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FOOD AND DRUG ADMINISTRATION

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NUCLEIC ACID TESTING FOR HCV AND OTHER VIRUSES IN BLOOD DONORS

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WORKSHOP

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WEDNESDAY, SEPTEMBER 16, 1998

The workshop took place in Conference Rooms D and E, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857 at 3:30 a.m., Edward Tabor, M.D., Chairperson, presiding.

PRESENT:

EDWARD TABOR, MD	Chairperson
JAY S. EPSTEIN, MD	Director OBRR
HARVEY J. ALTER, MD	Speaker
MIRIAM ALTER, PhD	Speaker

PRESENT: (CONT.)

CELSO BIANCO, MD	Speaker
JENS BUKH, MD	Speaker
ROGER DODD, PhD	Speaker
INDIRA HEWLETT, PhD	Speaker
STEVEN KLEINMAN, MD	Speaker
NICO LELIE, PhD	Speaker
MICHA NUBLING, PhD	Speaker
BETTY ROBERTSON, PhD	Speaker
BUY RAUTMANN, PhD	Speaker
JOHN SALDANHA, PhD	Speaker
LEONARD SEEFF, MD	Speaker
PETER SIMMONDS, MD, PhD	Speaker
MEI-YING W. YU, PhD	Speaker

INDUSTRY PRESENTERS:

ANDREW CONRAD, PhD	National Genetics Institute
CHUCK FRISBIE	Alpha Therapeutic Corporation
SUSAN CUSHING, MD	Baxter Healthcare Corporation
ELIZABETH DRAGON, PhD	Roche Molecular Systems, Inc.
BARBARA MASECAR	Bayer Corporation
CHARLES WATSON, PhD	Centeon L.L.C.

PRESENT: (CONT.)

INDUSTRY PRESENTERS (CONT.)

CHRISTINA GIACHETTI, PhD	Gen-Probe
SUSAN STRAMER, PhD	Amer. Red Cross
GERALD ZERLAUTH, PhD	Immuno AG
RICHARD FLANDERS, PhD	Abbott Labs.
MILTON PSALLIDOPOULOS, PhD	North American Biologicals, Inc.

C-O-N-T-E-N-T-S

PAGE

Opening Remarks 7

Overview and Epidemiology of HCV 11

Prevalence of HCV and Other Viruses in
Blood Donors 26

Nucleic Acid Detection of HCV and Other
Viruses in Blood Donors 43

Genetic Diversity of HCV 56

Application of PCR to Molecular
Epidemiology of HCV 70

Discussion 77

Industry Presentations: Test Methodologies
and Experience with Nucleic Acid Testing
of Plasma Pools

National Genetics Institute
85

Alpha
94

Baxter
99

Roche
106

Bayer
112

Centeon
118
Gen-Probe
122
ARC
129
Immuno AG
140
Abbott Laboratories
149

C-O-N-T-E-N-T-S (CONT.)

PAGE

Industry Presentations: Test Methodologies
and Experience with Nucleic Acid Testing
of Plasma Pools (cont.)

Nabi
154

Regulatory Issues in Assay Validation and
Standardization

Assay Validation: Regulatory Perspective

U.S. FDA
159

PEI
168

EU
176

Standardization of HCV RNA Assays

NIBSC
187

CLB
196

CBER
203

PEI
210

Discussions 214

Issues in Implementation of Nucleic
Acid Testing for HCV and Other Viruses 227
Panel Discussion 239
Closing Remarks 260

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

2 (8:35 a.m.)

3 DR. EPSTEIN: Good morning. I know we
4 still have people queued up in the hall trying to
5 get registered and get their packet, but in the
6 interest of having an on-time agenda I'd like to
7 open the meeting.

8 I'm Jay Epstein, Director of the Office
9 of Blood Research and Review in CBER, and it's my
10 pleasure to welcome you to this FDA-sponsored
11 workshop on Nucleic Acid Testing for HCV and Other
12 Viruses in Blood Donors.

13 First I'd like to congratulate all of
14 you for getting through the building security and
15 for finding the conference room. I suspect that the
16 rest of the day's activities will seem easy by
17 comparison.

18 As you know from the published agenda,
19 this workshop has been convened to explore the
20 current state of the technology and implementation
21 of nucleic acid testing for screening of blood
22 donors, particularly for hepatitis C, and to assist
23 FDA in making determinations that will influence
24 U.S. regulatory policy on such testing.

