make sure I understand a couple of things. One, when
2 you -- is it fair to say that if you look at the
3 Sichuan/418 antisera that it reacted as well with
4 Sydney/05/97 as the homologous antisera? That is the
5 way your table reads. So it actually does as well as
6 Sydney?

7 DR. COX: So you are looking at the
8 reciprocal?

9 DR. BREIMAN: Yes.

10 DR. COX: Yes.

11 DR. BREIMAN: Okay. And then you do get
12 with the Sichuan/418 added reactivity against these
13 new SI strains that you have mentioned like the 417,
14 the 420 and the 320.

15 DR. COX: Yes.

16 DR. BREIMAN: That wasn't there before
17 with the Sydney. And then there is only one dilution
18 difference from all of the other antigens when you
19 compare the two antisera except for that
20 A/Okinawa/289, which I don't know if you felt that
21 that was clinically important. The A/Okinawa/289
22 looked like it substantially reacted differently with
23 the Sydney antisera versus the Sichuan/418 antisera.

24 DR. COX: I am sorry, which one?

25 DR. BREIMAN: It is the A/Okinawa/289/98.

1 It had a 2560 titer against Sydney and 640 against
2 SI/418. Is that -- am I --
3 DR. COX: Yes. I mean, that is just a
4 strain that is well inhibited by all the antisera.
5 DR. BREIMAN: Yes. So I mean -- I guess
6 what I am asking is is that a meaningful difference,
7 the fact that there was 2560 for one and 640 for the
8 other?
9 DR. COX: No. No.
10 DR. BREIMAN: So it looks based on that
11 table that you provided that the -- you are only
12 gaining by looking at the Sichuan/418 -- as an
13 example, you are only gaining additional reactivity
14 against these new strains that you identified this
15 year, the SI/417 and 420.
16 DR. COX: That is right, Rob. And when we
17 look at these patterns year after year, what we are
18 looking for is where the virus is going. You know, we
19 are trying to see where is it going to be next year at
20 this time. And it is a really difficult -- it is
21 tremendously challenging, first of all, to get through
22 all the data. And then to try to understand what you
23 are seeing this year within the context of what you
24 have seen in other years. So if you are just looking
25 at the data that you are seeing this year, you would

1 say, well, it is a no-brainer. Stay with the Sydney.
2 The majority of the strains are Sydney. You are not
3 going to gain very much. But if we look over the past
4 10 years, you see that it is unlikely -- it is not
5 impossible because influenza viruses behave as they
6 will, not as we wish they would. But it is unlikely
7 that there are going to be Sydney-like viruses
8 circulating next year. So we need to try to see what
9 -- so we are looking for a virus that is going to give
10 us an advantage next year. So we are looking for the
11 patterns and so on. So --
12 DR. BREIMAN: I guess I was just wanting
13 to confirm that that was my impression from looking at
14 your table. That it looked like you would actually
15 only gain by switching to say the Sichuan/418 type of
16 antisera. I mean a vaccine that would give you that
17 kind of coverage.
18 DR. COX: That is right.
19 CHAIRPERSON FERRIERI: Dr. Greenberg? We
20 don't have -- I think everyone knows where we are from
21 now. So we don't have to announce our institutions.
22 DR. GREENBERG: Harry Greenberg. If a
23 decision is made to move to a new H3N2, a Sichuan-
24 like, what is the time table to pick the ideal? You
25 have a whole bunch of new viruses that have just come

1 in and you alluded to the fact that you wanted to test
2 some more of these viruses from China. How much -- I
3 mean, I don't have a real feeling for what that drill
4 is. I just heard from the manufacturers how important
5 it is to identify the specific strain, and I just
6 wondered where one would be in picking a new H3N2
7 strain, if that was the decision made.

8 DR. COX: We face this dilemma every
9 single year. And one of the disadvantages -- we are
10 really operating at a bit of a disadvantage. I am
11 sort of answering in an indirect way. We are
12 operating at a disadvantage this year because
13 influenza activity in the United States and Europe was
14 a bit late getting going. And so the strains weren't
15 coming in as quickly. We are getting a tremendous
16 number in now, and likewise there was a delay in
17 getting the strains from China. So that really puts a
18 lot of pressure on us to get these strains out quickly
19 -- to get them analyzed as quickly as possible and to
20 get them out quickly. Now there are a variety of
21 shortcuts that are taken when we find ourselves in
22 this kind of a situation. Sometimes we have often
23 funneled viruses through FDA and they are sent out
24 through FDA to the manufacturers at exactly the same
25 time so that they get exactly the same passage level

1 and so on. If we are really pushed, we can send the
2 strains directly to the manufacturers. In some years,
3 it has been impossible to get a high growth
4 reassortant, so the manufacturers were forced and
5 actually able quite successfully to use Influenza A
6 strains that weren't high growth reassortants. I thin
7 the last time that occurred was in 1986 with the
8 Taiwan strain. So it is a bit removed from our
9 current experience. But there are a whole variety of
10 things that can be done if they need to be done, and
11 we just try to expedite things the best way we can.
12 I know that both Ed Kilbourne and Roland Levandowski's
13 lab are working very hard on some high growth
14 reassortants now and we will just do everything we can
15 to expedite things.
16 CHAIRPERSON FERRIERI: Dr. Daum and then
17 Dr. Hall and then we are going to quit for lunch.
18 DR. DAUM: Bob Daum. I am intrigued by
19 this observation that the B/Victoria-like viruses
20 remain in a small part of the world and despite
21 several people admonishing us to listen to the fact
22 that there is a growing number of susceptibles
23 elsewhere. And I would like some clarification from
24 influenza experts as to whether this kind of behavior
25 is typical of influenza viruses and whether we should

1 really consider that issue in advising about
2 formulating the B vaccine.
3 DR. COX: We have been watching the
4 B/Victoria viruses circulate in China and a few other
5 countries in Asia exclusively for -- since about 1991.
6 I think in the 1990/91 season, there were some
7 B/Victoria-like strains isolated in Europe. And then
8 they just died out. But since then, those viruses
9 have continued to circulate and evolve in China. So
10 we would wait, I think, to see their spread to other
11 continents as we did for the H1N1 A/Beijing/262/95-
12 like strains before we got terribly excited about
13 putting them in. But nevertheless, we have to keep in
14 mind that these viruses are evolving and our
15 population is changing.
16 CHAIRPERSON FERRIERI: Dr. Hall?
17 DR. HALL: Caroline Hall. Nancy, from --
18 obviously from the H3N2, it would be ideal to have a
19 candidate that gave very good reactivity to both the
20 Sichuan and the Sydney components. And a couple of
21 your isolates, at least from the serology that you
22 have given us, do look like they can provide that.
23 The recent ones from Japan, et cetera. Even the
24 Alaska. Do we know anything about those in terms of
25 growth or potential candidate vaccines?

1 DR. COX: The Fukuoka is a cell isolate.
2 And you are talking about the Alaska -- yes, those are
3 all cell isolates.
4 DR. HALL: Is the Okinawa also a cell
5 isolate?
6 DR. COX: Yes. They are all isolated in
7 NBCK cells.
8 DR. HALL: Have they been tried?
9 DR. COX: No, no. We haven't pursued
10 those -- getting egg isolates. And perhaps Dr. Nerome
11 would have some egg isolates available in Japan. I am
12 not sure just what he has available.
13 DR. HALL: But they do look like they give
14 broader reactivity.
15 DR. COX: They may. The real test is the
16 cross -- once you have your ferret antiserum to that
17 particular virus. But, yes, it is possible that one
18 of those would.
19 CHAIRPERSON FERRIERI: Related to that,
20 though, Nancy, am I misinterpreting data that would
21 suggest that the current antibody to A/Sydney does
22 cross react with Sichuan/418/98 in some of the
23 vaccinees at least from Europe -- some of the data
24 from Europe and Australia?
25 DR. COX: Yes. There is a much greater

1 difference in reactivity in the human serologic
2 studies with the Sichuan/436 strain than the
3 Sichuan/418 strain.
4 CHAIRPERSON FERRIERI: Well, let's break
5 now. Thank you very much, Nancy. We have an hour and
6 15 minutes this afternoon approximately for further
7 discussion and recommendation to CBER on the strain.
8 So we will reconvene at 1:40.
9 (Whereupon, at 12:38 p.m., the meeting was
10 adjourned for lunch to reconvene this same day at 1:42
11 p.m.)
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1 A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

2 1:42 p.m.

3 CHAIRPERSON FERRIERI: Well, I am sure
4 that others will soon join us, but this is an
5 opportunity to have a very thorough discussion of the
6 issues that were presented today and coming up with
7 recommendations for next year's vaccine. Ms. Cole,
8 are you with us? Ms. Cole? Rebecca? I want her to
9 be tuned in with us before we get going.

10 MS. CHERRY: Rebecca, are you there?

11 MS. COLE: Yes, Nancy, I am here.

12 CHAIRPERSON FERRIERI: Can you hear me
13 too?

14 MS. COLE: Yes.

15 CHAIRPERSON FERRIERI: I thought I would
16 start by seeing if there were any members of the
17 committee who think we should take the path of least
18 resistance and just keep all the current components.
19 Do I have a show of hands? Any hands? None.
20 Rebecca, how do you feel about it?

21 MS. COLE: No.

22 CHAIRPERSON FERRIERI: Very good. We are
23 all in consensus then. I am sorry, Dr. Slusaw. I am
24 sure you are not too surprised, though. Well, let's
25 start discussing then perhaps. I want to do it one by

1 one. But before we start, is there anyone who feels
2 there hasn't been enough information or you feel there
3 is one last important question you must ask that would
4 influence your decision making? Yes, Dr. Hall?
5 DR. HALL: I almost hesitate to mention
6 this, but I just wondered has there ever been a
7 consideration of adding a fourth component so that if
8 you could not get the ideal virus to cross react
9 between two, if the fourth component -- if there were
10 two, say, candidates that grew well. So this would
11 mean -- I can't imagine what it means in terms of the
12 manufacturing, but at least in terms of the
13 immunogenicity. If this has ever been a consideration
14 or should be potentially one.
15 CHAIRPERSON FERRIERI: The closest thing
16 to that, perhaps -- and then I will let someone answer
17 it -- is when someone last year who came to the
18 meeting and spoke from the audience proposed a
19 separate vaccine for children, and that wasn't a real
20 popular suggestion for a number of scientific reasons
21 as well. Roland, would you like to address this
22 briefly and then maybe someone from the audience?
23 DR. LEVANDOWSKI: Okay. I will take a
24 stab at it, but I think it is something for the
25 manufacturers to talk about. I think it is a very

1 practical thing. The manufacturers, I guess, go
2 through campaigns for each of the strains, and they
3 only have so many days that they can be in
4 manufacturing with all of the strains. So if there is
5 another component that is added to that, it takes away
6 from that total number of days that can be used to
7 manufacture the vaccine. So if we just thought about
8 it in terms of simple numbers, if three strains take
9 12 months, more strains still take 12 months and so
10 you get that much less of each one of the components
11 as a result and less vaccine. I guess my understanding
12 is that not every day in manufacturing is a day that
13 leads to a product that results in distribution, so
14 there some of that that goes on also. I think it
15 would be possibly feasible for manufacturers to do
16 that, but not under the current understanding of how
17 they are distributing vaccines and how much vaccine is
18 being demanded. There would have to be some give
19 somewhere in the system, either more strains or more
20 vaccine. I guess it is a choice that has to be made.
21 Apart from anything to do with whether it really would
22 add anything to the immunologic responses.
23 CHAIRPERSON FERRIERI: Dr. Sluslaw, do you
24 want to add anything here? While you are deciding, I
25 would like to announce that we are going to have an

1 open public hearing starting immediately now. So if
2 you wish to speak, you can be part of the open public
3 hearing as well as addressing the question. And
4 anyone else who would like to come forward on the
5 subject that we are discussing. Could you please
6 announce your name for the recorders, please?
7 MR. WHITAKER: Charles Whitaker, Pasteur
8 Merieux Connaught. In relation to the discussion of
9 a fourth strain, as we saw earlier, the increased
10 doses of vaccine which have been manufactured over the
11 last few years have been made possible because of
12 anticipation of manufacturers to meet the demands that
13 have been increasing. The problem with introducing
14 the matter of a fourth strain, such as to cover I
15 guess the B situation where we have two circulating
16 families of B viruses -- introducing that complication
17 into the formula produces a ripple down effect for
18 manufacturing which, of course, now in order to meet
19 the current demand, we are producing early in the
20 year, starting in January as described. The problems
21 of an additional strain now of course compounds the
22 addition of the growth time and development, first of
23 all for a suitable candidate seed, and then the
24 production of that fourth strain to be included,
25 including the reagents and other things. So at this

1 time, with the demands that we have on our production
2 facilities to meet current demands with the trivalent,
3 it is really not possible for us to produce the same
4 amount of doses required with the fourth additional
5 strain, and we would certainly want to defer that to
6 a time when the strain might be more widespread than
7 we see it right now. So far the B has been isolated
8 and has not spread further, the B/Victoria-like
9 strains. So we would really take a serious step back,
10 I think, in our production capabilities if we were
11 forced to do that.
12 CHAIRPERSON FERRIERI: Thank you. The
13 issue is much broader than that. FDA may have an
14 official line on this, but this introduces a
15 completely new product with no data to support it
16 immunologically. You would have to launch massive
17 large-scale trials to do this. So it brings forward
18 more complications than the burden that would be
19 imposed on industry. Brief comments now before I hit
20 my agenda. Dr. Eickhoff and Dr. Greenberg?
21 DR. EICKHOFF: Dr. Hall, I think, has
22 raised an interesting point. And if I may reflect for
23 just a little bit. There was a time in history, and
24 Dr. Kilbourne knows this problem much better than I
25 actually, when in the late 1950's and early 1960's the

1 vaccine used -- and it was made primarily for military
2 use rather than civilian, it had five or six
3 components in it. But it does raise the issue of do
4 we need a trivalent vaccine. We have had a trivalent
5 vaccine now since about 1977 or 1978 or thereabouts.
6 So we have the tradition of 21 years behind a
7 trivalent vaccine, simply because all three major
8 virus types were cocirculating.

9 Listening to Dr. Cox present her data this
10 morning, I wondered transiently, why don't we just
11 dump in H1N1. And there are a couple of good reasons
12 why we should not just do that. Even though it is not
13 a big, big cause of morbidity and mortality in adults
14 and there is generally little or no excess mortality
15 with H1N1 strains, but it does disenfranchise
16 children, and that was the genesis of the comment last
17 year. And particularly it disenfranchises high risk
18 children who would probably suffer from such a move on
19 our part. But there is no federal regulation that
20 says we have to have a trivalent vaccine. Indeed we
21 don't. We are operating from the background of
22 tradition.

23 One option -- and I don't think we should
24 spend a whole lot of time discussing this issue today
25 -- but one option to consider at some point when it

1 might be appropriate is should we take out a
2 component, like an H1N1 or a B, and instead substitute
3 two cocirculating H3N2 antigens, for example. I don't
4 think this is the year to do that, but at least we are
5 not limited to an H1N1, H3N2 and B. That is purely
6 tradition.

7 CHAIRPERSON FERRIERI: Harry?

8 DR. GREENBERG: I was simply going to ask
9 historically is there data when two sort of types of
10 either an H1 or H3N2 are given together, does that
11 work as well? Traditionally the trivalent vaccine has
12 been three different viruses basically. But if you
13 made it two different with two sort of subtypes, do
14 you get appropriate immune responses? For example, if
15 you had two B's in this current vaccine, would you get
16 a good response to both of them? Is that known?

17 DR. KILBOURNE: No, it is not known. And
18 I thought Ted was going the other way with his
19 comments of the early multiple virus vaccine. Because
20 one concern at that time came up when we were
21 challenged with pandemic viruses and we still wanted
22 to be stuck with a multi-component vaccine at that
23 time. It was a matter of antigenic competition, which
24 is a real business. So as our chairman was reminding
25 us a minute ago, the new immunologic problems that are

1 going to arise with this are not just regulatory and
2 involved with the vaccine manufacturers. I think you
3 will have to reexamine the whole vaccine in terms of
4 responses to all antigens.
5 CHAIRPERSON FERRIERI: Well, let's move
6 forward. Anything else? I would like to start with
7 H1N1. What I think would be best for us to do today
8 is do one at a time and come up with our
9 recommendations and we will vote on them. I asked if
10 anyone wanted to speak in the open public hearing and
11 no one stood up. Would you like to?
12 MR. RUBEN: Yes.
13 CHAIRPERSON FERRIERI: Please. Announce
14 your name and association.
15 MR. RUBEN: My name is Fred Ruben. I am
16 with Pasteur Merieux Connaught. I just wanted to make
17 a comment about what happened this past year. There
18 were some delays in getting vaccine out to the public
19 due to manufacturing difficulties. And what I am
20 thinking -- I am one of these people that like to
21 think of the worst case scenario, and I am thinking
22 that we do need some time this year to pick an H3N2
23 that is going to be appropriate for our vaccine. We
24 have got a lot of unknowns given the strains that are
25 coming over from China. So I am thinking that it may

1 take well into the spring to come up with the right
2 decision about that.
3 I am also thinking how horrible it is to
4 be running a public health clinic and not have vaccine
5 when you've got your clinic scheduled and patients are
6 clamoring for it. The media picks up on it and says
7 there is going to be a vaccine shortage. It just
8 creates a nightmare. And I think as an influenza
9 thinking body and all of us are on the same page, we
10 want to continue to have the public to have confidence
11 in what we are doing. I think we ought to allow for
12 time to make the correct decision about the H3N2. But
13 if we are going to do that, there is always a counter
14 -- there is always an opposite effect. I think we
15 have to make a decision about the other antigens a
16 little bit sooner in order to have time to produce
17 those so you have the luxury of having the time to
18 make the H3N2.
19 So in the best of worlds, what I would
20 recommend for consideration today would be to make
21 some decisions about the H1N1, the B strains that have
22 been going around for five years in spots here and
23 there, and to go ahead and play a little guesswork
24 with those, but not guess on the H3N2 and allow
25 manufacturing to know what to make with those first