25 FDA has been focused on promoting the
26 development of gene-based testing of blood donors

1 ever since the discovery of gene amplification
2 methods.
3 Although blood products have become
4 quite safe with the progressive introduction of
5 multiple donor screening and testing methods over
6 the last two-and-a-half decades, the effort to
7 introduce gene-based testing has taken on a sense of
8 urgency since the recognition several years ago that
9 such testing could improve blood safety by
10 substantially shortening the window period of
11 infectivity prior to serologic detection in donors
12 acutely infected by a number of transfusion
13 transmissible agents.
14 This point was emphasized at an FDA
15 scientific workshop four years ago in September
16 1994, which was on the feasibility of genetic
17 technology, to close the HIV window in donor
18 screening. Proceedings of that workshop were
19 published in the March 1997 issue of Transfusion.
20 Since then FDA has approved
21 investigational studies of gene amplification of
22 donor samples using mini-pool protocols for several
23 agents. And let me remark parenthetically that if
24 any of you has not heard what a mini-pool is you're
25 probably at the wrong meeting -- although you'll
26 certainly hear it by the end of the day.

1 FDA also recently published a guidance
2 in the Federal Register on July 10th of this year on
3 the manufacture and clinical evaluation of in vitro
4 tests to detect nucleic acid sequences of HIV-1.
5 And this is a paradigm then, for us putting forward
6 the review and approval criteria which we then think
7 will provide the framework for product approvals in
8 this area.
9 Gene-based testing of individual
10 donations remains an ultimate goal pending further
11 technology development, and we are aware that the
12 NHLBI has sponsored some of the technology
13 development in that area.
14 Now at this workshop this morning we
15 will explore the current state-of-the-art regarding
16 the epidemiology of hepatitis C and the sensitivity,
17 specificity, and reproducibility of nucleic acid
18 tests for hepatitis C and other viruses of concern
19 in donor screening.
20 We will also hear about the clinical
21 experience with nucleic acid base testing, including
22 the prevalence of detection in donors and the issue
23 of genetic variation for HCV. In the afternoon
24 attention will shift to implementation issues and
25 questions pertinent to standardization and
26 regulatory control.

1 We anticipate and we encourage
2 discussion of a large number of practical issues,
3 such as the requirements for test sensitivity,
4 possible limitations to the size of many pools, the
5 definition of a reactive and a confirmed screening
6 test result, strategies for inventory control, and
7 look-back, and the prospect for replacing some
8 existing tests with nucleic acid-based tests.
9 We know that these questions are on many
10 people's minds. I would ask however, that our
11 meeting participants accord the FDA representatives
12 the opportunity, both to express their personal
13 opinions as well as when appropriate, to summarize
14 FDA's current thinking, while recognizing that the
15 official policy statements will be developed and
16 published in good time based on thoughtful
17 reflection.
18 At this point I would like to thank the
19 members of the organizing committee who all have
20 worked very hard to bring about this meeting. I
21 would particularly like to acknowledge both the
22 scientific and the regulatory contributions of Dr.
23 Indira Hewlett who has been FDA's leader in this
24 area and who not surprisingly, is also a chairperson
25 of this meeting.

1 Additionally, I would like to note the
2 high level of international interest and cooperation
3 that has characterized the development of methods
4 and standards in this field, and to thank our
5 international participants for making the effort to
6 join us today.
7 Now, I hope that I've succeeded in
8 conveying to you a sense of FDA's purpose in
9 convening this workshop. I'm confident that the
10 sharing of information and opinion will accelerate
11 our effort to establish nucleic acid-based donor
12 screening for hepatitis C and other agents.
13 So now let me turn over the meeting to
14 Dr. Edward Tabor, who's Director of our Division of
15 Transfusion Transmitted Diseases, who will introduce
16 our first, distinguished speaker.
17 CHAIRPERSON TABOR: Good morning. We'd
18 like to ask that each of the speakers, when they've
19 completed their talks, please sit at the central
20 tables in front of the podium.
21 The first speaker is Dr. Harvey Alter
22 from the Department of Transfusion Medicine at the
23 National Institutes of Health.
24 DR. HARVEY ALTER: Decisions about
25 nucleic acid testing are really very easy -- of HCV
26 -- are really very easy, and I think we're on that

1 course and there will be no question about it. Hard
2 decisions are really going to come up, for instance,
3 the BPAC meetings in the next few days -- things
4 about CJ disease.
5 For instance, the FDA is proposing that
6 we add this new question to the donor screening
7 process. This question will ask, have you ever,
8 even once in your life, had intimate contact with
9 the brain or spinal cord of a cow? This is going to
10 be difficult to decide on.
11 My job today is to give you background
12 on hepatitis C. These are data from the CDC, from
13 Miriam Alter's work. In acute hepatitis in the U.S.
14 only about 15 percent of cases are hepatitis C-
15 related. So it's not a big player; doesn't cause a
16 lot of acute, severe hepatitis.
17 The problem with hepatitis C is that it
18 is the primary cause of chronic hepatitis in the
19 United States. And that's because about 85 percent
20 of the people who develop hepatitis C infection
21 become persistently infected; although 15 percent do
22 resolve, as we'll discuss later on. And that's an
23 important distinction from other persistent
24 infections such as HIV.
25 But the real question is, why does such
26 a large number have persistent infection? And we

1 don't really know that answer, but one of the
2 possibilities is the so-called, quasi-species nature
3 of the hepatitis C virus that Dr. Bukh is going to
4 talk about in detail later.
5 And that is that the virus can exist
6 simultaneously as a series of closely-related but
7 immunologically-distinct variants. And this is a
8 real case -- our famous Hutchinson patient -- in
9 which Patricia Farci cloned in sequence, 105 clones
10 from this patient during acute phase of hepatitis.
11 At that time he had a predominant
12 strain, which is 57 percent of the clones with the
13 same, but in addition there were all these other
14 clones. A total of 20 different variants present in
15 his serum at a single point in time.
16 So that even if this major strain were
17 contained by an appropriate, neutralizing antibody
18 response, any one of these other strains would be
19 ready to emerge as the predominant strain and escape
20 the immune attack.
21 This is some more interesting work from
22 Dr. Farci. We've looked -- from our post-
23 transfusion hepatitis studies where we have serial,
24 acute phase samples -- we've looked at the changes,
25 the viral variation during the acute phase of
26 hepatitis C infection.

1 I think this is the only study that has
2 looked at it early on -- infection -- and samples
3 were taken here just after the first PCR of
4 positivity; here at the first ALT elevation; here
5 just at the rising ALT; and here at the peak of ALT.
6 So all within 16 weeks.
7 There were ten clones at each point that
8 were sequenced. And you can see here at week 3,
9 early in the infection, there were three variants
10 which I've labeled A, B, and C. By week-8 none of
11 those were still detectable, but now D was the
12 predominant strain but there was also E, F, and G.
13 By week-13 one could detect A again, so
14 it was still around, but now there was H, I, J, K,
15 L, M, N, O, P, and by week-16 G, L -- all the way
16 through U. So an entire -- in 16 weeks an entire
17 alphabet of hepatitis C viral strains. And any one
18 of these would be capable of becoming the
19 predominant strain during an immune attack.
20 So I think this is one of the major
21 reasons for this being a persistent infection, and
22 HIV has a similar occurrence.
23 Now, I'm going to pre-empt my talk by
24 showing this overview to say that there are really
25 three major forms of hepatitis C infection. Shown
26 here in blue are the ALT elevations; in purplish

1 color the RNA levels; and in yellow, the antibody
2 levels.
3 And what we know is that now, at least
4 from our studies, that 15 percent of the people
5 recover; that is, they are persistently antibody-
6 positive and persistently PCR-negative over many
7 years. And their ALT is normalized.
8 And another 15 percent go on to rather
9 severe disease within the first six to 10 years of
10 their infection and die from hepatitis C within that
11 timeframe. Those people have chronic ALT
12 elevations, persistent HCV RNA, and persistent
13 antibody.
14 And then there's the majority of people
15 which I think is about 70 percent, who have this
16 very benign, longstanding infection that we'll talk
17 about throughout this talk, where again they're
18 persistently PCR-positive, persistently antibody
19 positive, and have these very low-level ALT
20 elevations that are intermittent.
21 And you can see in all these scenarios
22 that HCV RNA appears very early in the infection in
23 weeks 1, 2, or 3 post-exposure, and antibody doesn't
24 appear until week-12 or so. So this is the window
25 that this whole meeting is about; the period from

1 here to here. I don't have my other window slide
2 but I'm sure somebody else will show it today.
3 But it's this nine weeks or so -- 9 to
4 12 weeks -- that HCV RNA testing will possibly
5 interdict and virtually prevent all transfusion-
6 associated HCV.
7 Now, the clinical cause of hepatitis C
8 I've alluded to. This is the famous single case
9 from Kendo Kiyosawa in Japan, but it shows what can
10 happen in the worst-case scenario. And that is, one
11 gets a blood transfusion, here's acute hepatitis
12 that was biopsy-proven so there was no underlying
13 chronic liver disease -- this was acute hepatitis.
14 A year later this patient has chronic,
15 persistent hepatitis, a mild form of chronic liver
16 disease; four years later chronic active hepatitis;
17 three years later briding necrosis, which is a pre-
18 cirrhotic region; two years later cirrhosis; and
19 three years after that, hepatocellular carcinoma
20 leading to death.
21 So within 18 years this patient went
22 from blood transfusion to death. There's no doubt
23 that this sequence can occur. I'm going to paint
24 the other side of the picture as well but I don't
25 dispute this occurrence; it happens. And hepatitis