1 two antigens, the H1N1 and the B. Then I think we are
2 all going to come out ahead because I think we will
3 probably have vaccine on time next fall.
4 CHAIRPERSON FERRIERI: Thank you very
5 much. We can now adjourn the meeting. Well, let me
6 continue. We will start with H1N1. For those of you
7 in the audience who have been here past years, year in
8 and year out, I think this panel has been very
9 prudent, appropriately cautious when necessary, and
10 very sensitive to the needs of industry. It has not
11 always permitted us to give you all your answers at
12 once, as you know. And FDA and CDC and other
13 regulatory bodies, I think are similarly prudent. And
14 to move in and make some rash decision would be
15 certainly not in keeping with our past performance and
16 record here.
17 So with H1N1, let me summarize just a tiny
18 bit what we have heard then. The current strain, the
19 A/Beijing/262/95 induces very broadly cross-reactive
20 antibody that is able to interact with a number of
21 other strains. The beauty of the current strain, the
22 one in the current vaccine, is it is well
23 characterized and we certainly have a lot of
24 information about it. We have no information that is
25 substantive on other strains. So if we could just

1 focus on this now, the H1N1. I would like to open it
2 up now to our advisory panel for a reaction and a
3 motion and then we will come to a resolution on H1N1.
4 To start, Dr. Estes and then Dr. Greenberg.
5 DR. ESTES: Well, I actually recommend
6 that we keep the current vaccine strain.
7 CHAIRPERSON FERRIERI: Thank you, Mary.
8 That is one of the most beautiful and succinct
9 sentences that I have heard today. Dr. Greenberg?
10 DR. GREENBERG: I second the motion.
11 CHAIRPERSON FERRIERI: Beautiful. We are
12 into discussion of the motion that has been seconded.
13 Any comments? Ms. Cox, do you wish to add anything?
14 Ms. Cole, sorry. Rebecca, do you have any comment on
15 the motion?
16 MS. COLE: No, Pat, I agree with it.
17 CHAIRPERSON FERRIERI: Okay. Thank you.
18 Any further discussion? Then we will have a formal
19 vote, yes or no in favor of the motion to retain
20 A/Beijing/262/95 in the new vaccine.
21 DR. DAUM: I was intrigued by one of the
22 options that Dr. Cox put forward, which would be to
23 update to a more recent virus in the Beijing lineage,
24 but then dismayed that there is no good candidate to
25 do that updating with. And I guess that therefore I

1 am coming around to the same conclusion, which it
2 sounds like others have expressed. But if such a
3 candidate could be developed, it might be a time to
4 reconsider my view on this.
5 CHAIRPERSON FERRIERI: Nancy, do you have
6 any comment on that? She may not be in the room right
7 now. Well, there is a motion here. I call for the
8 questions. We will start with Dr. Greenberg, yes or
9 no in favor of the motion.
10 DR. GREENBERG: I vote yes.
11 CHAIRPERSON FERRIERI: Okay. Dr. Daum?
12 DR. DAUM: Also yes. I hope that comment
13 would be noted.
14 CHAIRPERSON FERRIERI: Dr. Huang?
15 DR. HUANG: Yes.
16 CHAIRPERSON FERRIERI: Dr. Kohl?
17 DR. KOHL: Yes.
18 CHAIRPERSON FERRIERI: Dr. Snider? Is he
19 in the room?
20 DR. SNIDER: I pass.
21 CHAIRPERSON FERRIERI: Pardon me?
22 DR. SNIDER: I pass Madam Chairman. I
23 just got back from lunch.
24 CHAIRPERSON FERRIERI: Thank you.
25 Abstention. Dr. Estes?

1 DR. ESTES: Yes.

2 CHAIRPERSON FERRIERI: And Dr. Edwards?

3 DR. EDWARDS: Yes.

4 CHAIRPERSON FERRIERI: Dr. Kim, I think,

5 had to leave for his plane. Dr. Eickhoff?

6 DR. EICKHOFF: Yes.

7 CHAIRPERSON FERRIERI: Dr. Hall?

8 DR. HALL: Yes.

9 CHAIRPERSON FERRIERI: Dr. Hoke?

10 DR. HOKE: Yes.

11 CHAIRPERSON FERRIERI: Dr. Poland?

12 DR. POLAND: Yes, with the one concern to

13 note that if I read it right, the only U.S. isolate so

14 far would not be covered well by this vaccine strain.

15 CHAIRPERSON FERRIERI: Dr. Breiman?

16 DR. BREIMAN: Yes.

17 CHAIRPERSON FERRIERI: Dr. Kilbourne?

18 DR. KILBOURNE: Aye.

19 CHAIRPERSON FERRIERI: Aye, great.

20 CHAIRPERSON FERRIERI: I think I have

21 covered -- and my vote is yes, Nancy, for the record.

22 Okay. I wanted to start with the easiest. That gives

23 us more inspiration to tackle the others. I would

24 like to move to B now, so that we separate the A's

25 from the B's, and then we will tackle H3N2 last. So

1 the current strain, the B/Harbin/7/94, this past
2 several years has been included in the vaccine, four
3 years or so. For the record, I want to be accurate.
4 Roland, how many years has the B/Harbin been in the
5 vaccine? Four?
6 DR. LEVANDOWSKI: Since 1995, so I think
7 this is coming on to the fifth season it would be --
8 the fourth or fifth.
9 CHAIRPERSON FERRIERI: Okay.
10 DR. LEVANDOWSKI: One of those.
11 CHAIRPERSON FERRIERI: Fine. We have
12 heard that in Asia we have some B/Victoria-like
13 strains and B/Yamagada, and we have also heard that
14 there are lower titers reacting with the newer United
15 States viruses with decreased inhibitory activity of
16 ferret sera. So among the possibilities today would
17 naturally be, number one, to keep the current one, and
18 there are other candidate strains that have been
19 studied a little bit -- the B/Shangdong/7/97 and
20 B/Foshan/396/98. The former, the Shangdong, has been
21 studied to a certain extent and apparently has a
22 moderate yield. But there are unknowns about Foshan.
23 But we heard that among the possibilities also would
24 be updating B/Harbin of the same lineage or update the
25 B/Vic lineage. We will start some discussion on it,

1 but I do think that I have concerns, grave concerns,
2 about continuing with B/Harbin, and there has been
3 considerable B activity here and there around the
4 world and we might be in for another B year next year.
5 We haven't been hit hard by B for a little while, for
6 a few years. Who would like to launch a little more
7 analysis and moving us closer to some decision making?
8 Dr. Poland?
9 DR. POLAND: I guess from review that we
10 heard this morning and looking at the data, and I
11 can't recall what the yield was for this, but the
12 B/Beijing/184 strain looked to me to be the most
13 appropriate given the isolates that we have so far.
14 CHAIRPERSON FERRIERI: Which one, Greg?
15 DR. POLAND: B/Beijing/184. But Roland,
16 could you remind us, was that one that we could grow
17 in moderate or better yield?
18 MS. COLE: Pat? Pat, it is Rebecca.
19 MS. CHERRY: Hold on, Rebecca.
20 DR. LEVANDOWSKI: That particular strain,
21 the B/Beijing/184/93 was the strain that was
22 recommended. It was chosen for the reasons that have
23 been commented on already, but it was a strain that
24 did not grow at all. It would not have been
25 appropriate for manufacturing. Maybe I should comment

1 that it was a situation that put the manufacturers in
2 somewhat of an awkward situation because it was a late
3 decision. Nevertheless, it was possible to find an
4 alternate strain that seemed to be appropriate
5 antigenically with the information that was available
6 at the time.

7 DR. POLAND: And is that the strain that
8 WHO is using?

9 DR. LEVANDOWSKI: Everybody in the world
10 is using the B/Harbin/7/94 strain for vaccine. Nobody
11 in the world is using B/Beijing/184/93, at least not
12 to anyone's knowledge here.

13 CHAIRPERSON FERRIERI: Nancy, would you
14 mind -- this has been a huge amount of data, as usual,
15 and it has been hard to retain all of it. But could
16 you give us some notion of how big a deal it would be
17 to update the current B/Harbin, so we would keep this
18 lineage rather than go back to the B/Vic lineage. And
19 then maybe Dr. Slusaw would like to comment on my
20 question. I see him nodding his head. So updating,
21 how big a deal? And how good will it be then?

22 DR. COX: I think we have evidence using
23 a number of antisera for viruses that are related to
24 Beijing 184, which is a little -- it is a sublineage
25 separate from viruses related to B/Harbin. We have

1 evidence that antisera to those strains tend to cover
2 well, just as the Beijing/184 antisera tends to cover
3 better. We have -- the viruses that we have now that
4 are egg isolates are the B/Yamanashi/166/98,
5 B/Bucharest/311, there is a Romania strain, and then
6 of course we have the very recent B/Georgia/4/98
7 strain. And so what we would like to do is some
8 additional serologies and further explore some of
9 those strains.

10 CHAIRPERSON FERRIERI: Dr. Slusaw, do you
11 have a comment on this aspect of updating? The
12 hardships? The pros and cons?

13 DR. SLUSAW: My only concern about the B
14 is that as of this point to consider other B/Harbin-
15 like B candidates, the manufacturers don't have
16 alternate B strains in our hands yet to begin working
17 with to make working seed for production. So given
18 the time tables I have shown, it would be a number of
19 weeks or perhaps a month or a month and a half until
20 we would have a working seed. So that is our concern.

21 CHAIRPERSON FERRIERI: Last year, of
22 course, we made two major changes, two different
23 components. Both A's changed and you coped very well
24 even though there might have been some ultimate delay.
25 Dr. Kohl? And then I will have Ms. Cole on the line.

1 DR. KOHL: I guess I want to get back to
2 Bob Daum's question, I think, and plus a question by
3 Caroline Hall. I would like some more reassurance
4 that the B/Vic is going to stay where it is. What I
5 have heard so far is that it has been there for 9
6 years or so and hasn't moved and I should be happy
7 about that. Not being a flu expert, is that good
8 enough?
9 CHAIRPERSON FERRIERI: Dr. Cox?
10 DR. COX: Well, its been good enough for
11 us for the past five or six or however many number of
12 years it has been. I think we -- our feeling is that
13 we really need to keep close tabs on the B/Vic-like
14 viruses. The Australians certainly are very nervous
15 about the B/Victoria strains because they are right on
16 their doorstep being in Thailand and Singapore. But
17 we have faced very much the same situation that we
18 have seen now for the past number of years and so far
19 we have been okay going with the B/Harbin lineage.
20 DR. KOHL: And what is going to trip the
21 switch? What do you have to see before you advise us
22 to go with B/Vic?
23 DR. COX: We would be looking for a
24 pattern similar to what we saw for the H1N1
25 A/Beijing/262 virus, which moved to additional

1 continents. Last year we had detected it in Africa,
2 Europe and North America.
3 CHAIRPERSON FERRIERI: Rebecca Cole,
4 unrelated to Steve Kohl here. Ms. Cole?
5 MS. COLE: I was going to ask the same
6 question that Dr. Poland did.
7 CHAIRPERSON FERRIERI: Okay. Did you get
8 a sufficient answer, Rebecca?
9 MS. COLE: Yes. Thank you.
10 CHAIRPERSON FERRIERI: Thanks. Dr. Hall
11 and then Dr. Kilbourne.
12 DR. HALL: I could think that probably the
13 consensus thus far would be that we would like to
14 obviously recommend potentially an update on the B
15 antigen such that it would give better coverage for
16 the B/Beijing/184. The question being obviously that
17 there is no really good candidate. But, Nancy, I
18 thought that you had mentioned B/Georgia/4/98 as
19 potentially good. It is at least an egg isolate. Do
20 you know more about that? Is it a candidate, even if
21 it is not quite in the hands of the manufacturers yet?
22 DR. COX: We would actually like to send
23 it out because we just put it into ferrets. We just
24 got the egg isolate a week ago or so and have just put
25 it into one test. But given the time constraints that

1 we now have, we would like to get it sent out right
2 away. We will have a cross test in ten days or so and
3 we will have the sequence analysis as well. So I think
4 this would be a potential candidate.
5 CHAIRPERSON FERRIERI: Dr. Snider? I am
6 sorry, Dr. Kilbourne, forgive me, and then Dr. Snider.
7 DR. KILBOURNE: Yes. It is relevant to
8 this because I think that on the table here should be
9 the consideration that Roland introduced earlier, and
10 that is that the A and B viruses are different. They
11 are different not only structurally to some extent,
12 the size and so forth, but I think that they are
13 epidemiologically different in the sense that we don't
14 always have the continuous sequential antigenic drift,
15 unidirectional drift that we have seen with the A
16 viruses. You may get pockets of these strains that
17 are sort of isolated like the Victoria seems to be,
18 and I think we can be less secure about predicting the
19 future with that. So that I think that ought to be on
20 the table in the discussion.
21 CHAIRPERSON FERRIERI: Dr. Snider?
22 DR. SNIDER: Yes. I guess I just want to
23 reiterate what several people have said. I have a
24 great deal of concern about Influenza B for next year
25 given the fact that we haven't had that much activity

1 in the past. It seems possible that given the fact
2 that it was almost 20 percent in some places this
3 year, it could be even more dominant next year. So I
4 am very concerned that we have coverage and I am
5 concerned about the drift that we have seen and would
6 like to see a better match. I think what is difficult
7 for us at the present time is that we have just a
8 small amount of data. It seems to me that what we
9 would have to do if we want to make a change is make
10 it contingent on the availability of a strain that
11 would be able of appropriate levels of replication in
12 eggs as well as elicit the appropriate antibody titers
13 or immunologic responses we are looking for, and we
14 are working in a very tight time frame. And I think
15 we are talking about, although we haven't gotten to it
16 yet, a potential change in two of three components.
17 And one of the things we may have to do at the end of
18 our discussion is give some indication of the priority
19 of each of the two changes. Because for a lot of
20 practical reasons, it may or may not be possible to
21 make both of those changes this year.
22 CHAIRPERSON FERRIERI: I appreciate your
23 comments very much on that point, Dixie. When we have
24 finished later with H3N2, I think we can then address
25 the prioritization. All of you have heard many people

1 speak here. There is unrest here on the ranch. We
2 don't feel comfortable moving forward with the current
3 B. Dr. Greenberg?
4 DR. GREENBERG: I am still not clear --
5 since once before, I guess, a B was picked as ideal
6 but then had poor growth characteristics, I would hope
7 -- how many candidates are moving forward now of the
8 new Harbin subtype? I don't know what you would call
9 it. How many are moving forward in eggs so that the
10 likelihood will be that one of them will have the
11 correct serology and the correct egg phenotype? And
12 ideally when will that emerge?
13 DR. LEVANDOWSKI: Me? Oh, thanks.
14 CHAIRPERSON FERRIERI: Roland.
15 DR. LEVANDOWSKI: Well, obviously Nancy
16 Cox said that there were several strains that were
17 possibilities. Some of these we have already. Some
18 of these were discussed last fall but had not been
19 distributed to manufacturers yet. The only strains
20 that we have distributed to manufacturers are the ones
21 that Dan indicated. We have not sent out a new
22 B/Harbin-like strain in the last several months. So
23 we would be at some what -- the manufacturers would be
24 at somewhat of a disadvantage even at this time trying
25 to produce a seed virus for a new strain. Having said

1 that, I think they are pretty good at handling things
2 very expeditiously. And although I don't think I want
3 to put them on the spot either, I think they expect us
4 to do more than we can sometimes also.

5 However, having said that, there are some
6 strains. As Dan mentioned, we are prepared to send
7 out the Foshan strain. We do have the Romania strain
8 in our repository. Some of these other strains could
9 be sent directly from CDC to the manufacturers. So
10 all these things could be in the pipeline immediately
11 if not sooner.

12 CHAIRPERSON FERRIERI: Could the panel --
13 the panel is not going to be able to make a
14 recommendation on what the substitution could be, but
15 we could come up with a recommendation that very
16 expeditiously strains should be provided of the ones
17 that have just been cited -- Romania/318/98, is that
18 one of them, Roland? And then the Foshan/396/98. And
19 maybe Nancy Cox, the Georgia strain as well? At least
20 those three? And try to rev up and see what kind of
21 growth characteristics we might have. But I think that
22 we are at a point where we can make a motion that is
23 along the lines of what I have stated and that
24 includes a provision for fall-back if they should
25 fail. Who would like to make such a motion? Steve

1 Kohl, please?

2 DR. KOHL: Yes. I would like to make a

3 motion that appropriate updated seed stock or whatever

4 you call it -- appropriate updated B virus be

5 forwarded to the manufacturers for initializing the

6 vaccine for an update of the Harbin/B.

7 CHAIRPERSON FERRIERI: And the provision

8 that if it fails --

9 DR. KOHL: I guess I am being prompted?

10 And the provision that if it fails, we would I guess

11 have to fall back on the current isolate.

12 CHAIRPERSON FERRIERI: Yes. A second on

13 that? Who seconded that? It is multiples. For the

14 record, Dr. Daum seconded it. We are open for

15 discussion now. Excuse me, we are open for discussion

16 now. Dr. Daum?

17 DR. DAUM: Excuse me, Dr. Ferrieri. I

18 wondered whether Dr. Kohl might like to specify the

19 lineage in his motion?

20 DR. KOHL: I would think that the people

21 from the CDC and the FDA would be better at that than

22 me -- than I.

23 CHAIRPERSON FERRIERI: Well, they are --

24 DR. KOHL: The non-Victoria group is what

25 we are talking about.

1 CHAIRPERSON FERRIERI: They are Beijing
2 lineage. They are Beijing lineage.
3 DR. KOHL: Fine, Beijing lineage.
4 CHAIRPERSON FERRIERI: Other discussion
5 before we have a vote on the motion?
6 DR. BREIMAN: Pat?
7 CHAIRPERSON FERRIERI: Yes, Dr. Breiman?
8 DR. BREIMAN: Is failure well enough
9 defined? Do we have to define what failure is?
10 CHAIRPERSON FERRIERI: Well, as a non-
11 virologist but someone who has worked with
12 bacteriophage, failure would be inability to propagate
13 the virus and to have a low yield. Is that a good
14 enough one for industry? Inability to prepare
15 reassortants. I mean, that would be part of it.
16 DR. LEVANDOWSKI: Currently there aren't
17 any reassortants being made for Influenza B viruses,
18 so the manufacturers are really stuck using whatever
19 they get from nature at this point.
20 CHAIRPERSON FERRIERI: Okay.
21 DR. BREIMAN: I am sorry, the level of
22 immunologic response is not part of the measurement of
23 failure?
24 CHAIRPERSON FERRIERI: Well, they wouldn't
25 have that information except empirically based on

1 ferret cross reactivity with the strains and human
2 reactions -- human sera from last year's component
3 from the classic B/Harbin. It would be based on that
4 immunologic data unless there is something that is
5 being stashed away that we haven't heard about. Okay.
6 Now further comments? Dr. Edwards and then Dr. Estes.
7 DR. EDWARDS: I have a question. What if
8 one manufacturer is capable of generating a high yield
9 product and another is not? Would we have two
10 different kinds of vaccines made by two different
11 companies or is there some sharing of these stocks
12 from the manufacturers?
13 DR. LEVANDOWSKI: Manufacturers each make
14 their own seed virus. We actually don't use the term
15 seed virus to describe what we send to them. It is a
16 reference strain that is distributed to the
17 manufacturers and they each make their individual seed
18 based on their experience and capabilities and
19 knowledge of how to handle the viruses. I shouldn't
20 speak for the manufacturers again, but I suspect that
21 there probably are differences in the yield of the
22 same strain for different manufacturers because
23 something as simple as a half a degree Centigrade of
24 temperature can have a significant effect on how much
25 virus is recovered and duration and the process

1 itself. Actually, some of this doesn't even relate to
2 -- we are getting into things that don't relate just
3 to the virus itself but the capabilities of the
4 manufacturing process to tolerate differences in the
5 strains. It is a little bit unpredictable which way
6 that would go. But I guess the short answer is that
7 there could be differences in yield for different
8 manufacturers and their probably are differences in
9 yield for different manufacturers for the same strain.
10 DR. KILBOURNE: Each so-called virus
11 strain is in fact a quasi-species, so a new mutant may
12 pop up in one lab that isn't present in the other.
13 Even though you think you are all dealing with the
14 same thing. So even at that level, they may be even
15 slightly different antigenically.
16 CHAIRPERSON FERRIERI: Dr. Estes?
17 DR. ESTES: I wondered -- this actually
18 relates to Dixie's earlier comment. Do we need to set
19 an outside time frame for this decision to be made?
20 Because the manufacturers will have H1N1, but they
21 will need a second strain to get started with. And
22 because of the issues we are going to discuss about
23 the third component.
24 CHAIRPERSON FERRIERI: I am not
25 comfortable with our setting a time limit on it here.

1 I think that would be unwise. I think that the
2 regulatory agencies should be working, as we move
3 forward now, very, very closely, and we will be
4 hearing an update on this within 8 weeks. So we will
5 have information, Mary. That would be my goal. I
6 don't want that to be a rigid rule. I think there has
7 to be active communication and information and then we
8 move forward based on the preliminary data from the
9 attempts to propagate the virus. Dr. Greenberg?
10 DR. GREENBERG: I would like a little
11 clarification on the B/Victoria. I find myself in
12 agreement with the analysis that it has stayed where
13 it is and so that is the best data you have. I would
14 like to know whether that picture could change in the
15 next three or four months, and if it could, are you
16 prepared for that? Should you have an egg isolated
17 strain that is ready to go sort of in the bank?
18 DR. COX: I can answer that. I take the
19 easy questions. We do have a strain. It is the
20 Shangdong/7 strain, which is actually being used in an
21 experimental vaccine trial in Australia. We
22 previously used the Beijing/243 Victoria-like virus in
23 an experimental trial. So we would have both of those
24 strains, potential vaccine strains, that had already
25 been put into humans.

1 DR. LEVANDOWSKI: And those have been
2 distributed to manufacturers as well. So everybody
3 should have that in their repository now.
4 CHAIRPERSON FERRIERI: We have a motion to
5 deal with now. I think we have discussed it
6 sufficiently and we have a lot of discussion that we
7 need to apply to H3N2. So if someone would please
8 call the question and we will vote on the motion,
9 which is to provide at least these three strains we
10 have heard about, the Foshan, Romania and Georgia of
11 the Beijing lineage to industry as soon as possible
12 and to find out their characteristics for propagation
13 and yield. And if all fails, then the contingency
14 fall-back plan is to retain the B/Harbin strain that
15 is in the current vaccine. So we will start voting on
16 the opposite side of the room today. Dr. Kilbourne?
17 DR. KILBOURNE: Is this a vote?
18 CHAIRPERSON FERRIERI: Yes.
19 DR. KILBOURNE: Yes.
20 CHAIRPERSON FERRIERI: Thank you. Dr.
21 Breiman?
22 DR. BREIMAN: Yes.
23 CHAIRPERSON FERRIERI: Dr. Poland?
24 DR. POLAND: Yes.
25 CHAIRPERSON FERRIERI: Dr. Hoke?

1 DR. HOKE: Yes.

2 CHAIRPERSON FERRIERI: Dr. Hall?

3 DR. HALL: Yes.

4 CHAIRPERSON FERRIERI: Ms. Cole?

5 MS. COLE: Pat, my vote is yes.

6 CHAIRPERSON FERRIERI: Yes?

7 MS. COLE: Yes.

8 CHAIRPERSON FERRIERI: Thank you, Rebecca.

9 Dr. Eickhoff?

10 DR. EICKHOFF: Yes.

11 CHAIRPERSON FERRIERI: Dr. Edwards?

12 DR. EDWARDS: Yes.

13 CHAIRPERSON FERRIERI: Dr. Estes?

14 DR. ESTES: Yes.

15 CHAIRPERSON FERRIERI: Dr. Snider?

16 DR. SNIDER: Yes.

17 CHAIRPERSON FERRIERI: Dr. Kohl?

18 DR. KOHL: Yes.

19 CHAIRPERSON FERRIERI: Dr. Huang?

20 DR. HUANG: Yes.

21 CHAIRPERSON FERRIERI: Dr. Daum? Sorry,

22 Bob?

23 DR. DAUM: Yes.

24 CHAIRPERSON FERRIERI: And Dr. Greenberg.

25 DR. GREENBERG: Yes.

1 CHAIRPERSON FERRIERI: And my vote is yes.
2 Thank you all very much. We will move on then to the
3 H3N2. We have heard a lot of data on this. There has
4 been a lot of activity and there are many, many
5 strains -- a couple of hundred -- 200 Chinese strains
6 that have arrived for study in CDC. So there has been
7 expressed up to now grave concerns about the
8 possibility of retaining the current H3N2 strain. So
9 we have various candidates that have been mentioned.
10 A couple of Sichuan strains, 436 and 418/98, and we
11 have some information on their yield even -- moderate
12 yield. But we need, perhaps, more data. We don't
13 have the serologic data that is very vast to address
14 this specifically. But now having stated sort of the
15 briefest summary of the problem, I would like very
16 much for the panel to share their assessment analysis
17 of this issue, H3N2. The problems and could we work
18 a solution or not? Who would like to start? Dr.
19 Eickhoff, thank you.
20 DR. EICKHOFF: Excuse me. I recall Dr.
21 Kilbourne last year at this meeting, when we selected
22 the A/Sydney variant, after we had gotten through the
23 selection process and we agreed to do it, he allowed
24 as how maybe we are locking the barn door after the
25 horse is out. And indeed the same comment would apply

1 even more so this year. As Nancy has pointed out, the
2 A/Sydney H3N2 variant has circulated widely for two
3 years now. I think we would be pushing our luck to
4 expect that to be a stable situation for this coming
5 year. I don't think we are at a stage yet where we
6 can pick and choose individual strains, but I would
7 think it would be time to move on in the direction of
8 one or more of the A/Sydney variants that are
9 currently being isolated and would hope that we wind
10 up with a motion to do very similar to what we just
11 did with influenza B, namely to ask for further data
12 regarding growth characteristics of some of these
13 variant strains and defer a decision for the time
14 being.
15 CHAIRPERSON FERRIERI: Dr. Snider and then
16 Dr. Huang.
17 DR. SNIDER: I just wanted to be reminded
18 of how many strains that are available yet to be
19 characterized and get some idea of how long it will
20 take to get, if not all, a substantial proportion of
21 those strains characterized. Because clearly the
22 season this time has been mentioned late, so we are
23 just now in many parts of this country in the midst of
24 the outbreak and we don't really have as good a handle
25 on it as we would like. It sounds like we have got to

1 make a decision by March on the last component of this
2 vaccine, so it is really a question for me of how much
3 information we can get between now and the time the
4 last component has to be decided upon. And it may
5 very well be that given a lot of other considerations
6 about Influenza A and so forth, that the H3N2 ought to
7 be the last component to go in so that we can get the
8 maximum data about it. But if Nancy and others could
9 tell us where we stand with regard to how much is
10 outstanding in the way of isolates.
11 CHAIRPERSON FERRIERI: Nancy?
12 DR. COX: We did a quick check yesterday
13 and have another 200 strains that had already been
14 logged into the computer and another 7 packages had
15 arrived just yesterday that contained viruses that
16 hadn't been logged in yet. So I have no idea how many
17 were in there. But some of the viruses are newly
18 arrived and haven't gone into tissue culture, but
19 others have gone into tissue culture or eggs,
20 depending on their substrate of origin. So we can
21 actually test as many as 75 antigens in a test. So
22 providing they are growing well, which they have been
23 doing pretty well this season, we can crack through
24 quite a few viruses in a two to three week time
25 period. So we could have another 150 tested if they

1 are growing well in another three weeks.

2 CHAIRPERSON FERRIERI: I hope that is

3 maximum. Actually, Dr. Estes, we will be rediscussing

4 this issue in approximately 41 to 42 days, not 8 weeks

5 in order to come up with very firm recommendations on

6 B and H3N2. So I am hoping that everything goes well.

7 Someone in my lab asked me what we were going to be

8 deciding today, and I said, well, I didn't know yet,

9 but a lot of information was very contingent on what

10 comes out of China. And she said, but why China? And

11 I said, well, historically a lot of trends and

12 antigenic changes have emerged from strains from China

13 and similar parts of the world. Would either of you

14 like to say something a little more sophisticated than

15 that? Or Dr. Kilbourne, if you would like to say

16 something on that point.

17 DR. KILBOURNE: Something sophisticated?

18 CHAIRPERSON FERRIERI: As you wish. Our

19 definition here is very broad. We have a lot of

20 leeway.

21 DR. KILBOURNE: The conventional wisdom --

22 and I have some unconventional wisdom -- is that China

23 is an area where you have a combination of both

24 enormous population, particularly in urban centers and

25 the opportunity for many generations of virus and

1 therefore many mutants to be thrown off. You also
2 have rural areas where there is exposure to animals,
3 whence some of these come. And the other question I
4 think is really the chicken and the egg business of
5 where did it all start eventually, and this may have
6 to go back to the origins of man. So I don't know what
7 more you can say than that, sophisticated or
8 unsophisticated.
9 CHAIRPERSON FERRIERI: Thank you. I
10 wanted us to have a little levity here because it
11 doesn't get any lighter.
12 DR. KILBOURNE: This is as good as it
13 gets.
14 CHAIRPERSON FERRIERI: It is as good as it
15 gets. Yes, Dr. Hoke?
16 DR. HOKE: Well, it seems to me there are
17 two bits of information that we need. One is
18 regarding any possible strains that are on the menu
19 here that might be better. And I think that the
20 A/Sichuan/346/98 was mentioned as a possibility. So I
21 guess there is information regarding whether that is
22 ready to go as well as information on this new
23 collection that CDC has and whether there are any
24 surprises in the isolates to be made. But is this
25 strain one that would do the job and is it ready to go

1 or --

2 CHAIRPERSON FERRIERI: Well, we have some

3 information on it. We know that the yield has been

4 moderate with that one as well as with 418/98. Is

5 that correct, Nancy? On one of the slides that you

6 showed or someone showed it -- Roland did.

7 DR. LEVANDOWSKI: Well, these are strains

8 that we were able to get out to manufacturers and they

9 were able to get at least some minimal amount of

10 information back to us. I guess I would say that

11 moderate is probably a generous description for these

12 strains, the Sichuan/418 and Sichuan/346, and they are

13 not equivalent. The Sichuan/418 inherently seems to

14 grow better, and other people have found this besides

15 our lab, than the Sichuan/346. The Sichuan/346 is one

16 of those that could be a problematic strain just

17 because it yields relatively poorly to begin with. It

18 probably on its own would not be a strain that would

19 be useful for manufacturing and even a high growth

20 reassortant could be somewhat lower yielding than some

21 of the other high growth reassortants are. They are

22 not all equivalent. So we have just that kind of

23 information.

24 CHAIRPERSON FERRIERI: And could you

25 clarify for me again, either you or perhaps Dr. Cox,

1 the difference serologically? It was my impression
2 that the current antisera against A/Sydney and from
3 immunized individuals and ferret sera have better
4 neutralization or reactivity with 418 than with 346,
5 Nancy? And so that we have more information that
6 makes it a superior strain than simply its growth
7 characteristics. But again, there may be something
8 better out there. Would you want to comment on that?
9 Everyone wants to feel secure that we are not holding
10 back and denying industry a go-ahead on something like
11 Sichuan 418/98.

12 DR. COX: Right. I think the complicating
13 factor is that we do know that there are some sequence
14 differences between these two strains which probably
15 account for the difference in reactivity that you see
16 on two of the tables. And we noted that in the human
17 serologic studies, the responses to Sichuan/346 were
18 lower than to 418. So this strain is more different
19 and therefore possibly a strain that should be given
20 greater consideration. Unfortunately, it is not the
21 strain that grows well, so we have both sides of the
22 coin that we are trying to look at.

23 CHAIRPERSON FERRIERI: And what you will
24 be doing with these roughly 200 strains or so is to
25 characterize them and see how close they may be to

1 this. Will one of them emerge as being broadly very
2 well neutralized by antisera that we have available,
3 human and ferret and so on? Trying to come up,
4 again, with best choice that covers all ground.
5 DR. KILBOURNE: Nancy, can we really say
6 at this point that there is any significant difference
7 between the two viruses, the 418 and the 346? Because
8 it may just be a matter of antibody affinity at this
9 point, isn't that correct? In terms of judging the
10 reactivity when you talk about lower titer? Just
11 because they have a lower titer with the original
12 immunizing strain doesn't necessarily mean that they
13 are antigenically dissimilar in an important way.
14 DR. COX: They are not really terribly
15 different from each other. They are not terribly
16 different from each other. So you are right in that
17 sense. There could be a difference in avidity. But
18 we do see some sequence differences between them that
19 could account for -- some four-fold differences that
20 we see on some of these tables.
21 CHAIRPERSON FERRIERI: For the record, I
22 have been -- I wrote it down as 346 and then somewhere
23 else I saw it as 436/98.
24 DR. COX: I apologize.
25 CHAIRPERSON FERRIERI: Is it 346?

1 DR. COX: 346.

2 CHAIRPERSON FERRIERI: Thank you. For the
3 record, we have been talking about Sichuan/346/98 and
4 contrasting it with 418/98. Further comments from the
5 audience? Yes, Dr. Huang?

6 DR. HUANG: I would like to second
7 everything that Dr. Eickhoff said much earlier, which
8 summarized basically, in that we don't have enough
9 information right now to really specify strain.
10 However, I think most of us feel that we should not go
11 ahead with the current A/Sydney. And if it is at all
12 helpful, we may perhaps vote on that and then delay
13 the decision until we get more information on the
14 Sichuan strains.

15 CHAIRPERSON FERRIERI: Thank you, Alice.
16 Would you be so kind as to make that a motion that we
17 do not support the use of the current A/Sydney and
18 want to encourage further study of these other strains
19 before -- with data to be presented to us as soon as
20 possible so we finalize the decision.

21 DR. HUANG: So moved.

22 CHAIRPERSON FERRIERI: Second on that?

23 DR. EICKHOFF: Second.

24 CHAIRPERSON FERRIERI: Thank you, Ted.
25 Now we can have discussion on the motion. We don't

1 want anyone to feel swept away. We are not being --
2 I hardly think this is being impetuous today, but I
3 think we have to -- for FDA and for everyone in the
4 audience, permit them to understand our thinking.
5 This is not a surprise, what we have arrived at.
6 Other comments on the motion? Yes, Dr. Poland?
7 DR. POLAND: I guess my only concern,
8 while I sympathize with the intent of the motion, is
9 that what if these 200-some odd viruses yet to be
10 characterized turn out to either be uncharacterizable
11 or worse, A/Sydney?
12 CHAIRPERSON FERRIERI: Well, I could
13 answer that, but I would like the pros here to do that
14 -- the influenza pros. Who would like to tackle that
15 question?
16 DR. POLAND: Because now -- I guess the
17 idea I am getting at is now the numerator for non-
18 A/Sydney viruses becomes much smaller. And I don't --
19 I guess in part it may be a question of is it
20 unprecedented to have the same strain of H3N2
21 circulate three years in a row?
22 CHAIRPERSON FERRIERI: It is highly
23 unlikely, but has it ever happened before?
24 DR. KILBOURNE: It is highly unlikely, but
25 I am not myself persuaded of a need for change just on

1 the basis of something never happened before. On the
2 one hand, if you look at the ferret data, the strains
3 are 50 percent unrelated. If you look at the human
4 data, they are 50 percent related. So we are really
5 at that hinge point, I think. And I think we always
6 forget how much overlapping, inter-heterovariant
7 immunity there is. I think that is a point that Ted
8 Eickhoff established years ago working with the early
9 H2N3 variants.
10 CHAIRPERSON FERRIERI: Other comments on
11 the motion? Yes, Steve Kohl?
12 DR. KOHL: A question. Am I reading the
13 data wrong? It looks to me like the Sichuan strains
14 effectively neutralize the A/Sydney. So even if
15 A/Sydney continued to be a problem, the Sichuan
16 strains would still be a reasonable answer, is that
17 correct, Dr. Cox?
18 DR. COX: Yes. If you look at the
19 homologous titers, they do. And if you compare the
20 titers that you get with homologous strains to the
21 titers that you get with Sydney-like viruses, you see
22 that they do a good job in an HI test. So you are
23 reading it correctly.
24 CHAIRPERSON FERRIERI: But how poorly is
25 the reverse, though? Is the reverse sufficiently poor

1 to warrant going with one of the A/Sichuan's rather
2 than staying with A/Sydney? Will that -- do you feel
3 it will be based on the study of all the new isolates
4 that have come in?

5 DR. COX: I think that is one of the
6 things that we are going to be looking very carefully
7 for is how well do these two new Sichuan antisera
8 inhibit the most recently isolated strains. That is
9 one of the key pieces to the puzzle.

10 CHAIRPERSON FERRIERI: Okay. Dr.
11 Greenberg, and then we are going to vote.

12 DR. GREENBERG: Is 418 not acceptable? Or
13 if your data shows that the antisera to 418 works well
14 with your new isolates in HI, do you have to -- is
15 another isolate needed? Because I heard that 346 was
16 a poor grower, but I couldn't quite figure out the
17 other one.