1 C is a leading indicator for liver transplantation
2 here in the U.S. and throughout the world.
3 In our studies in fact, although most of
4 the people on initial biopsy are very mild to
5 moderate disease and even on follow-up biopsy either
6 improved or had stable histology, about a quarter of
7 the patients progressed over time. And we wound up
8 with 20 percent of our patients having cirrhosis,
9 and that 20 percent figure, histologic cirrhosis in
10 about 20 percent of HCV patients comes up from study
11 to study.
12 And in that group who had cirrhosis,
13 three of them died of liver failure, three of them
14 had very severe liver disease but died of their
15 underlying heart disease, but one had cirrhosis for
16 ten years and was still very well compensated and
17 died of something else. And we have another patient
18 now has cirrhosis for 22 years and is very well
19 compensated. So you have this spectrum even among
20 those who have cirrhosis.
21 This is another picture of the bad side
22 of this infection. And these are data from Myron
23 Tong who works in a tertiary care center so he's
24 getting the worst cases referred to him. But in his
25 population, of those who are referred for hepatitis
26 C, 44 percent had chronic hepatitis but 46 percent

1 of his population had cirrhosis and 11 percent had
2 hepatocellular carcinoma.
3 Part of that was because he had a large
4 Asian population who seemed to be more prone to
5 hepatocellular carcinoma. So they had a
6 disproportionate amount of carcinoma among the
7 Asians. But nonetheless, this overall 11 percent
8 carcinoma and 46 percent cirrhosis is about the
9 worst numbers that I have seen, and I think it
10 reflects the severity of the cases when they're
11 referred to him and the Asian population.
12 But now the other side of the coin.
13 Perhaps the -- and I think almost certainly, the
14 bigger number. These are the summary data from the
15 multi-center study headed by Leonard Seeff. You've
16 all seen these before and Leonard is here in the
17 audience. But I think it's a critical study, and I
18 say it with some bias because we're participating in
19 it.
20 But it was very well controlled. And
21 shown here in white are the cases, and in orange and
22 green the two control groups -- very carefully
23 matched control groups.
24 And now after 22 years, when one
25 compares the overall mortality among the patients
26 who had hepatitis which turned into -- the most

1 would be hepatitis C in the 1970s, compared to the
2 controls who were transfused but did not develop
3 hepatitis, the overall mortality is identical.
4 After about now, I think 22 years in the latest
5 analysis.
6 There was a slight increase in liver-
7 related mortality among the hepatitis C case, but it
8 was very slight; about one-and-a-half percent
9 increase over the controls. So this is a fairly
10 benign picture over at least the first two decades
11 of this infection.
12 And we now have studied -- not a very
13 good slide and I don't know if it can be cleared up
14 -- but these are data from the Irish study of women
15 who received contaminated lots of Rhogam. They were
16 followed up for 17 years at this time. It's a
17 little bit longer now.
18 But just the bottom line of this is when
19 you look for fibrosis in these patients 17 years
20 later, 57 percent had no fibrosis and only two
21 percent had cirrhosis -- I'm sorry, two percent had
22 cirrhosis and two percent had severe hepatitis. So
23 maybe wind up with four percent with cirrhosis in
24 the first two decades.

1 These were young women when they were
2 infected, but I think again, this says the first two
3 decades for most people are relatively benign.
4 And here's our own data from our
5 hepatitis C positive donors that we now have in
6 long-term follow-up. These studies were dealing
7 with Jay Hoofnagel's group and one can see that
8 these are liver biopsies.
9 And the vast majority of these patients
10 who are being biopsied on an average of 18 years
11 after the onset of their infection -- and we know
12 that from when they took drugs or had a transfusion
13 -- have very mild, histologic lesions. And only
14 this 13 percent have either cirrhosis or severe,
15 chronic hepatitis that might lead to cirrhosis. So
16 we're getting the same picture from all of these
17 studies.
18 Poynard has done a very nice study to
19 look at the progression of cirrhosis to -- the
20 progression of fibrosis to cirrhosis in a large,
21 multi-center study conducted in Europe. And
22 basically he developed a fibrosis unit that I won't
23 go into, but essentially one unit equals one stage.
24 And so it takes about seven-and-a-half
25 years to go from stage zero, which is no fibrosis,
26 to slight fibrosis, 15 years to go to more advanced

1 fibrosis, another seven-and-a-half years -- or 22
2 years in total -- to get to a lot of fibrosis, and
3 30 years to get to cirrhosis.
4 So this is a slowly evolving disease in
5 those who do develop cirrhosis and maybe a lot of
6 these patients who are doing well in 20 years are
7 going to suddenly do badly, but I don't think so.
8 And here's another interesting study by
9 Fattovich which shows that even when you get
10 cirrhosis with hepatitis C, you can stay compensated
11 for a very long time. And here's the 5-year
12 survival in people who have cirrhosis -- is 91
13 percent. And the 10-year survival probability is 79
14 percent, after you have documented cirrhosis. So
15 this is a very, very slowly evolving process.
16 Now what makes some people go on to
17 cirrhosis and other people not, or what makes some
18 people have very severe cirrhosis and others lesser?
19 Well, we don't know all the co-factors but one of
20 them clearly, is alcohol.
21 And this is an interesting study by
22 Correo in which they compared teetotalers and
23 alcohol abusers -- defined by 175 grams of alcohol
24 per day. And two major findings from this study.
25 One, if you are HCV-negative, even if
26 you were an alcohol abuser, the relative risk of