18 DR. COX: Well, it is a little bit
19 difficult to explain all the nuances. And, of course,
20 I am sure you all realize that we are trying to work
21 very closely with WHO. There is really no scientific
22 reason to have different vaccines in North America and
23 in Europe. The strains that circulate are very, very
24 similar. And there are sort of -- the criteria that
25 are used for making the recommendations at WHO are

1 similar, but we sort of talk about things in a
2 slightly different way. And when you look at the
3 human serologies, you see that the titers to Sichuan
4 346 are reduced to a greater extent than they are to
5 418, and that sort of moves one towards thinking that
6 this is a strain that has greater antigenic
7 differences when it comes to the way human beings
8 respond to these strains. So it is just unfortunate
9 that the 418 -- the 418 strain does grow better. I
10 think we just need to gain more experience with these
11 two strains and the antisera to them and really weigh
12 all the different components of the decision making
13 and see what works and what the experience is of the
14 vaccine manufacturers. Because sometimes a strain
15 that initially grows poorly, once there is a high
16 growth reassortant, it does behave satisfactorily for
17 the manufacturers. So I think it is sort of a wait
18 and see kind of situation.
19 CHAIRPERSON FERRIERI: I'd like to restate
20 the motion that the committee seconded. We do not
21 recommend retention of the current A/Sydney/5/97.
22 There is insufficient evidence or data to recommend a
23 substitute strain at this time. And we recommend
24 expeditious study of the approximately 200 Chinese
25 strains and submission of further data in the very

1 near future for us to make a final recommendation as
2 a panel. Will start the vote. The discussion ended.
3 Is there something that is vital? Dr. Eickhoff?
4 DR. EICKHOFF: Do we wish to include a
5 fall-back position?
6 CHAIRPERSON FERRIERI: Well, the original
7 motion did not have a fall-back. The Chair entertains
8 a revision or whatever you would like to have as an
9 add-on to the motion.
10 DR. KILBOURNE: My problem with your
11 motion, Madam Chairman, has to do with the statement
12 that we do not recommend. Because I don't think that
13 we have enough information. I think it becomes sort
14 of self-contradictory in the rest of the motion. But
15 I am not sure that we want to say -- maybe some would
16 not want to recommend Sydney. I would have
17 reservations about whether or not the ensuing data we
18 are going to get might reverse our position on that.
19 Also, I would like to bring up a point that Dr. Cox
20 brought up with me over the phone the other day and
21 remind her of it. It was my understanding, Nancy,
22 that as yet the leading candidate strains to replace
23 this have not been epidemiologically very significant.
24 Is that right in China? The Sichuan viruses?
25 DR. COX: That is right. I mean, we have

1 a cluster of these viruses from Sichuan, and actually
2 there was fairly extensive activity from May to
3 September. But we don't have Sichuan-like strains
4 identified yet in this current package from China.
5 But we are working -- as we work our way through, we
6 will see what we see.
7 CHAIRPERSON FERRIERI: Would you like me
8 to restate the motion? We are not strict
9 parliamentarians here. But the sense of what was
10 earlier moved and seconded was that we do not
11 recommend retention of the current A/Sydney strain and
12 based on the data we have, we have no recommendation
13 for a substitute. And part of the motion was that we
14 expeditiously study these additional strains and
15 gather more data. That is as benign as the motion
16 gets.
17 DR. BREIMAN: It could be a little bit
18 more benign and just say we are concerned about it or
19 something. Because it seems to me that once you are
20 on record saying that you do not recommend use of that
21 particular antigen, that if the other ones turn out
22 not to work or they have problems, I believe you have
23 closed the door.
24 CHAIRPERSON FERRIERI: If we insert the
25 phrase based on the information that we have at this

1 time, then I think that it might accommodate
2 everyone's ideas and it leaves it completely open. To
3 be more specific than that I think would be unwise.
4 Because we need more information. Dr. Kohl?
5 DR. KOHL: Again, Dr. Cox, clarify this
6 for us. It looks like there are strains from
7 California from Sichuan, from again California from
8 Korea that are low HI with the A/Sydney.
9 DR. COX: That is correct.
10 DR. KOHL: So there actually has been --
11 I don't know if the word widespread is correct, but
12 there has been multiple sites of isolation of H3N2's
13 that are not covered -- not well covered by the Sydney
14 isolate.
15 DR. COX: That is correct. And I made a
16 comment, which I went over very quickly. We were sent
17 quite a bit of information from the WHO collaborating
18 center in Melbourne, and they have a fairly
19 substantial proportion of strains which are reacting
20 -- which are four-fold down or more -- actually, 30
21 percent of their viruses are four-fold or greater
22 reduced with the Sydney antiserum. And of some recent
23 viruses from Thailand which they had analyzed,
24 approximately 50 percent of those strains were reduced
25 four-fold or greater. So I think we can say that

1 there is a wide geographic distribution of strains
2 which are reduced in titer to the Sydney.
3 DR. KOHL: And I think we are starting to
4 get widespread, albeit anecdotal reports, of immunized
5 individuals in this country who are having documented
6 Influenza A not further characterized. So I am not
7 convinced that the A/Sydney is a solution to next year
8 under any situation.
9 CHAIRPERSON FERRIERI: Thank you, Steve.
10 I think this is a very important point that you
11 unearthed in the data presented earlier and we didn't
12 go back and reemphasize that. This is why the motion
13 was presented originally as it was. There was great
14 unrest about accepting the adequacy of the A/Sydney in
15 the new vaccine to come on board. Is it relatively
16 rare that we are going to get boxed into a corner,
17 Roland, and end up with nothing better? I mean, we
18 have other strategies. We always can pray and do --
19 and I mean that very seriously. There are other
20 things that can be done in the laboratory hopefully
21 that will solve this problem. We will have something
22 better. We are determined there will be something
23 better.
24 DR. BREIMAN: Can I ask one more question?
25 CHAIRPERSON FERRIERI: Yes, and then we

1 will vote.

2 DR. BREIMAN: Those strains that Steve

3 referred to, though, if you look at the ferret

4 antisera data, and maybe I am reading these wrong, but

5 for several of those strains, the dilution was not

6 that impressive for 418 either. So that I guess I am

7 a little confused as to why we think that that would

8 be the solution. Am I reading it wrong?

9 DR. LEVANDOWSKI: Are you asking me?

10 DR. BREIMAN: Well, anybody.

11 CHAIRPERSON FERRIERI: Yes. Do you wish,

12 Roland, to start answering it?

13 DR. LEVANDOWSKI: I am not sure I even

14 heard it because I was thinking about your last

15 comments and wondering if I was supposed to respond to

16 those things.

17 CHAIRPERSON FERRIERI: No. I was just

18 waxing philosophical. Sorry, Roland. What is going

19 through my mind is that of course we don't have

20 anything really in hand at the moment for any of these

21 things. And just as we were talking about for

22 Influenza B, the same situation applies here. There

23 is the vaccine strain that we have. It may not be

24 ideal, but at least we know quite a lot about it and

25 how it works and how it can be handled. I guess I

1 would hope that part of the fall-back position would
2 be that if everything else falls through, at least we
3 still have Sydney to use.
4 CHAIRPERSON FERRIERI: That is not part of
5 our motion, but I think we must keep that in mind that
6 that may be the only thing that we will be able to
7 fall back on. I like to think of things as having
8 partial immunity. A little bit of the antibody goes
9 a long way and may be better than having no antibody.
10 So a modified illness to me is a positive strike in
11 any disease. If you can modify illness not so extreme
12 that you don't have a superimposed staphyloccal or
13 pneumococcal pneumonia and die in a nursing home.
14 There is a full spectrum of influenza clinically from
15 having at the most minimal, mild myalgias, no fever or
16 very low grade fever and a little sore throat and you
17 get better over three or four days and you may even be
18 going to work and even spreading it all around, but it
19 responds to mild anti-inflammatory agents. At the very
20 middle of the spectrum, you stay home in bed for a
21 week and you have high fever and full blown systemic
22 symptoms. At the very far end of the spectrum, you are
23 80 years of age and you were unimmunized and you come
24 down with influenza and succumb to a superimposed
25 bacterial pneumonia. But in-between, in all of those

1 three spots, a little bit of antibody could make you
2 go from third phase into the middle or the low end in
3 terms of clinical severity of disease. So we are not
4 considering that in any of our thinking or discussion.
5 We are trying to be pristine in what we are invoking
6 immunologically, and that may not always be possible.
7 So I agree. If we can't have a perfect strain here,
8 then I would say let's go with A/Sydney. But that is
9 not part of the motion. We will have a chance to come
10 back to it in six weeks, I hope. Does anyone feel
11 differently about the clinical activity? Okay. Let
12 us vote then. We will start with Dr. Greenberg. The
13 motion in its most simple is that we do not recommend
14 retention of the current A/Sydney/5/97 at this time
15 based on the data presented and that we have
16 insufficient data to recommend a substitute strain and
17 that further studies will be done on the 200 Chinese
18 strains to gather more data.
19 DR. GREENBERG: I would ask whether saying
20 rather than we do not recommend, saying we have
21 substantial reservation would be just almost as strong
22 and give us slightly more of a back door. I am sorry.
23 CHAIRPERSON FERRIERI: A revision to the
24 motion has been accepted. Is there a second?
25 DR. EDWARDS: Second.

1 CHAIRPERSON FERRIERI: Very good. I am
2 not sure if that is parliamentarily acceptable.
3 DR. GREENBERG: I am sure it is not, but
4 it works.
5 CHAIRPERSON FERRIERI: It will be fine.
6 None of us is a lawyer or parliamentarian at this
7 table that I know of. Thank you very much, Harry. I
8 think that is quite acceptable to me.
9 DR. GREENBERG: I vote yes.
10 DR. DAUM: I vote yes.
11 DR. HUANG: Yes.
12 DR. KOHL: Yes.
13 CHAIRPERSON FERRIERI: Dr. Snider?
14 DR. SNIDER: Yes.
15 CHAIRPERSON FERRIERI: Dr. Estes?
16 DR. ESTES: Yes.
17 CHAIRPERSON FERRIERI: Dr. Edwards?
18 DR. EDWARDS: Yes.
19 CHAIRPERSON FERRIERI: Dr. Eickhoff?
20 DR. EICKHOFF: Yes, with the provision
21 that I would not limit consideration of variant
22 strains to the 200 strains that most recently arrived
23 from China.
24 CHAIRPERSON FERRIERI: Dr. Hall?
25 DR. HALL: Yes.

1 CHAIRPERSON FERRIERI: Dr. Hoke?

2 DR. HOKE: I -- is it fair for me to ask

3 to hear what it is that we are voting on? I mean it

4 was changed but --

5 CHAIRPERSON FERRIERI: It was the motion

6 that we have grave reservations -- it has been

7 reworded so that instead of saying that we do not

8 recommend retention of the current Sydney/5/97, it has

9 been reworded that we have grave reservations about

10 retaining the strain.

11 DR. HOKE: I actually would be opposed to

12 that.

13 CHAIRPERSON FERRIERI: Well, you have a

14 choice at this point of voting against the motion then

15 or abstaining.

16 DR. HOKE: I vote against it.

17 CHAIRPERSON FERRIERI: Fine. We have a

18 no.

19 DR. POLAND: Would you accept a friendly

20 amendment to remove the word grave?

21 CHAIRPERSON FERRIERI: Was that --

22 DR. GREENBERG: I said substantial rather

23 than grave.

24 CHAIRPERSON FERRIERI: I am sorry, Harry,

25 I thought you said grave.

1 DR. POLAND: Could we just --

2 DR. GREENBERG: I actually like

3 substantial more than grave.

4 DR. POLAND: Could we even then remove

5 substantial? We just have reservations.

6 DR. GREENBERG: Reservations is fine.

7 CHAIRPERSON FERRIERI: You accept that,

8 Harry?

9 DR. POLAND: In that case, I can vote yes.

10 CHAIRPERSON FERRIERI: Fine. Dr. Breiman?

11 DR. BREIMAN: Yes.

12 CHAIRPERSON FERRIERI: Dr. Kilbourne?

13 DR. KILBOURNE: Yes.

14 CHAIRPERSON FERRIERI: And I vote yes.

15 Dr. Hoke, I do want -- Mrs. Cole?

16 MS. COLE: I vote yes.

17 CHAIRPERSON FERRIERI: Thank you. I would

18 like for the record for us to understand your

19 objections, though, Dr. Hoke. We went through so many

20 revisions of it that I thought it would be a step back

21 to start over. But I want to know what the objection

22 was.

23 DR. HOKE: I agreed with the comment that

24 someone made earlier that if we made an excessively

25 negative comment about the current vaccine strain and

1 we are left with nothing else, then we would have a
2 difficult time rationalizing why in the final analysis
3 we might go back to that. And so, I mean, we have a
4 bird in the hand and we have no bird in the bush in a
5 sense.

6 DR. KILBOURNE: Charlie, the motion was
7 modified.

8 DR. HOKE: Well, yes. Well, I just grew
9 confused by the multiple modifications.

10 CHAIRPERSON FERRIERI: Yes. I sympathize.

11 DR. HOKE: I felt that it would be better
12 to have a motion that reflected that really there is
13 nothing wrong with this as a vaccine strain. I mean
14 the only thing that is wrong is that we have used it
15 for a couple of years and the strain is now dominant
16 and it is not likely to be -- or there is a
17 possibility that it won't be the dominant strain in
18 the next year. So that there is nothing -- I wanted
19 to leave us a comfortable position in case we are left
20 with nothing else.

21 CHAIRPERSON FERRIERI: I understand. But
22 actually it is a very serious issue that we have been
23 using it for all of these years and that there may be
24 sufficient drift that has taken place that this isn't
25 adequate and that this has occurred in past years here

1 in these deliberations as well. It would be nice if
2 we had something more substantial to say this looks
3 like this is our trump card that is in the wings now
4 and we are just going to refine it. But we don't
5 today.
6 DR. KILBOURNE: Perhaps if the motion as
7 modified could be read back to Dr. Hoke, it would be
8 reassuring to him.
9 DR. HOKE: Okay.
10 CHAIRPERSON FERRIERI: Dr. Eickhoff? Do
11 you have a point? We have voted on the motion as part
12 of the record and so we also have on record Dr. Hoke's
13 reservations about the motion and I think all of that
14 will be taken into account. I have no doubt that the
15 right thing will be done eventually, Dr. Hoke.
16 DR. HOKE: Neither do I.
17 CHAIRPERSON FERRIERI: Thank you. That is
18 part of the public record too. Dr. Eickhoff?
19 DR. EICKHOFF: Well, I hesitate to say
20 this now, but I think the committee is almost surely
21 unanimous in what its sense is and I think some artful
22 wordsmithing of the motion or editing of the motion
23 might be appropriate.
24 CHAIRPERSON FERRIERI: Other comments? I
25 propose we take a break because we are moving into the

1 next session, which is also an open session on update
2 of H5N1. I know some of you may have to leave early.
3 And for members of the committee whom I have been
4 working with for the past several years, I want to
5 thank you all. It has been a great joy intellectually
6 and personally for me to have served with you all, and
7 I hope we will see each other again.
8 (Whereupon, at 2:58 p.m. off the record
9 until 3:17 p.m.)
10 CHAIRPERSON FERRIERI: I'd like everyone
11 to take a seat now. If the panel could return to the
12 table. We would like to start as soon as possible
13 this afternoon. I want to really thank everyone who
14 plans to stay all afternoon. Some members of the
15 advisory panel have an unavoidable early flight
16 because of commitments back at their own institution.
17 But as we move into this session, the speakers are
18 going to have to be extremely strict with their time.
19 Any ability to condense your presentations will be
20 appreciated. The reason I say this is that there are
21 individuals who are speaking today who have asked to
22 leave early. So that has complicated things.
23 So will announce the speakers and keep you
24 on time. And I am going to be here quite late because
25 my flight was canceled. So I have no urgency. But I

1 don't want everyone else to have to be inconvenienced
2 with traffic problems and getting to the airport and
3 so on. So Dr. Roland Levandowski will start. We need
4 the intro and I hope that the reorganization that we
5 were just asked to do will not be confusing to those
6 of you who know nothing about H5N1 viruses. Roland?
7 DR. LEVANDOWSKI: Okay. Great. Thanks
8 very much. I thank you for indulging us some. I
9 apologize. It is actually not the fault of the
10 speakers. It is really my fault that there is some
11 confusion about the timing for the meeting and how I
12 had instructed people. So I guess you have to blame me
13 for that gap. But we will get started and we will try
14 to be expeditious and stay on time.
15 I just want to give a very brief
16 introduction. We wanted to take the opportunity to
17 let the committee know about activities that have been
18 going on since the H5N1 viruses became apparent in
19 Hong Kong. As you know, there really has not been any
20 H5N1 activity in people that we know about to this
21 point since December of 1997. In spite of that, we
22 have many activities that are ongoing in terms of
23 design and production of vaccines, and we did want to
24 have an opportunity to let you know some of the things
25 that are going on.

1 Just briefly, you are familiar with the
2 fact that there are a number of Influenza A subtypes.
3 I put this slide up here to simply illustrate that if
4 we use the H1N1 virus as the root virus for people, in
5 the past there have been events where reassortment has
6 occurred in nature that has resulted in pandemics and
7 widespread of new Influenza A subtypes. And what I am
8 showing here in one instance is the H3N2 strain, that
9 has mostly human gene segments. And since influenza
10 is a segmented virus, it can exchange freely these
11 gene segments. And the H3N2 virus that is in people
12 today actually seems to have acquired three of the
13 gene segments from an Avian strain at some time in the
14 past.
15 Now there is plenty of information that
16 has been published about the H5 strains, and I only
17 wanted to point out to you here that the difference
18 between the H5N1 strains and other strains that have
19 been in people relates to the fact that these strains
20 truly are avian in nature and all the internal genes
21 really derive from an avian source and do not
22 represent a humanized strain. That may be some
23 explanation for why these strains have not gone any
24 further than they have.
25 The activities that are related to the

1 vaccines and investigations have really been global in
2 nature. Just as everything else for influenza is and
3 must be, the understanding of how to deal with the new
4 subtypes as they appear, how to identify them, and how
5 to do manufacturing is a very complex situation. And
6 partly it is complex because as you know from today
7 there is something going on related to producing
8 influenza vaccines all the time for the licensed
9 manufacturers, and there are some very active
10 manufacturers who will be seeking licenses no doubt
11 for other types of vaccines.
12 But what I am showing here is that much
13 information stems from the organization of
14 international centers that have been backed by the
15 World Health Organization and also many national
16 laboratories that collaborate to supply information,
17 not the least of which have been the laboratories in
18 Hong Kong during this last year in relation to the H5.
19 Industry also has had a large part to play in
20 investigations. And as we will discuss, you will see
21 that there are collaborations that are going on
22 between government and industry to evaluate H5
23 vaccines and candidate strains. And finally, within
24 the United States here, of course all the different
25 arms of the Public Health Service in collaboration

1 with USDA and the Department of Defense have been
2 actively involved in identifying ways that we can
3 complete some evaluation of vaccines that are in the
4 process now.

5 Now obviously there are lots of issues
6 that could be addressed, and I don't intend to address
7 many of them here. There are, of course, a number of
8 strains that could be considered for use in vaccines
9 in the classical sense for the inactivated vaccines.
10 The prototype strains from Hong Kong were the
11 nomenclature of the Hong Kong/156/97 as one prototype
12 of one variant, and Hong Kong/483/97 as the prototype
13 for another variant of what is really the same virus
14 but were easily distinguished antigenically. They
15 both share in common that they were highly pathogenic
16 for birds, and there are strains that came out of
17 birds that are probably the direct precursors for the
18 human strains. But that pathogenicity is something
19 that has made work with these strains a little bit
20 difficult. Because we know from the clinical
21 experience in Hong Kong that these strains can be very
22 severe in terms of morbidity and mortality in people.
23 You will hear about activities that have been going on
24 to make use of molecular techniques to bypass that
25 sort of problem.

1 Finally, there always may be other strains
2 that could serve the purpose and there have been at
3 least one strain that may be a non-pathogenic
4 surrogate that could be used for production of
5 vaccines, the A/duck/Singapore/97. It has a problem
6 on its own in that it has a neuraminidase that is
7 different from the prototype strains that we are
8 mostly interested in, but there are ways to handle
9 this, including reassorting techniques and other
10 things.
11 Now the kind of vaccines that could be
12 contemplated, of course, are the whole panoply of
13 vaccines that have been discussed in various forums
14 over quite some time. Obviously a plasma DNA vaccine
15 sounds like it might be a good idea because of the
16 simplicity of production of the vaccine and delivery.
17 Nevertheless, that is not something that is really in
18 the works at the moment except in animal studies. A
19 purified hemagglutinin vaccine, you all are aware that
20 there are companies that are interested in producing
21 these vaccines. One in particular, Protein Sciences,
22 has been very active in clinical trials over the past
23 several years with not only the H5 subtype but also
24 with the more typical H1 and H3 subtypes and have a
25 substantial body of clinical information that has been

1 published.

2 Not to belittle the inactivated vaccines,

3 they of course have a very important role to play, but

4 the inactivated vaccines require, as we have been

5 talking about earlier in the previous session, a seed

6 virus that is adequate to support manufacturing. This

7 is not always a simple thing to get a virus that can

8 grow in eggs well or grow in tissue cultures well and

9 that has all the characteristics that you would like

10 it to have.

11 And finally, last but certainly not least,

12 live attenuated vaccines are possible candidates for

13 H5. And what we are going to hear from the speakers

14 who come next are bits of information for all of

15 these. Now just by way of background, and this

16 information won't be necessary until some later

17 speakers, but I just want to mention that there are

18 some clinical trials that have been made very great

19 progress over the last year, and have been in not only

20 Phase I but Phase II trials. The CDC, of course,

21 produced a plasmid from the original Hong Kong/156/97

22 prototype strain and provided that to Protein

23 Sciences, who made a purified hemagglutinin vaccine

24 that was suitable for doing clinical trials. Those

25 clinical trials were sponsored by NIAID, and I would

1 like to take credit at FDA as being one of the
2 volunteers for the study, since that may be my only
3 way to take credit for being in this. But those
4 studies -- there were Phase I studies that were done
5 to evaluate the utility of the purified hemagglutinin
6 vaccine in laboratory workers to give the possibility
7 of some protection at a time when it was not really
8 possible to produce in that very limited space of time
9 an inactivated vaccine or any other kind of vaccine.
10 Those studies have now gone on, and we
11 will hear more about them from later speakers. The
12 studies -- we will also hear something about the Phase
13 II studies, which have been pursued to try to identify
14 exactly what the dose of the vaccine of those
15 inactivated vaccines should be and to glean some
16 information that could be useful for other studies of
17 inactivated vaccines. I think I should stop there and
18 turn it back to you, Dr. Ferrieri.
19 CHAIRPERSON FERRIERI: Thank you very
20 much, Roland. The order has been reversed. So the
21 European vaccine's trial scheduled for 4:00 by Dr.
22 Wood from the UK will be presented now.
23 DR. WOOD: Thank you very much. Most of
24 my talk will be about European vaccine -- attempts to
25 produce an H5 vaccine and plans to do clinical trials

1 of this vaccine. But I will talk about some more
2 general issues at the beginning.
3 If we can go back to December of 1997,
4 what were our objectives at that time in terms of
5 vaccine? I have identified three main objectives
6 here. To identify a safe, practical vaccine strain.
7 To develop reagents to measure the potency of any of
8 those vaccines. And to evaluate those vaccines by
9 animal studies and if we have time to do limited
10 clinical studies.
11 Roland has alluded to some of the
12 strategies that could have been followed, but at that
13 time there were three main strategies that were
14 followed. First of all, to attenuate the Hong Kong/97
15 virus, this pathogenic virus, by genetically modifying
16 the hemagglutinin so the virus would not be pathogenic
17 for chickens and hopefully not for people as well. To
18 identify a surrogate H5N1 virus that resembled the
19 Hong Kong virus yet was not pathogenic. And thirdly,
20 to express the H5 hemagglutinin in a expression system
21 such as a baculovirus system. And we will hear about
22 these two, the attenuation and the baculovirus system
23 in later talks.
24 I am really going to concentrate on the
25 surrogate H5N1 virus from now on. This is a

1 phylogenetic tree of the HA1 region of the H5
2 hemagglutinin provided by Jill Banks of the Central
3 Veterinary Laboratory at Weybridge in the UK. Here we
4 have the chicken Hong Kong virus and the human Hong
5 Kong virus, and quite closely related to this is
6 Duck/Singapore that Roland just mentioned. In fact,
7 its HA shares about 93 percent sequence homology.
8 When we look at the Duck/Singapore virus
9 antigenically and compare it with the Hong Kong
10 viruses, we see it also is antigenically quite closely
11 related. This is an HI test with post-infection
12 ferret sera to two Hong Kong viruses, Group I virus
13 and Group II virus and Duck Singapore virus. And you
14 see although these HI titers are not identical, they
15 are quite close, showing that the Duck/Singapore virus
16 is quite closely related antigenically.
17 So this was in fact one of the leading
18 vaccine candidates back in about January of 1998. It
19 had advantages and it had some disadvantages. The
20 advantages I have listed here are that first of all it
21 was non-pathogenic. So a vaccine manufacturer could
22 work with this virus in safety. The hemagglutinin was
23 similar. A conventional vaccine could be made from
24 Duck/Singapore virus and there would be no licensing
25 issues.

1 On the downside, it had the wrong
2 neuraminidase. It has N3 neuraminidase and it grows
3 extremely poorly in hens eggs. So to try and rectify
4 both of these disadvantages, a number of labs
5 throughout the world tried to produce reassortants to
6 increase the growth and substitute the N1
7 neuraminidase. The basic strategy was quite similar
8 in most of the labs that tried. It was to reassort
9 Duck/Singapore with PR8 or a virus with PR8 internal
10 proteins. And to try and substitute the N1 either
11 from PR8 or from another virus such as a swine virus
12 isolate in Ireland which had an even more closely
13 related N1 neuraminidase.
14 The first virus -- I should say first of
15 all that no one has been successful. All of these
16 attempts have yielded H5N3 virus in using conventional
17 techniques in eggs. The first virus that was produced
18 was produced in my lab in May of 1998, NIB-40. It had
19 a growth advantage. It was not a reassortant. It had
20 all the genes from Duck/Singapore. So it was probably
21 a high growth variant we just selected out in the
22 process. Alan Hampson in Melbourne produced ARIV-1,
23 which grew much better than both the Duck/Singapore
24 parent and NIB-40. I don't know whether this is a
25 reassortant. I haven't been able to find this out.

1 And then we have two viruses which were derived, one
2 in Roland Levandowski's laboratory here and one in Ed
3 Kilbourne's laboratory. And these are still being
4 characterized.
5 But it has shown how difficult it was to
6 produce reassortants from the Duck/Singapore virus.
7 Now you can speculate as to the reasons for this.
8 There may be that there is a basic incompatibility
9 between the human PR8 virus genome and the avian virus
10 genome. It may be that the selection process in eggs
11 wasn't efficient or our antiserum reagents weren't
12 good enough to do the selection. But whatever the
13 reason is, we weren't very successful.
14 NIB-40 was the virus that in fact was
15 chosen in Europe to do some limited clinical studies
16 with. As well as having a seed virus, we also need
17 reagents to measure the vaccine potency. The test
18 that is used worldwide is a single radial diffusion
19 test. And we have developed antigen -- you need a
20 pair of reagents, a calibrated antigen and an
21 antiserum against the hemagglutinin. We have made
22 antigen reagents to Hong Kong/489 and Duck/Singapore
23 and three different antiserum reagents. One was
24 produced a long time ago against Chick/Scotland/59, an
25 H5N1 virus. The other two were more recently

1 produced. One against the baculovirus HA. This was
2 the Hong Kong/156 virus. And the other against
3 purified HA from Hong Kong/489. We evaluated these
4 three sera in the test with these antigens, and by far
5 the best was this old antiserum, Chick/Scotland. And
6 I must say that delighted by director, Geoffrey
7 Schild, because at the time this was produced, he was
8 actually working in the lab. So he produced this
9 serum. So he is delighted that one of his old serum
10 has come good.
11 The next thing we did was to evaluate the
12 Duck/Singapore virus as a vaccine in mouse efficacy
13 studies. We produced inactivated whole virus vaccines
14 from Hong Kong/156, from 489, from NIB-40, which is
15 Duck Singapore here, from an irrelevant H3N2 vaccine,
16 Shanghai/90, and we had an unvaccinated group. There
17 were groups of 10 mice and they each received two
18 shots of 15 mcg, a human dose. And on this left-hand
19 side of the slide, we see the antibody response as
20 measured by HI test. In red, we have the antibody
21 responses to H5 measured using the Hong Kong/489
22 virus, and in yellow we have antibody to H3 measured
23 with a Shanghai virus. So you see the Shanghai virus
24 was just as immunogenic as the H5 viruses. You may
25 want to comment on the fact that the Duck/Singapore

1 vaccine was maybe not so immunogenic. But I think
2 this is probably due to antigenic differences between
3 the Hong Kong/489 virus and Duck/Singapore. Because
4 if we use Duck/Singapore virus in the HI test, we have
5 equivalent antibody levels. So I think we can quite
6 safely say that each of these vaccines was equivalent
7 in terms of immunogenicity.

8 Two weeks after the second shot, we went
9 on to challenge these mice with a lethal challenge
10 dose of Hong Kong/156 virus. And then we monitored
11 survival of the mice. In both of the control groups,
12 the unvaccinated group and the H3N2 vaccine group,
13 there was no survival at all. The mice died within 8
14 days. Yet complete survival in each of the H5 vaccine
15 groups. So this is very encouraging that the
16 Duck/Singapore vaccine had some potential to protect
17 against the pathogenic Hong Kong virus. I have since
18 seen quite similar data from Jacquie Katz using the
19 Hong Kong/489 -- or is it 483 -- the Hong Kong/483 as
20 a challenge virus. So it protects against the other
21 group of the Hong Kong viruses as well.

22 So we now move into plans for clinical
23 studies. In the UK, Carol Nicholson and I have been
24 interested in doing clinical studies in naive
25 populations to ask the question what would happen if

1 you put a vaccine into naive populations in a pandemic
2 situation. And we have been thinking about different
3 models, such as an H7N7 vaccine or an H2N2 vaccine.
4 But then H5N1 came along and we had an ideal model and
5 we could also use this to evaluate the Duck/Singapore
6 as a candidate vaccine.
7 But these were some of the questions that
8 we thought we should try and answer. What is the
9 minimum immunogenic dose of the three types of
10 vaccine, whole virus, split and surface antigen
11 vaccine? Is one better than the other? There is a
12 school of thought that says the whole virus vaccine
13 would be actually more immunogenic in naive
14 populations, but it has never been satisfactorily
15 answered. Past studies -- the vaccines have not been
16 standardized as efficiently as the vaccines are
17 standardized now. So you would always question the
18 actual dose that was administered back in 1957 or 1968
19 or 1976 or 1977, when similar studies were done.
20 There is no consistent agreement from trial to trial
21 on the results as far as I could see, and there was
22 also no direct comparison of each of these three
23 vaccines.
24 Another question you might want to ask is
25 does a PR8 reassortant induce antibody more

1 effectively than a completely foreign avian virus with
2 no human influenza genes at all? So that may be an
3 issue for the future. How effective are adjuvants?
4 We know that a variety of adjuvants have been tested
5 in animal models and some of them have shown to be
6 very effective. Yet, when we go into clinical trials,
7 some are less effective. It may be that in naive
8 populations in human trials, the adjuvant would behave
9 more like it does in an animal model. And it may
10 actually be very useful. It may be antigen sparing,
11 where an antigen is a precious commodity in a pandemic
12 situation. And finally, would alternative strategies
13 be better in a pandemic -- DNA vaccines, live
14 vaccines, et cetera.
15 Two companies in Europe have made vaccines
16 from Duck/Singapore, from NIB-40. Chiron, Italy have
17 made 14,000 doses of surface antigen vaccine. This is
18 at a level of 15 mcg. Medeva in the UK have made
19 7,000 doses of surface antigen vaccine and 1500 doses
20 of whole virus vaccine. And these are intended to be
21 clinically evaluated.
22 The plan for the UK trial as it existed
23 back in January of 1998 was to do a direct comparison
24 between a vaccine made from Hong Kong/489 virus, a
25 pathogenic virus, and a vaccine made from

1 Duck/Singapore to compare their immune responses. And
2 to have three different dose levels, 7.5, 15 and 30
3 mcg., and to give the subjects two shots and then
4 evaluate the immune response. And to compare their
5 response with the Duck/Singapore whole virus vaccine.
6 There will be Phase I and followed very quickly by
7 Phase II, should the Phase I have been shown to be
8 that the vaccines were safe.
9 This was -- to put this together, we had
10 a consortium of a number of organizations. CAMAR down
11 in the UK, NIBSC, Medeva, Maria Zambon at the PHLS,
12 and Carol Nicholson, the clinician at Leicester Royal
13 Infirmary. The biggest challenge, as you can imagine,
14 was to produce this arm of the study. The virus was
15 produced in containment under conditions which were as
16 close as possible to good manufacturing practice. And
17 a limited amount of inactivated allantoic fluid was
18 made in containment. The big problem was when we came
19 to process this at the site of manufacture. Because
20 normally, as many of you know in the audience, you
21 deal with hundreds of liters or thousands of liters.
22 Yet here in containment, I think we produced about 30
23 liters of inactivated fluid. So there is a big scale
24 difference between what we produced and what is
25 normally processed by a manufacturer, with the result

1 that we lost most of this. It was a big
2 disappointment, but we lost it. It just didn't
3 survive the processing to produce the surface antigen
4 vaccine. So that meant we had to drastically change
5 our plans.
6 So the current plan is that we will look
7 at the Medeva surface antigen Duck/Singapore vaccine
8 compared with the Medeva whole virus vaccine at these
9 three dose levels. And also taking place in the UK is
10 a collaboration between Chiron and Carol Nicholson to
11 evaluate their Duck/Singapore vaccine. This time the
12 comparison will be surface antigen versus adjuvant at
13 surface antigen to compare the effect of the MF-59
14 adjuvant in naive populations.
15 One problem in evaluating the immune
16 responses is that the HI test, which is the gold
17 standard for serology, has been shown not to be very
18 sensitive in measuring antibody to H5 in human sera.
19 This is one reason that we are not relying on this
20 test. We are going to use the virus neutralization
21 test that Maria Zambon has developed. But we are also
22 going to evaluate another test, the single radial
23 hemolysis test. And for those of you not familiar
24 with this test, this is an agarose gel containing
25 erythrocytes -- sheep or turkey erythrocytes -- to

1 which is bound the virus of interest, in this case
2 Duck/Singapore virus. Also in the gel is incorporated
3 guinea pig complement. And then if we introduce serum
4 containing antibodies to Duck/Singapore in the wells,
5 the antibody binds to the virus and initiates the
6 complement mediated lysis of the cells. So you have
7 a zone of lysis and the size of that zone depends on
8 how much antibody was in that particular serum. This
9 test is used routinely in Europe for evaluating
10 vaccines. It is allowed by the regulatory
11 authorities. So we have a lot of experience with this
12 test.
13 So we tried to develop a test for H5 using
14 Duck/Singapore. These are two ferret sera, post-
15 infection ferret sera to Hong Kong/483 and to
16 Duck/Singapore. And you see very big zones of lysis
17 here. We also looked at a range of human sera, pre
18 and post-conventional vaccine, 1997 trivalent vaccine
19 no zones. But unfortunately we saw two very faint
20 zones with random human sera. And what we think is
21 happening there is that these sera contain some
22 antibody to nuclear protein, Influenza A nuclear
23 protein, which Duck/Singapore is recognizing. So it
24 does initiate a faint, non-specific zone of lysis. We
25 can remove this entirely by adsorption with another

1 Influenza A virus. So these along the bottom line
2 here are the sera adsorbed with Influenza A viruses.
3 No non-specific zones, but the specific H5 zones are
4 left intact. So with just one extra step, adding an
5 Influenza A virus, I think we have a test which is
6 worthy of evaluation in our clinical studies.
7 This is my last slide, and I think many of
8 us view the Hong Kong episode as really a rehearsal
9 for what will come in the future. And I think at this
10 stage it is very useful to look back. It hasn't been
11 an easy year for quite a few of us. And to ask
12 ourselves how could we prevent some of these problems
13 happening in the future? What lessons can we learn?
14 And the first one, I have put up here is a dialogue
15 with veterinary authorities. This was one of the
16 first hurdles we had to overcome. We had to have
17 approval in the UK from the Ministry of Agriculture,
18 and I am sure this was the same here with the USDA.
19 They had to grant us a permit and they had to inspect
20 our facilities. And I think it is important that this
21 dialogue continues for the future and veterinarians
22 are involved in many aspects of pandemic planning.
23 We had to work in containment laboratories
24 for the Hong Kong virus. For some of us, we already
25 had containment laboratories. For others, they had to