1 developing cirrhosis was 15 compared to the
2 reference of a person who was HCV-negative and a
3 teetotaler.
4 But if you were HCV-positive -- if you
5 had hepatitis C plus alcohol -- the odds ration went
6 up to 147. You greatly increased the risk of
7 developing cirrhosis. And that's been a consistent
8 finding from study to study.
9 Well, a long time ago I developed this
10 guess as to -- I developed this sort of an estimate
11 of what would happen if you took 100 people who had
12 hepatitis C, virus positive, and what I've already
13 shown you is that 15 percent resolve the infection
14 so you're left with 85 percent, 85 people who
15 develop chronic infection.
16 Of those, 20 percent will develop
17 cirrhosis and 80 percent will have a stable disease
18 for a very long time, or will die of a non-liver-
19 related death. So of the 17 patients now who have
20 cirrhosis, let's say if even 75 percent of them have
21 a mortal cirrhosis before they die of something
22 else, the total number of people who die of their
23 hepatitis C would be only 13 percent within the
24 first two decades of their infection.
25 Now ultimately, some of these stable
26 people will move over here into the mortal group,

1 but most of them will live out their normal lifespan
2 or will die of whatever they were going to die of in
3 the first case. So less than 15 percent mortality
4 was my guess based on our own studies and some other
5 preliminary studies.
6 Well, the Seeff study has now advanced
7 to looking at morbidity, and Leonard has given me
8 permission to show this slide in advance of
9 publication -- of what we hope will be publication.
10 And it's a complicated slide so I'm just going to
11 focus on a very brief area.
12 In 1974 there were 103 people who were
13 hepatitis C-positive. Most of these developed their
14 hepatitis C as a result of blood transfusion but
15 there were a segment of these who were already HCV-
16 positive when they entered the study.
17 But what I want to focus on is what
18 happened to these 103 people -- almost the same
19 number as in my schematic. Well, what happened was,
20 there was just like the 15 percent I predicted that
21 were anti-HCV-positive, HCV RNA-negative that
22 appeared to have recovered.
23 But here's a group that was
24 unanticipated: ten percent of these patients not
25 only were now RNA-negative but they were also
26 antibody-negative. They had no evidence of their

1 prior HCV infection. So the natural recovery rate
2 could be as high as in the recovery range of 25
3 percent.
4 But for this purpose I want to focus on
5 this 73 percent who are persistently antibody-
6 positive and persistently RNA-positive. Only half
7 of them -- or actually 40 percent of them -- had
8 biochemical evidence -- 40 percent of the total
9 population had biochemical evidence of chronic
10 hepatitis. So 60 percent had normal ALT levels on
11 at least two samples.
12 Some of these in this group who had
13 chronic hepatitis and were HCV RNA-positive were
14 biopsied, and based on those biopsies we made some
15 extrapolations: that 30 percent of the people in
16 this group would develop cirrhosis; that less than
17 five percent in the group who had no biochemical
18 evidence of hepatitis but were RNA-positive would
19 develop cirrhosis; and less than one percent of
20 these who recovered would develop cirrhosis -- and
21 it really would probably be zero.
22 If you add all those numbers together
23 you come up with a cirrhosis figure again, of less
24 than 15 percent in the first 20 years.
25 So the question then is what's going to
26 happen after the first 20 years? If this is where

1 we are now, at around 15 to 17 percent, will the
2 curve continue such that even after 60 years no more
3 than 50 percent of people will develop cirrhosis?
4 Or will the curve for some reason accelerate so that
5 by 60 years everybody will have cirrhosis? Or even
6 by 40 years everybody will have cirrhosis.
7 But there's no reason to suspect the
8 sudden acceleration. And in fact, the curve may
9 actually asymptote here. I didn't show that but
10 it's possible that people who haven't developed
11 cirrhosis within 20 years may never develop
12 cirrhosis. They may be selected out as being those
13 who are not going to progress. So I think the curve
14 will be here or lower rather than anything higher.
15 So when you look at hepatitis C it's
16 like the blind man looking at the elephant. It
17 depends on where you see it; from what angle you see
18 it as what you think it is.
19 If you're a blood banker or a primary
20 care physician you're seeing people who are totally
21 asymptomatic, who are anti-HCV-positive, who would
22 have no clue that they had hepatitis C if you hadn't
23 picked it up on a blood test. So we're seeing the
24 very mild spectrum of this rear -- the rear end of
25 the elephant. And I think as even shown on the
26 slide, this is the biggest end of the HCV story.