1 either find a colleague who did have a containment
2 laboratory or actually build one. So there problems
3 with actually getting funding to build containment
4 laboratories. There were problems in writing codes of
5 practice for a new virus, in getting stocks of
6 antivirals, and getting approval from safety and
7 veterinary authorities. All kinds of hurdles we had
8 to overcome to actually get up and running and working
9 with the pathogenic virus.
10 Now we have containment laboratories in
11 quite a few laboratories throughout the world and it
12 is really important that these are maintained. One
13 possibility that we may want to consider is that we
14 have at least one or two containment laboratories
15 within a site of manufacture. So that without any
16 change at all, a completely novel possibly even
17 pathogenic virus can be grown and produce a few
18 thousand doses of vaccine that could be used for
19 people in surveillance laboratories and for other
20 vaccine manufacturers and for the staff in those
21 laboratories. And we have learned that if we are
22 going to do this, we certainly need small scale
23 production plans.
24 We have had problems in producing
25 reassortants. Normally this process is very

1 efficient. We produce them more or less to order with
2 the normal Influenza A strains. But with the H5
3 virus, we have problems. So maybe it is time to
4 reflect and ask ourselves are we doing this the right
5 way. Are there other methods we could look at such as
6 using plaquing in mammalian cells or maybe using
7 reverse genetics to produce the reassortants in the
8 future.
9 Rob Webster has, for a number of years,
10 put forward the idea that we should be working up
11 banks of seed viruses for likely pandemic strains. I
12 think with our recent experience, I really believe it
13 is time to start working with this. H5's and H9's and
14 H4's and maybe other possible subtypes -- H2's. And
15 start to build up banks of reassortments and SRD
16 reagents to measure the vaccine potencies.
17 Fast track regulatory systems. We never
18 actually got the marketplace with H5 vaccines. There
19 was no need for it. So there were no licensing
20 problems. But you can envisage that with some of the
21 strategies that were being followed to make H5
22 vaccines, there would have been problems. Because
23 some of those were genetically modified products,
24 which are not currently licensed. We have had
25 preliminary discussion in Europe, a brainstorming

1 session back in March of last year, to try and think
2 of methods to fast track novel vaccines in a pandemic
3 situation, and also to fast track the mandatory
4 testing of those vaccines. And I think that is
5 something that should be encouraged.
6 And finally, we should continue developing
7 alternative technologies to produce vaccines. We
8 shouldn't rely on the whims of a chicken laying eggs.
9 We should keenly pursue cell culture vaccines,
10 adjuvanted vaccines and DNA vaccines, just to name
11 few. Thank you very much.
12 CHAIRPERSON FERRIERI: Thank you, Dr.
13 Wood. I don't know, Roland, if you had built in time
14 for questions from the panel. How do you feel about
15 the afternoon? The ones who have stayed are to be
16 congratulated, but I guess we could have some room for
17 at least a question. I understand the next presenters
18 have to leave at 4:30. So we will keep that in mind in
19 the time we take for any questions. But if you have
20 questions for Dr. Wood at this point? I guess not.
21 So we have another presentation called additional
22 vaccine activities by T. Mabrouk from Biochem Pharma
23 in Canada and Dr. Li from Aviron. Will both of you be
24 presenting? Only one presentation, okay.
25 DR. LEVANDOWSKI: Dr. Ferrieri, there are

1 actually two separate presentations.

2 CHAIRPERSON FERRIERI: So there will be

3 two presentations. Dr. Mabrouk?

4 DR. MABROUK: Thank you very much.

5 CHAIRPERSON FERRIERI: Thank you. I would

6 advise then that we keep it with two thirty minutes

7 maximum total for the two of you.

8 DR. MABROUK: Sure. First of all, I am

9 very pleased to be here to present some results

10 regarding the H5N1 project. This study was done at

11 Biochem Vaccines, which is a subsidiary of Biochem

12 Pharma. First of all, I have to thank very much Dr.

13 Levandowski to give this opportunity to Biochem

14 Vaccines to present some results regarding this

15 project.

16 So we have talked today about the H5N1

17 project, and this is the plan of my presentation.

18 First of all, I will do a very small introduction

19 because I think the most important things was done by

20 Dr. Levandowski and Dr. John Wood. The second one,

21 the objective of the study. And after that, what is

22 the strategy to do that and the results.

23 So as you see, the results is divided into

24 three parts. The first, how we assess the identity of

25 the clone H5N1, and the second one, how we assess the

1 purity of the clone and some immunogenicity data that
2 we have already now.
3 So I have to clarify one point before I
4 start the presentation. Biochem Vaccines is
5 developing now with our new partner, which is Smith-
6 Kline Beecham, a new technology for the production of
7 the vaccine using cell technology. At Biochem
8 vaccine, we have now a new clone, which the name is
9 BV5F2. It is MTCK derived cell line, but this cell
10 line is not a mutagenic cell line. So during all
11 these studies, I will talk about BV5F1 and the clone
12 was isolated using this BV5F1, which is, as I said,
13 not a tumorigenic cell line.
14 So as you know, H5N1 is subdivided into
15 two groups, 156-like virus and 413-like virus. It is
16 the first time that we showed a direct transmission
17 from the poultry to humans. We had 18 cases of
18 infection reported and we have 6 persons who died. So
19 the virulence of this H5N1 is associated with a
20 stretch of basic amino acids as the cleavage site
21 between H1 and H2. And the other particularity of
22 this clone is that we have deletion of 19 amino acids
23 on the neuraminidase protein.
24 So the objective of this study is to
25 develop a non-pathogenic H5N1 in order to produce

1 split H5N1 vaccines using the cell technology and
2 without using PC facility. That is the objective of
3 this study. And as you know and as it has been
4 mentioned, Protein Sciences has good results regarding
5 the complement H5 proteins and Aviron is working on
6 the itinerary to H5N1.
7 So here in our strategy we used the Duck/
8 Singapore virus and the swine virus. And as it had
9 been mentioned by Dr. John Wood, we used the Singapore
10 virus because the H5 of this virus is very close with
11 the H5 of Hong Kong and the N1 of swine virus is very
12 close to the N1 of the Hong Kong virus.
13 So what we did is we infect the virus by
14 these two viruses and after that we purified the
15 clones by using the technology of plaques technology.
16 So here the first step is the plaque purification.
17 And after that, we do the immunoselection using the
18 antibody against a New Jersey/876 to neutralize H1 of
19 the swine virus. So we isolate some clones here. In
20 this case, we have 50 clones. And we grow these
21 clones and after that we select only the clones that
22 show very high growth on the cell lines.
23 So after that, the first step is to be
24 sure that our clones is H5N1, and we do that by PCR.
25 So we have four clones positive for H5 and positive

1 for N1 segments. And the second step is to do the
2 same selection using the same antisera. After that,
3 we have to do the exercise of plaque. I mean, to make
4 another selection, and we have to analyze by PCR and
5 we have to be sure that at the first passage, we do
6 not have any contamination -- I mean H1 and N3.
7 After the first passage, we have to do
8 five passages on the cell line to be sure that our
9 virus is not contaminated. So we do the same exercise
10 after the five passage. We do identity. We look for
11 the H5N1. We assess the purity by looking for the
12 contaminant H1N3. So in this case here, we have the
13 clone 1A, 1B, 5A, 10B, and 27A positive. When I say
14 positive, that means that H5 and N1, and we do not
15 have any contaminant, N3 and H1.
16 So I don't think that I have to go through
17 all these techniques. I think that you know the
18 techniques. The first step is to do the reverse
19 transcription for cDNA and after that we have to
20 amplify the segment hemagglutinin or neuraminidase,
21 and this depends on the kind of primer that you use.
22 So this primer is used for the identify.
23 So to assess the purity of the clones, we have to do
24 the first PCR. And then the second time, we have to
25 do the nested PCR. So we use two microliters of the

1 first PCR reaction to do another PCR to be sure that
2 we do not have any contaminations. So this is the
3 primer that has been used for the nested PCR.
4 So here are the conditions of the first
5 reverse transcription. So as usual, you know it very
6 well. And we did only one cycle to amplify and to
7 synthesize cDNA. Here are the conditions of the PCR
8 reactions. So we use two primers, 5 prime and 3
9 prime, depending on the segment. And we did 30
10 seconds, 55 degrees for 30 seconds and 70 degrees for
11 one minute. All of these conditions I think is
12 already published on the papers. So here is the
13 nested PCRs that I did. As I mentioned before, we
14 used only 2 microliters from the first PCR and we do
15 the same reaction of PCR, 30 seconds.
16 So here we show you the results that we
17 had. So for the passage 1 and for the identify, we
18 have here the results of the PCR reactions. We put
19 here all the positive clones and all the counter swine
20 virus, the dog virus, and the negative as a mark. And
21 we amplify the segment by using primers, which
22 normally does give to us a fragment of 295bp. So as
23 you see here, only for the H5 we detected that for the
24 dog. We do not detect anything for the swine. And we
25 have 1A, 1B, 5A, and 10B and 27A positive for H5. And

1 for the N1, we have all these clones are positive for
2 neuraminidase. So we pursue these studies using only
3 the clones which are positive for H5.
4 So this is after passage 5. We get the
5 same reactions using the same primers. And we do see
6 that we have the same positive results for the clones
7 1A, 1B, 5A, 10B and 27 A, and we have nothing for the
8 negative and we have nothing for the swine virus. And
9 for the neuraminidase, we have the same pattern of
10 results.
11 Now it shows here that after passage #10,
12 we have the same kind of results after passage #1. So
13 this is to be sure that our clone is H5N1 by doing
14 PCR. Now to be sure, we present a lot of veracity to
15 support the idea and to support the results that it is
16 H5N1 and not another clone. So the first is the PCR
17 and the second is the sequence. So we sent the
18 sequence to another lab and after that we sent the
19 sequence to the Internet, and we have here as you see
20 93 percent of identity between our clones and the Hong
21 Kong sequence. And we did that for the hemagglutinin
22 molecule and for the neuraminidase protein.
23 So this is the nucleotide sequence. It
24 doesn't mean that for the amino acid sequence we will
25 have the same percentage of identity. Maybe we could

1 have higher than that. But we did not have these
2 results now.
3 So here is some serological results where
4 we used post-infection ferret sera against
5 Duck/Singapore H5N3. This is lot number 1, if I can
6 say, and Duck/Singapore #2 and A/Texas and Nanchang.
7 And after that, we react these post-infection ferret
8 serum against these viruses and these clones. So
9 here, as you see, all the clones are very -- they give
10 us a high titer when we use the Duck/Singapore post-
11 infection ferret serum. But we do not understand why
12 when we use the A/Texas and the Nanchang, we have a
13 very high titer here in this case. This was repeated
14 many times and we have almost the same kind of
15 results.
16 So now to be sure that our clone is pure
17 and is not contaminated, so we have to assess the
18 purity of the clones at passage #1 and passage #5. So
19 here I am showing the results of the purity after
20 passage #1. This is for the hemagglutinin H5. So
21 here we detect H1 only for the swine, but we do not
22 detect anything for the other clones. This is the
23 nested PCR, and we have the same pattern even after
24 the second PCR. So it does mean that our clones, 1A,
25 1B, 5A, 10B and 27 A are pure at passage #1.

1 And we did the same thing for the
2 neuraminidase and we see that here we have one for the
3 Duck/Singapore, but it is not clear here on the photo.
4 But on the gel, we have it. It is very hard to
5 convince you. And we did the same thing for the
6 neuraminidase here at the second PCR, what we call
7 nested PCR, and we detect only one band here for the
8 Duck virus. And we have the same results after
9 passage #5. So we have no contamination with our
10 clone.
11 So based on these results, we can say that
12 the clones that we have -- clones 1A, 1B, 5A, 10B and
13 27A -- are H5N1-like virus, and we cannot find any
14 contaminant on these clones. So we have the same
15 results.
16 So we did some immunogenicity activity
17 testing by doing hemagglutination inhibition tests.
18 So we used post-infection sera against Hong Kong
19 viruses. We have here the lot number. This is against
20 the 156-like virus and this is against 483 and this is
21 against 491. So actually you can see here for the
22 clone 1B, we have a high titer with all these post-
23 infection ferret sera. And for the B/Harbin, we
24 detect the titer was less than 20.
25 We could not really do -- I mean, to

1 complete these experiments, we have to use the post-
2 infection ferret sera against our clone, but we do not
3 have that antisera now. So we administer this
4 recombinant to the mice and here we have the results.
5 This is the number of the mice -- 1, 2, 3, 4, 5, 6.
6 Mice #'s 1, 2 and 3 received PBS and the mice #'s 4,
7 5, 6, 7, 8, 9, and 10 received the H5N1 vaccine. So
8 here as you see for our recombinant 1B, we have some
9 mice, #5, who has some immune response against H5N1
10 vaccine. For the A/Singapore, you have only two mice
11 who showed some immune response. But for the swine,
12 the level is very, very low and for B/Harbin.
13 So if I can summarize all of these
14 results, we can say that for the identity, we can say
15 that our clones are H5N1 based on the results of PCR
16 sequencing and immunoreactivity. And we can say that
17 our clones are pure based on the results of nested
18 PCR. And we can say that these clones are immunogenic
19 based on the results of immunogenicity in mice.
20 So I have to thank Dr. Janique Forget and
21 Francine Allard, who did this work, and I have to
22 thank very much Dr. John Wood from the NIBSC, and Dr.
23 Nancy Cox. Dr. John Wood sent to us all the Singapore
24 and swine virus, and Dr. Nancy Cox sent to us all the
25 reagents to assess the immunogenicity of these

1 viruses. Thank you very much.

2 CHAIRPERSON FERRIERI: Thank you, Dr.
3 Mabrouk. Roland, did you have any questions for him?

4 DR. LEVANDOWSKI: I guess maybe I would
5 make just a comment that it is remarkable considering
6 what the rest of us have been trying to do working in
7 eggs to make reassortants. It seems that there was no
8 problem at all using this technique of picking out the
9 clones and then working with them separately rather
10 than having to work with a complete mixture. And
11 actually, this is something that Dr. Kilbourne has
12 been talking about for years already, and it seems
13 like a technique that we should be moving toward
14 adopting. I don't know if Dr. Kilbourne has some
15 thoughts on that.

16 DR. KILBOURNE: You have expressed them
17 well.

18 CHAIRPERSON FERRIERI: Other comments from
19 the panel here? Otherwise, we will move on then to
20 Dr. Li from Aviron. And then when he is through, we
21 will go back to the purified HA vaccine trials
22 originally slated for 3:15.

23 DR. LI: We have been working very closely
24 with the CDC, NIH and FDA to develop vaccine
25 candidates against the H5N1 influenza viruses. I am

1 going to summarize the preparation and the
2 pathogenetic testing of two candidates which can be
3 used to prepare live attenuated vaccines and which can
4 also be used as a substrate for manufacture of
5 inactivated vaccine.
6 We know from the previous introduction by
7 Dr. Levandowski, we started the vaccine development
8 approach in January of 1998. We know from work done
9 at the CDC and by others that there were highly
10 pathogenic viruses. We knew that all genes of the
11 H5N1 isolate are of avian influenza origin. We knew
12 there were two antigenic subgroups coexisting with 156
13 and 483, the prototypes respectively. We knew that
14 the HA cleavage site contained much of the basic amino
15 acids which might contribute to the pathogenesis seen
16 in chicken and possibly in humans.
17 Based on this information, we needed to
18 find a vaccine approach which not only can result in
19 a safe, efficacious vaccine for human use, but also is
20 safe for personal immune development and the
21 protection of the vaccine. You already heard about a
22 vaccine approach by using baculovirus system or by
23 using apathogenic antigenic strain. I am going to
24 present to you a vaccine strategy which is illustrated
25 here by applying genetics.

1 The strategy is to modify the HA cleavage
2 site by deleting most of the basic amino acids and
3 then to produce a recombinant 6:2 reassortant virus
4 which contains the genetic modified HA agent and an
5 unmodified NA agent from the H5N1 Hong Kong isolates
6 and the remaining genes for a very long life itinerary
7 of ours, the ca A/Ann Arbor/6/60 strain which was
8 developed many years ago by Dr. Massab for the
9 University of Michigan.
10 For this purpose, we have cloned the HA
11 and NA gene ofboth the 156 and the 483 strain. In the
12 HA construct of our vaccine candidates, we deleted the
13 five basic amino acids at the HA cleavage site you can
14 see here. In addition, we inserted a 1 sera reduce
15 bag to mimic the low pathogenic HA or NA gene. The
16 remaining arginine codon of the vaccine construct was
17 changed to appear medium codon to increase the
18 stability of the construct.
19 Then we applied the recombinant technology
20 to introduce the cloned HA and NA genes into the coded
21 WR spectrum. To prepare candidates in 483, we were
22 forced the transfect the modified 458 HA and NA gene
23 into the coded WR spectrum to generate the 7:1
24 intermediate. Subsequently, we have transfected the
25 wild type unmodified 483 NA gene into the 7:1

1 intermediate to generate the 6:2 vaccine candidates.

2 Candidate MVS/156 was generated in a

3 slightly different way in which we explore the

4 possibility of transfecting HA and NA genes

5 simultaneously but in two different reactions. In

6 fact, we were able to generate two intermediates, one

7 bearing the HA gene and one bearing the NA gene.

8 Subsequently, we applied this classical reassortant

9 technique to generate the 6:2 MVS/156. I should have

10 mentioned that we also use a condition similar or very

11 close to the GMT condition to produce the vaccine

12 strain, including a useful for doing the transfection

13 of for vaccine production.

14 Here our colleagues at the CDC test the

15 antigenicity of the vaccine candidates by using ferret

16 sera risked against 12 different H5N1 Hong Kong

17 isolates, including against the wild type 156 strain

18 and the 483 strain. Overall, we can see that the

19 antigenicity of the MVS/156 were similar to the wild

20 type of 156, and the MVS/483 is antigenically more

21 similar to the wild type 483 virus.

22 We did some of the original

23 characterization of the vaccine candidates. We show

24 here both candidates reduplicated a reasonable titer

25 in eggs with the MVS/156 up to $10^{9.4}$ EID 50 per ml.

1 This is an important finding because the production
2 capacity of the vaccine is largely dependent on the
3 replication of the worst titer in eggs, currently the
4 only substrate approved for production of the
5 influenza virus vaccine.
6 Duck/Singapore/97, as you know from the
7 previous speaker, is another potential vaccine
8 candidate. However, it doesn't replicate unless in
9 eggs. In addition, we have shown here that both
10 vaccine candidates replicate at a 25 degree
11 temperature. So they are cold adapted. They are also
12 temperature sensitive with a shut-off temperature of
13 39 degrees. Also, they are parenteral cold adapted
14 Ann Arbor/60 strain.
15 As we know, the virus has much of its
16 basic amino acids at the HA cleavage site. They
17 require trypsin for every replication in many cells.
18 Whereas viruses with single basic amino acid HA can
19 require trypsin for efficient replication. Here we
20 show that the vaccine candidates, after depleting most
21 of the basic amino acids, now require trypsin for
22 efficient replication. In CEF and MDBK cells, we can
23 see the vaccine candidates, like the cold adapted
24 parenteral virus and like the apathogenic or low
25 pathogenic Duck/Singapore strain, form plaque in the

1 presence of trypsin, but in the absence of trypsin, no
2 plaque was detected.
3 We know that type H5N1 viruses are highly
4 pathogenic in chickens and kill them more quickly. We
5 did two experiments in chickens to test how safe are
6 our vaccine candidates. We used two different chicken
7 species. The first experiment was done exactly
8 according to USDA guidelines. We show that like the
9 cold adapted parenteral virus and like the
10 Duck/Singapore/97 strain, the vaccine candidates did
11 not kill any chickens. So they are low pathogenic in
12 chickens.
13 In the second experiment, we increased the
14 inoculation dose by 10-fold. We had other different
15 inoculation routes including the intranasal and
16 intratracheal route. Again we show that like the cold
17 adapted parenteral virus, the vaccine candidates did
18 not kill any chickens. In contrast, the wild type 156
19 and 483 strains killed chickens within two to three
20 days after inoculation.
21 Here we show the vaccine candidates, when
22 distributed in live attenuated form, are capable of
23 inducing protective immunity in chickens. I should
24 tell you at the beginning that a chicken is not a very
25 good model for testing the immunogenicity of the cold

1 adapted virus presumably because of the high body
2 temperature or the temperature sensitive phenotype of
3 the cold adapted viruses. And this I should mention
4 to you that the sample size of this study is very
5 small, each group represented four chickens only.
6 However, we can say here that three of four chickens
7 which were inoculated with MVS/156 were protected from
8 wild type 156 challenge. All four chickens which were
9 inoculated with MVS/483 were protected from wild type
10 483 challenge. Now two out of the four chickens which
11 were inoculated with MVS/156 were protected from wild
12 type 483 challenge, and the two of the four chickens
13 immunized with 483 were protected from wild type 156
14 challenge. However, because of the small sample size,
15 we cannot draw much of a conclusion about whether this
16 caused protective immunity between these two
17 subgroups.
18 And as I expected, cold-adapted parenteral virus did
19 not initiate any protective immunity against both the
20 156 and the 483 strains.
21 Recently, Dr. Perdue from USDA did one
22 more experiment by using inactivated preparation for
23 the MVS/156. What was shown here is that all chickens
24 inoculated with this inactivated preparation were
25 protected from either wild type 156 or 483 challenge.

1 This clearly indicated the vaccine candidate is
2 immunogenic and caused protection in this animal
3 system.
4 The ferret is an excellent model for
5 mimicking the human influenza virus infection. We did
6 two experiments in ferrets to assess the safety
7 characteristics of the vaccine candidates. We show
8 that none of the inoculated ferrets developed flu-like
9 symptoms. In addition, the replication of the vaccine
10 candidates are mostly restricted in the upper
11 respiratory tract. We also cannot detect any virus
12 replication in the lung.
13 Notably, in the second experiment, we
14 didn't observe any replication of MVS/156 in both the
15 turbinate and lung. In terms of immunogenicity, we did
16 a preliminary study in ferrets. Again, because of the
17 limitations which we have to do to conduct the animal
18 trial, we used a single ferret for each of the groups
19 only. We show in the first experiment that the single
20 ferret which was inoculated with MVS/483 shows the
21 same HI antibody response to both wild type 156 and
22 483, whereas the singular ferrets which were
23 inoculated with MVS/156 did not show the same HI
24 response. However, when we repeated the study in the
25 second experiment, we showed that both groups show the

1 same HI response.

2 In summary, we have applied the
3 recombinant technology to generate a vaccine candidate
4 against the H5N1 virus. The candidates contain
5 genetically modified HA and unmodified NA gene from
6 the wild type H5N1 viruses and the remaining gene from
7 live attenuated cold adapted strain. We show that the
8 vaccine candidates are temperature sensitive, they are
9 cold-adapted, they are dependent on trypsin in some in
10 vitro cell lines, and they grow to very good titer in
11 chicken eggs. In addition, the recombinants are
12 nonpathogenic in chickens and safe in ferrets. They
13 are capable of inducing protective immunity in chicken
14 in both the live attenuated form and in the activated
15 form. And finally, we believe that the H5N1 vaccine
16 candidates have satisfied the preclinical material and
17 should be considered for further characterization in
18 humans.

19 First, I want to thank our collaborators
20 who contributed tremendously to this development on
21 pathogenic studies. Drs. Klimov, Subbarao and Cox
22 from CDC, Dr. Perdue from USDA, Dr. Hietala from U.C.
23 Davis, Dr. Liu from Aviron, and Dr. Bryant, who was
24 formally associated with Aviron. Thank you.

25 CHAIRPERSON FERRIERI: Thank you for a

1 very interesting presentation, Dr. Li. Any questions
2 for Dr. Li? His approaches have great promise for
3 other related viruses. I guess not. Dr. Kilbourne,
4 comments or questions?
5 DR. KILBOURNE: Well, the comment is one
6 of admiration for the accomplishment. But I would
7 like to know specifically, when you say this is high
8 yield in eggs, what does that mean in terms of
9 relation to PR8, for example?
10 DR. LI: You made a very good comment. If
11 I said high yield, I should take it back. I tried to
12 say it is a reasonable titer. And certainly we didn't
13 have a comparison. Maybe the inactive manufacture
14 could comment on this much better. We think -- I
15 don't have any idea how -- normally the high yield
16 reassortants, what kind of titer you achieve in the
17 eggs, but the titer, of one of the candidates of 10 to
18 the 9.4 ETD per ml, I thought that was reasonable.
19 DR. KILBOURNE: Okay. I hope you could
20 put some quantitative figure on it, but that is okay.
21 CHAIRPERSON FERRIERI: Yes, Dr. Breiman?
22 DR. BREIMAN: You mentioned removing the
23 5 basic amino acids to produce virulence and then a
24 couple of steps to increase stability of the strain.
25 With your live attenuated form, do you have any way of

1 evaluating the reversion?

2 DR. LI: That is a very good question

3 because why the H5 always have the pathogenic viruses

4 whereas the other sub types don't. Maybe it has

5 something to do with the HA structure itself. What we

6 did basically is, as I also mentioned before, we

7 changed the arginine codon to increase the stability.

8 In addition, we did a sera passaging of CEF cells in

9 the absence of trypsin to see if the phenotype

10 changed. In fact, after three to four passages, there

11 was still a requirement of trypsin for replication,

12 indicating that the modification didn't change.

13 DR. KILBOURNE: Does the neuraminidase

14 clone retain the deletion?

15 DR. LI: Yes. You are absolutely right.

16 The deletion, which is a characteristic of the

17 neuraminidase. At this time, we didn't see a direct

18 correlation of pathogenesis -- of this deletion to any

19 pathogenesis. We didn't do any. In the meantime, we

20 tried to generate mutants, which help to repair to say

21 what kind of phenotype we might have there.

22 CHAIRPERSON FERRIERI: Thank you very

23 much. Now we will move back up to the top. And we

24 will hear presentations on purified HA vaccine trials

25 from Dr. J. Katz, CDC first. The two presentations

1 were originally designed for 45 minutes, but I
2 understand that one or both of you volunteered to
3 contract your talks a bit.
4 DR. KATZ: I guess that is me. I have
5 just removed about five overheads. So hopefully we
6 can keep things shortened. This is just an outline of
7 what I was going to talk about, and I will just give
8 only one overhead as an introduction now. And then I
9 will move straight into a description of the serologic
10 assays that we have been using to evaluate antibody to
11 H5 avian viruses in humans, and then how we have used
12 those assays to determine the serologic results from
13 the Phase I trial that Dr. Levandowski introduced, and
14 then also I will give preliminary results of the Phase
15 II trial being conducted at the University of
16 Rochester.
17 This is my only introduction. Everything
18 else so far has been said by the other speakers. So
19 I just wanted to remind the audience that the 16 H5N1
20 viruses that were isolated out of 17 cases fell into
21 two antigenic groups. And just to remind you of the
22 severity of the disease in humans, with 6 fatalities
23 and two other severe cases, and both groups were
24 fairly equally represented in the fatal and severe
25 cases as you can see by this timeline of the case

1 distribution in May, November and December.

2 So I am going to move on right now into

3 looking at the different serologic methods. It became

4 very evident early on in the outbreak investigation

5 from the H5N1 outbreak in Hong Kong that the

6 traditional hemagglutination inhibition assay was not

7 going to be terribly useful for detecting antibody to

8 H5 viruses in humans, and I will show you a bit of

9 data about that in a minute.

10 So at the time the first case was

11 recognized, we went into development a

12 microneutralization assay to detect antibodies to H5

13 in human sera, and it is just basically an overnight

14 assay looking at the ability of antibodies to inhibit

15 about 100 TCIB 50 of virus, and we have recently had

16 a publication accepted which describes this assay and

17 its useful detection of antibody in human sera. And

18 it also relates to the other assays that we were

19 setting up concurrently. We also looked at the H5

20 indirect ELISA, and this was using the recombinant HA

21 protein prepared by Protein Sciences and expressed in

22 the baculovirus system. And we found that the ELISA

23 was useful and specific for sera from children, but we

24 had so grave limitations in using an IgG ELISA for

25 adult sera, and I will show you why in a moment.

1 So we also -- we wanted some sort of
2 confirmatory assay because the microneutralization
3 assay was new and being used for the first time in
4 this way. So we again used the Protein Science
5 recombinant HA as an antigen in Western blot, and we
6 used this to confirm results in the either
7 microneutralization assay for positive results from
8 adult sera or the ELISA in children.
9 This is just an overview of the
10 microneutralization assay. Basically two-fold
11 dilutions of serum are added to a 96 well plate and
12 then the virus. In our hands, we have been using Hong
13 Kong/156, which means we have been doing these in a
14 BSL-3+ containment facility. It is added and then
15 allowed to incubate for a couple of hours, after which
16 time a relatively low number of low passage MDCK cells
17 are added to the plates and the plates are incubated
18 overnight for 18 to 20 hours. The next day, the cells
19 monolayer is washed and fixed, and the readout is an
20 ELISA detecting the presence of the Influenza A
21 nuclear protein using a specific monoclonal antibody.
22 And the readout, the way we express the titers, is a
23 50 percent endpoint based on control positive and
24 negative wells.
25 So once we had identified several

1 confirmed cases that were confirmed by virus
2 isolation, we had S1 sera that were collected within
3 about 7 days of the symptom onset of the individuals,
4 and in some cases, we also got S2 sera, which were
5 collected about 14 days later. And so we wished to
6 compare the hemagglutination inhibition assay with our
7 microneutralization assay using -- this was Hong
8 Kong/156 H5N1 virus. And you can see for these first
9 two cases, we couldn't detect any sero conversion by
10 HI, but we could detect a nice 8-fold rise from the S1
11 to the S2 by the microneutralization assay. And this
12 was true for other single serum that we got at a
13 substantial time point after infection. And only in
14 situations where we had neutralization titers of over
15 1,000 could we detect any significant HI activity. So
16 we decided that we probably would go ahead and use the
17 microneutralization assay.
18 And this is just characterizing now the
19 primary antibody response to H5N1 infection in 16
20 individuals. This is a combination of all the serum
21 that we could obtain at different time points from
22 different infected individuals. And some of these were
23 just single time points and our ability to collect
24 sera at adequate time points was compromised by the
25 severity of the illness in a lot of individuals. But

1 you can see that the best fit curve pretty much
2 defines a very typical primary response to a primary
3 infection and this dotted line -- this is actually a
4 log scale, but this dotted line represents a titer of
5 80. And we could see that after about 14 days after
6 symptom onset, most individuals were making an
7 antibody response of 80 or more, and we decided to use
8 this as a cutoff for a level of positivity for the
9 presence of H5 specific antibody in human serum. And
10 you can see actually that out by day 20 or so onwards,
11 titers were actually as high as 640 to about 1280. So
12 they were making quite a substantial antibody
13 response.
14 So we could now go ahead and test the
15 sensitivity and specificity of the microneutralization
16 assay using these known confirmed cases of H5N1
17 infection, and we could use these to detect the
18 sensitivity of the assay by just seeing how many of
19 these we could detect antibody in. And then we also
20 used a fairly large number of controls which were
21 collected from individuals age-matched, either adults
22 or children, that were non-exposed, and these were
23 individuals that had come from Hong Kong blood donors
24 or other children's groups in Hong Kong and also
25 individuals in the U.S.

1
2 And we actually compared each of these
3 three serologic assays we had developed separately,
4 but we were really interested in a combination of
5 assays, since we didn't really consider the Western
6 blot was a feasible serologic test to be doing on
7 thousands and thousands of serum, which is what we
8 were looking at when we were doing the outbreak
9 investigation. So you could see -- and we broke this
10 down into children and adults, and it was mainly based
11 on the results we were getting with the ELISA. But I
12 will just go ahead and go through the results with
13 children. You can see that the ELISA was very
14 sensitive in detecting specific antibody to H5 in
15 children and also quite specific. And when we
16 actually combined either the neut or the Western blot,
17 the ELISA was slightly better in both cases. However,
18 this wasn't true in adults and we found that the
19 neutralization assay was superior, and the main reason
20 was the fairly significant reduction in specificity
21 that we found in the IgG ELISA in adult human sera.
22 And you will see more examples of that when we look at
23 the responses of individuals in the clinical trial. So
24 a combination of neutralization and Western blot
25 turned out to be the most specific and sensitive assay

1 that we could use to detect antibody to H5 in adult
2 populations.
3 So I will move on now to some results we
4 have obtained in the Phase I trial that Dr.
5 Levandowski introduced, which was organized by a
6 number of groups and used two doses of the Protein
7 Science baculovirus expressed HA from the Hong
8 Kong/156 virus. We used two fairly low doses, and
9 there were a lot of reasons for choosing these doses
10 at the time. And one of those was just the sheer
11 limitation of the amount of protein that was
12 available. So the decision was made to start out with
13 relatively low doses. So either a 10 or 20 mcg dose
14 were delivered. There were two doses delivered
15 intramuscularly at day zero and then at day 21. And
16 I will be showing you antibody responses detected at
17 pre-vaccination and at various time points post-
18 vaccination. We used all of these assays. I am not
19 going to show you the Western blot, but just to say we
20 have done Western blot results on all of these sera.
21 So the first Phase I trial so far has been
22 conducted at five sites for a total of 56 adult
23 volunteers with an age range of 28 to 66. The first
24 two sites received two doses of 10 mcg of the
25 recombinant H5 protein, 28 volunteers with a mean age

1 of 43. And I believe that this group has subsequently
2 been offered a third dose at the 20 mcg level,
3 although I won't be presenting any of those results
4 today. Then three other sites received 2 doses of 20
5 mcg, and again, there were 28 volunteers with a mean
6 age of 41.
7 And what I have done here is just compare
8 the different assays that we were using to evaluate
9 the immune response to the vaccine and the top is the
10 neutralization. And I have just marked with the
11 arrows the two individuals here that are by our
12 criteria making a response to the vaccine. This was
13 that they needed to have at least a three-fold rise
14 and at least achieve a titer of 80. Now you can see
15 these two individuals actually have high antibody, but
16 they started out with high pre-existing titers. And
17 what I forgot to mention with the sensitivity and
18 specificity of the microneutralization is that we have
19 noticed when we were setting up that in individuals
20 around 60 years of age and older, that we start to see
21 a lack of specificity. And we are presently working
22 to try and determine what this lack of specificity is,
23 but at least in some of these individuals, this is an
24 age-related effect.
25 Interestingly, we also went back to the HI

1 just to see if in this group of individuals, we could
2 detect antibody by the HI. And you can see that we do
3 get a small amount of antibody in these two
4 individuals that are reacting by neutralization. But
5 in this case, it is basically going from less than 10
6 to 20 and from here it is going from less than 10 to
7 40. So it is not really a terribly robust response.
8 And then we also looked at the ELISA, using again the
9 recombinant protein. And again, although we could see
10 many sort of four-fold increases after vaccination, we
11 were again starting out with very high levels, if you
12 look at this ELISA endpoint antibody titer here. We
13 are up in the range of thousands and millions of
14 titer. And so in some cases, we really -- we just
15 couldn't distinguish any significant antibody
16 responses over and above the background.
17 So we did the same sort of comparison with
18 a site that now received a 20 mcg dose, and here again
19 we have 2 individuals out of 12 in this situation that
20 were making a significant neutralizing antibody
21 response. And this time, the HA was -- the
22 hemagglutination inhibition test was absolutely flat.
23 We could not even detect these antibody responses by
24 HI. And because of the very high backgrounds we were
25 seeing with the ELISA, we decided to try something

1 different here. We thought that one of the problems
2 with the ELISA not being H5 specific was that we were
3 probably picking up some cross-reactive epitopes that
4 cross-reacted with other subtypes of Influenza A,
5 which as H3 or H1, which most individuals would have
6 antibodies to. But perhaps these were not very high
7 affinity antibodies for binding to H5. So we went to
8 the method of using various doses of urea, which is a
9 common method for looking at high affinity antibodies.
10 And we found that when we used 8 molar urea as a
11 washing stick in the ELISA, that we could dramatically
12 reduce the background. The scale is now about 10 to
13 100 fold lower. And that we could still see the same
14 sorts of relative increases in post-vaccination serum
15 that we were seeing without the urea in place.
16 However, when you look at these results on a one to
17 one level, you can see that these two individuals that
18 show neutralizing antibody also show a four-fold rise
19 in ELISA, but there are other individuals such as this
20 one which isn't giving any significant neutralizing
21 antibody response but is showing a whopping ELISA
22 antibody titer and there is a couple of other examples
23 of that down here.
24 So the feeling from our lab is that we
25 prefer the neutralization assay still as the most

1 specific assay and sensitive assay in the detection of
2 antibody to H5.
3 And this is a summary now of the sera that
4 we have tested so far from the Phase I trial. As I
5 said, there were five sites, and these are the numbers
6 of individuals in each of those sites. The 10 mcg
7 dose, ultimately only 2 out of 28 individuals gave a
8 greater than three-fold rise and achieved a titer of
9 80 or more for a total of 7 percent of the individuals
10 responding. And as we doubled the dose, we also
11 improved the response somewhat such that a total of 6
12 out of 28 individuals receiving the higher dose or 21
13 percent were showing a response. But that was still
14 far below what we had hoped, and it was somewhat
15 disappointing.
16 Before I go on to a more hopeful end to
17 the talk, I just wanted to briefly mention some of the
18 results we are trying to get now and also looking at
19 the lymphocyte proliferation response in individuals
20 who have received a 20 mcg dose. This was an
21 individual who didn't have an antibody response. This
22 individual did. And you can see when we look at their
23 proliferative response to the recombinant HA's, we get
24 a very nice -- this is just post-vaccination at this
25 point. We get a nice response to either the Hong

1 Kong/156 or the 483 recombinant protein, and that that
2 response is much higher than the background to other
3 recombinants such as the H3 or the H7.
4 This was just really a preliminary assay
5 for us to define the system, and we are going to go
6 back now and look at pre and post-vaccination
7 responses.
8 This is the more hopeful end to the talk.
9 Just a few days ago, we were able to get very
10 preliminary results from John Treanor's Phase II
11 trial, and I think he is going to speak about that and
12 give you a little more detail of that. But that was
13 a dose escalation trial, and we decided to start with
14 the highest dose and work our way down. Sort of
15 rationalizing that if we weren't going to get anything
16 at the highest dose, we could just forget about it.
17 But we have, in fact, gotten some very nice results,
18 and this is the preliminary results from 10
19 individuals. What I am showing here is zero time
20 point at the first vaccination and then 14 days after
21 the first vaccination. Zero time point at the second
22 vaccination and then 14 days after that. But the way
23 the clinical trial has been set up, there are three
24 different intervals between the first and second
25 vaccine, and John will explain a bit more than that.