1 However, if you're a gastroenterologist
2 or hepatologist you're having some of these patients
3 referred to you and you're seeing some of the more
4 severe cases, but you also realize that most of the
5 cases you see are really quite benign. But you have
6 a more balanced perspective.
7 On the other hand, if you're Myron Tong
8 in a tertiary care center, or you're a transplant
9 surgeon, you're seeing people with terrible, end-
10 stage hepatitis C and you think this is a horrendous
11 disease.
12 Well, the truth is, it is. It is a
13 horrendous disease and it is a mild disease, and
14 we're just talking about proportions. And it's my
15 estimate that the big proportion is the big rump of
16 this elephant and that most people -- 70 percent of
17 people -- will have a lifetime of relatively benign
18 hepatitis C.
19 But that's my guess and we don't have
20 the data yet because we haven't lived long enough,
21 and I clearly will not. In any event, you've been a
22 very attentive audience and I thank you for that
23 attention. Thank you very much.
24 CHAIRPERSON TABOR: We'll save questions
25 for the discussion period after the break. The next
26 speaker is Dr. Steven Kleinman from UCLA.

1 DR. KLEINMAN: Thanks, Ed. Formerly
2 from UCLA, I should say.
3 My task for today is to talk about the
4 prevalence and incidence of hepatitis C in U.S.
5 blood donors. I'm going to confine my remarks to
6 volunteer blood donors and I will mention at the end
7 of the talk a little bit about some of the other
8 viruses that we're concerned about.
9 So again, the objectives are to discuss
10 HCV prevalence and incidence in allogeneic donors
11 based on these data to project HCV genome
12 amplification testing yield, and then to contrast
13 the prevalence and incidence of HCV to other
14 transfusion transmitted viruses.
15 I'll be using two data sources: one is
16 the REDS database which encompasses the years 1991
17 to 1996, and the other is the database from the
18 American Red Cross. These data are for 1996 and
19 '97, and these data were supplied to me courtesy of
20 Dr. Susan Stramer of American Red Cross.
21 Now the database for REDS retrovirus
22 epidemiology donor study that most of you have heard
23 of, sponsored by NHLBI, consists of approximately
24 one million annual, allogeneic donations which have
25 been collected from five U.S. blood centers, and the
26 viral markers that we analyzed -- this slide's taken

1 from a previous presentation -- were for: HCV,
2 which we'll talk about today; antibody HBsAg; HIV
3 antibody -- which we did some modification by
4 excluding certain false positives; and HTLV
5 antibody, which I won't say too much about today.
6 And what we did was determine incidence
7 rate for four, overlapping 2-year intervals, and we
8 defined incidence as the number of incident cases or
9 sero-conversions. So negative anti-HCV tests
10 progressing to a positive anti-HCV test per 100,000
11 person-years, and all donors included in these
12 calculations gave at least two donations in the
13 specified interval.
14 And then we also calculated annual
15 prevalence in first-time donors, and that's pretty
16 straightforward. It's the number of positive, anti-
17 HCV positive donations in first-time donors per
18 100,000 first-time donations, and then we looked at
19 these over time.
20 Now, the tests that were used to
21 generate this data, we started generating the data
22 at the beginning of multi-antigen EIA testing or
23 EIA-2. Most of the centers use the Ortho EIA.
24 And the confirmatory tests for the first
25 year of data were first generation RIBA, and then
26 from July '93 through the end of '95 were second

1 generation RIBA. And the first half of '96 is the
2 same but the second half of '96 which I'll show you,
3 is based on version 3 testing, or EIA-3 and RIBA-3
4 testing.
5 Now the Red Cross database which is '96
6 and I think the first half of '97, are from American
7 Red Cross regions that comprise 38 percent of
8 American Red Cross collections. The data were
9 compiled for previous BPAC and so -- which was
10 looking at plasma production issues, and so it's for
11 data less than -- for donors less than 60 years of
12 age; which, since that's the large majority of
13 donors I don't think puts much of a skew in the
14 data.
15 Three of the regions in the Red Cross
16 database are also in the REDS database, and the HCV
17 assays from June '96 onward all use the EIA-3.0 and
18 RIBA-3.0.
19 So these are the data that I'll show.
20 Now, this first slide of data is basically, if you
21 just take a rate per donation. So all allogeneic
22 donations collected, how many are HCV antibody-
23 confirmed positive. So this is not really an
24 incidence or a prevalence; it's a frequency that
25 you'd see in everyday screening.

1 And as you can see here, if we take the
2 aggregate REDS data we get 152 per 100,000 -- or,
3 that's 1.5 per 1,000. If we take 1996 and look at
4 the REDS data you can see we get 105 per 100,000.
5 For the first half of the year we're going to use
6 2.0 and 125, so a slight increase when we use 3.0.
7 And if you take the Red Cross data which
8 is partially 2.0 and 3.0, we get 112. So for 1996
9 and 1997 we seem to have consistent rates of
10 slightly more than one per 1,000 donations testing
11 positive.
12 Now, this is prevalence in first-time
13 donors, so if we only look at not all donations but
14 donations from first-time donors, again taking the
15 comparable time periods you can see in aggregate we
16 had about -- these are 95 percent confidence
17 intervals here -- we had 541 per 100,000 during the
18 5-year period. If we stick to 1996 we have close to
19 400 per 100,000 using 2.0; and using 3.0 it
20 increases.
21 And as you can see here, this is
22 statistically significant. And I think it has
23 several potential explanations which are involved in
24 switching from 2.0 screening tests to 3.0, as well
25 as switching from 2.0 RIBA which generates some

1 indeterminant test results from infected people, and
2 those people are now positive by RIBA 3.0.
3 So I don't think this represents a
4 dramatic or even real increase in HCV in donors. I
5 think some of it may be part of changing tests. And
6 again, the Red Cross data is consistent, falling
7 within these same ranges. So we're talking about,
8 in first-time donors, 400 to 500 per 100,000.
9 And in the REDS database we took a look
10 at the temporal trends in HCV prevalence and you can
11 see here the reason the aggregate data is larger
12 than the '96 data is because of the first --
13 primarily the first year of HCV testing where we
14 were in first-time donors, finding rates between 600
15 and 700.
16 Now, I'm not -- in re-analyzing this
17 data I'm not convinced that this is really a
18 decrease in prevalence. It may be the fact that we
19 were confirming data with RIBA 1.0 at that point,
20 and so a positive on 1.0 may not always be a
21 positive on 2.0. But I think the major point here
22 is a fairly consistent picture of HCV in the first-
23 time donor population.
24 Now, here's trends in HCV incidents. So
25 this is in repeat donors, and you can see here that

1 hasn't changed over time. And we're looking at
2 levels of about four per 100,000 per person-years.
3 If we take a look at the incidence data
4 in 1996 we can see that again, we had four per
5 100,000 person-years. But again, when we were
6 converting from -- when some donors at one donation
7 tested by 2.0 and another one tested by 3.0, we're
8 not clear if that's a new infection or if it could
9 be an infection that was missed by 2.0 screening.
10 And so when we take a look at the
11 aggregate data we get an incidence of apparent
12 seroconversion of about 6.2 per 100,000 person-
13 years. And when the Red Cross did the same thing
14 they got an incidence of 11.6 per 100,000 person-
15 years.
16 And the point I want to make here is I
17 hope, illustrated on the next slide -- although I
18 have a feeling these are slightly out of order. But
19 let me go back. The point that I want to make here
20 is that we really need to talk about ranges of
21 incidence as we build models, and so I'll show you
22 some subsequent calculations.
23 We take a point estimate. Most people
24 have been quoting the number that REDS published
25 several years ago about the risk of HCV being one in

1 103,000 -- risk of a window period HCV unit being
2 one in 103,000 antibody-negative units.
3 And I just want to emphasize that that's
4 an estimate and that that estimate has a range
5 around it, and when we try to predict what will
6 happen in the future we're better off taking the
7 whole range and not thinking that the point estimate
8 represents absolute truth.
9 Anyway, if we now take a look at our
10 frequency of positive donations and we break it down
11 into whether the donor was a first-time donor or a
12 repeat donor, you can see here -- this recaps some
13 of the earlier data; this is in the Red Cross
14 database -- 4.25 per 1,000 donations in first-time
15 donors; only at 0.29 per 1,000 donations in repeat
16 donors.
17 Since first-time donors make up 21
18 percent of the database we can project that we'll
19 have in 12 million units collected yearly in the
20 U.S., we'll have a little more than 10,000 HCV
21 antibody-positive results coming from first-time
22 donors.
23 Repeat donors, 79 percent of the
24 database, we'll have about 2300 results coming from
25 repeat donors. Now the ratio of positive findings
26 in donations from first-time donors versus repeat

1 donors is 14.7 but the actual numbers in our yield,
2 the ratio goes down to 4.6 because it's a weighted
3 average.
4 But however you look at it most of our
5 antibody-positives come from first-time donors.
6 This should be no great revelation to anybody in the
7 room since first-time donors represent prevalence
8 and again, repeat donors represent new infections.
9 Now, in a manuscript that was published
10 a couple of years ago we looked at -- within the
11 first-time donors we looked at this slightly
12 differently. And this is '92/'93 data. And we
13 looked at people who make one donation to REDS, said
14 it was their first-time, only donation, and never
15 came back -- their rate was eight per 1,000 donors.
16 And so obviously, they didn't come --
17 one of the reasons they didn't come back was because
18 they were positive and couldn't come back -- for the
19 positives -- and then other people elected not to
20 come back.
21 But we had a group of first-time donors
22 who did come back and make subsequent donations in
23 those two years, so they started out as first-time
24 donors and became repeat donors, and their rate was
25 actually the lowest: it was 0.4 per 1,000 donors
26 HCV-positive. So the point is, when you look at a