1 But the encouraging thing is the number of responses
2 we are getting above this line. And in fact, you can
3 see that except for these two individuals, we are
4 getting quite substantial response and even the height
5 of the neutralizing antibody mean titer that we are
6 getting is upwards of 200 to 400, which is very
7 encouraging. You can see that some individuals are
8 responding right after the first vaccination, whereas
9 other individuals seem to require the two doses.
10 So just to put that in perspective with
11 the earlier study now, we can see that, again, for
12 only a very limited number so far examined, for 10
13 individuals we have 8 of them responding and we have
14 a very nice pre to post -- or at least a post-
15 vaccination GMT rise in the neutralizing antibody
16 response. So of course we will be working in the
17 future to go backwards now and look at the lower doses
18 and see what the minimal dose is that will give
19 satisfactory immunogenicity.
20 So in summary, I just wanted to remind you
21 that in a naturally infected individual, by 14 days
22 post infection, we can see titers of 80. And after 20
23 days or so, the typical neutralizing antibody titers
24 were as high as 640. The results I just showed you
25 are somewhere in-between that, and that would suggest

1 to me a pretty satisfactory response to a vaccine.
2 So we have used the microneutralization
3 assay with confirmation by Western blot. I should say
4 the last set of results I showed you have not yet been
5 confirmed by Western blot, but I am confident that
6 that is just a formality. And we have used it to
7 adequately detect antibody to H5N1 in adult sera.
8 When we vaccinated individuals at the 10
9 or 20 mcg dose using the recombinant HA, we show
10 fairly low antibody responses in only 7 of 21 who
11 responded respectively. However, I will leave it to
12 John Treanor now to spend a bit more time on his dose
13 escalation study, and I think the results from that
14 will be far more promising. Thank you.
15 CHAIRPERSON FERRIERI: Thank you, Dr.
16 Katz. Why don't we move on to Dr. Treanor. While he
17 is coming up front, if anyone at the table has a
18 comment or question for Dr. Katz. Dr. Hoke?
19 DR. HOKE: Do you have any
20 characterization of the confirmation of the antigen?
21 DR. KATZ: Yes. It is folded correctly
22 and I think Dr. Wilkenson, who is from Protein
23 Sciences, can probably address that. But it actually
24 has HA activity, so it has correct 3-dimensional
25 structure as far as we can tell, and actually forms

1 sort of a rosette that they can look at under the BM,
2 is that correct?
3 DR. WILKINSON: Bethanie Wilkinson from
4 Protein Sciences. And I didn't hear the question, but
5 I think it is about the structure of the protein and
6 whether it forms chimers? Is that correct?
7 DR. HOKE: Yes. I was -- you know, it
8 seems as though there is a specific activity -- there
9 is activity, but the specific activity is low,
10 suggesting that there are lots of -- there is a lot of
11 poorly formed protein.
12 DR. WILKINSON: I am sorry, I don't
13 understand where you would get that from.
14 DR. HOKE: Well, the dose is so much -- is
15 -- well --
16 DR. KATZ: One thing that we probably
17 should say is that this is on just a mcg of protein
18 basis, and I don't think you can exactly correlate
19 that to the 15 mcg of hemagglutinin that is used in
20 traditional vaccines which is measured by a different
21 method. And the point has been made in the past that
22 at some point we probably should compare those
23 directly. But I think the assumption was that if you
24 use the method that is used by the FDA, that 10 mcg
25 dose may actually be a little bit lower.

1 DR. WILKINSON: Actually, we have measured
2 the specific activity and it is very good. The doses
3 were too low and you do need a higher dose. But with
4 a vaccine where people aren't naive, you can use a 15
5 mcg dose and you get equivalent to what you get in a
6 current vaccine. So we think it is -- it is probably
7 just as biologically active. There are slight
8 differences in the glycosolation which may account for
9 some of the differences that we see.
10 CHAIRPERSON FERRIERI: Thank you. Dr.
11 Treanor, University of Rochester.
12 DR. TREANOR: I'm going to guess that
13 anybody who is still here probably missed their
14 flight, so I can just talk for as long as I want. But
15 we will try and go through this briefly.
16 We have been working with the recombinant
17 baculovirus for several years through the vaccine
18 evaluation units. And a number of years ago, just by
19 way of background, we did some very preliminary
20 immunogenicity studies which are shown here. This is
21 just a comparison of the HAI responses to various
22 doses of recombinant either H3 or H1 hemagglutinin or
23 bivalent vaccine. And I just want to show you that
24 the various doses of the Beijing, which is the H3, or
25 the Texas, which is the H1, did induce levels of HAI

1 antibody, which is a functional antibody, which were
2 comparable to those induced by sub virion vaccine in
3 that there was a dose response with relatively higher
4 doses of the recombinant hemagglutinin resulting in
5 progressively higher levels of post-vaccination
6 antibody, much more striking for the H3 responses than
7 the H1 responses in healthy adults.
8 Now the next slide. Those HAI responses
9 were also associated with the development of
10 neutralizing responses against H3 and H1 measured by
11 microneutralization tests very similar to the one that
12 Dr. Katz just described. In one of the studies, and
13 these studies were done in collaboration with Doug
14 Powers at St. Louis University and also with Peter
15 Wright at Vanderbilt. And in one of the studies,
16 although not designed as a formal efficacy trial, we
17 did follow the subjects during the flu season and we
18 did see some suggestion that the vaccine had
19 protective efficacy. This was one of the dose-ranging
20 studies in which individuals received 15 mcg or 15 mcg
21 with alum or 90 mcg, or received a trivalent vaccine
22 or placebo in a randomized double blind fashion, and
23 what you can see is that the rate of laboratory
24 confirmed influenza illness was 13 percent in the
25 placebo recipients and it was 1 percent in the

1 combined 77 subjects who had received recombinant
2 hemagglutinin in any dose, which is suggestive that
3 there would be some protective efficacy here, although
4 clearly this is not a definitive test of efficacy.
5 When the H5 situation arose, the
6 recombinant H5 product was ready very early, and so it
7 was available for testing. And we designed a study
8 which was mostly involved with objectives of
9 determining the minimum effective dose with the idea
10 that the strategy would be to try and stretch the
11 available quantities of vaccine as far as possible,
12 and also to try and determine what type of schedule of
13 administration would induce antibody the most rapidly,
14 believing that possibly this would necessary if a
15 pandemic were eminent. So that the study was designed
16 to look at dose-related antibody responses, the
17 effectiveness of low-dose boosting, the optimal
18 interval for boosting, and the kinetics of antibody
19 following the second dose.
20 This had results in a somewhat complicated
21 study design, but we looked at doses of 25, 45, 90 mcg
22 for an initial dose followed by boosting with either
23 25, 45, 90, or 10 mcg. And we randomly assigned
24 people to either a 21 day interval between doses, 28
25 days, or 42 days, with a corresponding placebo group

1 in each cohort so that people were, of course,
2 unblinded as to interval but were blinded as to dose
3 assignment.
4 The vaccine was administered in a total of
5 1 ml intramuscularly. Individuals filled out a diary
6 card for 7 days, and serum for antibody was drawn
7 before and 14 days after dose one and then before, at
8 7, 14, 21 and 28 days after dose 2. A lot of sera.
9 This is a lot of stuff to handle in the
10 microneutralization lab. The sera was tested by an
11 IgG ELISA against baculovirus expressed antigen, which
12 was done in protein sciences, and then in heroic
13 efforts at CDC by microneutralization against the Hong
14 Kong virus.
15 Now very briefly, the results of the
16 formal safety analysis has not been completed, but
17 there were no serious adverse events. There was one,
18 I guess significant event. One of the subjects became
19 pregnant after the first dose. This was a woman who
20 had had a tubal ligation and became pregnant anyway.
21 We don't know whether this was a vaccine-related
22 effect or something else. There were no complaints of
23 severe arm pain or swelling in any dose group. No
24 individuals had fever following vaccination, and
25 generally the vaccine was extremely well tolerated as

1 had been seen with the recombinant H1 and H3 products.
2
3 Now these are antibody responses in the
4 21-day interval group assessed by ELISA. And this
5 ELISA is done at a single dilution of 1:1000. This is
6 just sort of screening preliminary information. As
7 Jacquie mentioned, there is some background even in
8 these subjects who clearly have never been exposed --
9 well, I shouldn't say clearly, but probably have never
10 been exposed to H5 viruses before -- at a single
11 1:1000 dilution, OD readings in the pre-vaccination
12 sera ranged from undetectable to 0.6. And to clean
13 this up a little bit, what we have done is actually
14 subtract the first sera's value from all subsequent
15 sera to try to adjust everything down to a single
16 baseline.
17 And what you can see is that 14 days after
18 a single dose, there is a significant increase in the
19 amount of OD reactivity at 1:1000, and that with the
20 boost at 21 days, there is not much of an additional
21 increase. But the striking thing here was that
22 particularly at 45 and 90 mcg doses, there is a very
23 significant increase in reactivity against the
24 baculovirus expressed H5 antigen by ELISA.
25 Now the next slide shows the results from

1 the 28-day interval group, which are very similar.
2 Again, at the higher doses, 90 mcg and 45 mcg, there
3 is a very significant increase in OD reactivity. This
4 is a placebo group in which there is no increase, and
5 the 20 mcg dose in which there is a trivial increase
6 in OD reactivity. At 28 days, the antibody has
7 drifted down a little bit and there does appear to
8 possibly be a response to boosting. And then the next
9 slide shows the 42-day interval. Again, a response to
10 the initial vaccine, which is dose dependent, and then
11 possibly a response to boosting. Now I should point
12 out that this is preliminary and only has been
13 completed for about half the subjects in the trial.
14 And so it is really premature to draw conclusions
15 about the effect of boosting. But strikingly, there
16 is an increase in antibody, at least as measured by
17 ELISA, even after a single dose, and that is
18 summarized on the next graph.
19 This shows the pooled results for all
20 individuals who received any dose, regardless of
21 interval, and just looks at the OD reactivity 14 days
22 after the first dose. And you can see that there is
23 a dose related effect in terms of the amount of
24 antibody as measured by ELISA at 14 days, with the
25 highest levels at 90 and 45 mcg. These are the plus

1 and minus, the standard area. The results in terms of
2 OD, total increase in OD, is statistically
3 significantly different in the 45 and 90 mcg doses
4 than it is in placebo or 20 mcg, using as a crude
5 criteria an increase in antibody of 0.4 units or
6 greater. There were a total of 8 out of 16
7 individuals who appeared to have responded at 20 mcg,
8 10 out of 16 at 45, and 25 out of 30 at 90. This is
9 obviously a completely arbitrary criteria, but is
10 beyond the amount of variability from assay to assay
11 when assaying the same sera by several standard
12 deviations.
13 Now as Jacquie alluded to, we have very
14 preliminary data regarding neutralizing antibody
15 responses which are shown here. What we elected to do
16 to try and cut the work load down is to simply assay
17 the sera from individuals who had received 90 mcg or
18 placebo. These assays were done by Dr. Katz in
19 blinded fashion without knowing either the order in
20 which the sera were obtained or whether the volunteers
21 had received vaccine or placebo. And this looks at
22 the serum results just in the vaccinees. This is the
23 log 2 titer of neutralizing antibody plus or minus the
24 standard error. And what you can see is that there is
25 an increase, a significant increase, in antibody level

1 14 days after receiving 90 mcg, and another increase
2 after receiving a booster dose of 90 mcg.
3 Unfortunately, I can't count. This is really 10.
4 Using that criteria of a three-fold increase to a
5 level of over 80, there were 4 out of the 10
6 individuals who had responded by the pre-dose 2 sera
7 and 8 out of 10 individuals who had manifested a
8 neutralizing antibody response following two doses.
9 So the preliminary conclusions of this
10 study are that the recombinant H5 is well tolerated at
11 doses as high as 90 mcg. Serum binding and
12 neutralizing antibody were detected as early as 14
13 days following a single 90 mcg dose. Serum binding
14 antibody was detected after a single dose of 45 or 90
15 mcg, but was infrequent after 20 mcg, which is similar
16 to what was seen in the Phase I study. And the rapid
17 responses to a single dose may suggest that normal
18 U.S. adults are partially primed for responses by a
19 previous exposure to H1 or H3 influenza, although that
20 is really very speculative. But it is interesting to
21 see those very high responses to a single dose.
22 The study was sponsored by NIAID, Gina
23 Rabinovich and Bill Blackwelder played a major role in
24 the study design, especially Bill with the statistical
25 approach. Bethanie Wilkinson and Gale Smith are at

1 Protein Sciences. Bethanie has done the ELISA's.
2 Jacquie Katz and Nancy Cox from the CDC influenza
3 branch, and at the University of Rochester, the study
4 coordinator, Diane O'Brien.
5 CHAIRPERSON FERRIERI: Thank you very
6 much, Dr. Treanor. This is open now for comments or
7 questions. Dr. Levandowski?
8 DR. LEVANDOWSKI: I have got a question.
9 Thinking back to the data that you showed for the H1
10 and the H3, where there was, I guess, a pretty evident
11 difference in what could be called the dose response
12 curve, can we draw any conclusions from that for other
13 Influenza A subtypes or hemagglutinin subtypes? Do
14 you think that there is any validity to that
15 difference that you were seeing with the H1 and the
16 H3, and should we plan to -- or should we expect more
17 of that for other subtypes?
18 DR. TREANOR: My own opinion is that at
19 least in the way that the vaccine is currently being
20 vialed and delivered that it is likely that responses
21 to 45 mcg doses or in that range will be better than
22 to 10 or 15 mcg for the recombinant hemagglutinin.
23 And there are multiple possible reasons for that that
24 you could speculate about. But just in terms of the
25 practical observations, it seems to be fairly

1 consistent that 45 mcg doses are better just in our
2 experience.
3 CHAIRPERSON FERRIERI: Yes, Dr. Hoke?
4 DR. HOKE: Well, I would just like to
5 commend your whole group for their persistence, and it
6 really looks like you've got something. I did some
7 work some years ago in another system where there was
8 HAI and neut and it was Japanese encephalitis, and it
9 was very curious as to how ELISA and HAI and
10 neutralizing antibody responses to vaccine worked out
11 sometimes in rather paradoxical ways, but that the
12 neutralizing tests somehow always seemed to be the one
13 that was most clear in spite of the difficulty of
14 doing a biological assay.
15 I am puzzled a little bit by some
16 suggestion early on that there was -- that there is
17 some background antibody that you had to adjust for?
18 This is H5 and no one is supposed to have been exposed
19 to that.
20 DR. TREANOR: By ELISA, I think that
21 looking at the health laboratory workers in the Phase
22 I study and even in our healthy adults, there is a
23 certain amount of binding of that very highly purified
24 H5 antigen by sera from individuals who would not be
25 expected to have had prior exposure. And this may

1 reflect antibody which cross reacts with -- or which
2 is directed against cross-reactive epitopes. That is
3 a very interesting question which could be approached,
4 but right now I don't know what the answer to it is.
5 DR. HOKE: And even at higher doses, I
6 don't remember if you said, but was there ever any HAI
7 antibody?
8 DR. TREANOR: We didn't test it. Now that,
9 as Jacquie was explaining, is subject to a degree of
10 technical issues related to affinity with the
11 hemagglutinin and red cells and whatnot. But in this
12 assay, I don't think we have done HAI testing. That
13 is something that will be done. As far as the other
14 thing is concerned, someone mentioned earlier, I think
15 actually John Wood, that some of this cross-reactivity
16 can be eliminated by adsorption of the sera with H3
17 antigen. Now one possibility is that it is directed
18 against the common antigens. But because you can also
19 eliminate it in the ELISA to a certain extent by
20 adsorption with purified H3 hemagglutinin, my
21 suspicious is that at least some of it is directed
22 against cross-reactive epitopes on the hemagglutinin
23 itself.
24 CHAIRPERSON FERRIERI: Thank you very
25 much. It is now time for the open public hearing.

1 This is an official part of our posted program in the
2 Federal Register. Is there anyone in the audience who
3 would like to come forward and say anything? If not,
4 I would like to thank all the remaining members in the
5 room for staying. I thought the program was very
6 interesting and I want to thank you, Roland, for
7 organizing everything today. Thank you.
8 (Whereupon, at 5:07 p.m., the meeting was
9 concluded.)
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CERTIFICATE

This is to certify that the foregoing transcript in the matter of: MEETING

Before: VACCINES AND RELATED BIOLOGICAL PRODUCTS ADVISORY COMMITTEE

Date: JANUARY 29, 1999

Place: BETHESDA, MD

represents the full and complete proceedings of the aforementioned matter, as reported and reduced to typewriting.