1 first-time donor some of those people will go on to
2 be repeat donors. They seem to be as safe as -- or
3 have at least as low HCV infection rates as any of
4 our first-time -- as any of our repeat donors.
5 Now, to summarize the HCV temporal
6 trends here, the incidence has not varied over time
7 since we began collecting data in '92, through the
8 period of EIA-2 and RIBA-2 tests. The prevalence in
9 first-time donors has decreased. Most of that
10 decrease came subsequent to 1993 and has remained
11 fairly stable. And the first and only-time donors
12 show the highest prevalence.
13 Now turning to incidence for a minute, I
14 want to go through relative risk. Everybody's seen
15 this: the per unit risk is the incidence rate times
16 the length of the window period. And so when we get
17 to the window period for HCV, this is the window
18 period as determined by Correo's data of
19 transfusion-associated HCV cases, and I think it's
20 duplicated by several other people.
21 And you can see here from the time of
22 transfusion it takes about 53 days to elevated ALT,
23 about 70 days to EIA-3.0 positivity, and about 82
24 days -- these are average figures -- to 2.0
25 positivity. So it's only a donor in those fist 70
26 days of infection who would be HCV antibody-negative

1 and potentially capable of infecting recipients and
2 potentially capable of detection by HCV genome
3 amplification testing.
4 Now here's a slide taken from Mike
5 Busch's look at the older transfusion-transmitted
6 virus study repositories. And I show this because
7 of a couple of reasons. What it illustrates is --
8 and they determined time from transfusion to ALT
9 elevation of 90 in a series of -- I'm not sure how
10 many of it -- about 30 or so patients; and also time
11 to PCR detection.
12 And I want to emphasize the ALT thing
13 here because - and remember, ALT takes a couple of
14 weeks before -- it comes up a couple of weeks before
15 antibody. And this basically shows a distribution
16 of times to ALT development with about half of the
17 patients developing an elevated ALT at about 45 days
18 or so.
19 But there is a tail on this
20 distribution, and while it's not directly relevant
21 to this presentation it is relevant to issues of how
22 long the window will be, and certainly I think, came
23 into discussion when determining how long donor
24 retested plasma needed to be held in quarantine
25 before you can assure that 95 percent of the units
26 were beyond the HCV window phase.

1 So this is just to estimate -- this is
2 really in summary, to show you a range of window
3 periods for HCV antibody development, but all fairly
4 tight within a couple of months.
5 Now, if we then do our risk
6 calculations, again as I emphasized, we estimated
7 one in 103,000 donations might be infectious --
8 antibody-negative but infectious. But the 95
9 percent confidence interval was relatively wide.
10 Now all the newer incidence calculations
11 using more recent data are all within this 95
12 percent confidence interval; however, the original
13 point estimate shows a slightly lower risk than
14 point estimates using the newer data and that's why
15 I wanted to use the range in the next slide here.
16 And what I've done in this slide is try
17 to, based on both prevalence and incidence, estimate
18 how many positive PCR -- HCV-positive PCR tests we
19 would expect to generate when screening the U.S.
20 volunteer blood donor population -- assuming for a
21 moment we were doing single unit HCV GAT testing and
22 then making the extrapolation that our pool testing
23 will pick up everything that our single unit testing
24 would if it's sensitive enough -- as I'm sure other
25 speakers will talk about -- you get these numbers.

1 We have 13,440 HCV antibody-positive
2 donations per year, out of which we estimate about
3 90 percent will be PCR-positive -- both based on
4 data that Harvey showed about the ten percent who
5 resolve infection, and on studies that have actually
6 been done, both in the U.S. and Europe on HCV
7 antibody-positive donors.
8 So that would yield about 12,096 PCR-
9 positive units. We then go into the window period
10 units, those people who are antibody-negative but
11 infectious, taking the range of the lower 95 percent
12 confidence interval up to the point estimate of
13 103,000.
14 We'd get anywhere from 116 to 428
15 donations that were donated within this window
16 period of which, since it takes about 12 days on
17 average for the PCR to become positive, we can
18 estimate that we'd pick up 56, 58 out of the 70-day
19 window. So we'd pick up about 80 percent of these
20 as PCR-positive. And so we'd get an estimate of
21 somewhere between 93 and 342 PCR-positive units from
22 these potential, infectious, window period donors.
23 Now the third category that we've talked
24 about in the past are persons who are persistently
25 antibody-negative, who have viral nucleic acid, and

1 who are therefore chronic carriers who are never
2 picked up by antibody testing.
3 A number of years ago we thought that
4 these people are quite common, but I think most of
5 us now believe, looking at some of the data that's
6 come out of nucleic acid screening programs, both in
7 Europe and in the plasma industry here, that these
8 people are in fact, rare, and I don't think will
9 have much of effect on the overall calculations.
10 So you can see here that if we summarize
11 here PCR-positive donations, 97.3 to 99.2 percent
12 will be from antibody -- HCV antibody-positive
13 donors. And the ratio of PCR yield in antibody-
14 positive donors to that in antibody-negative donors,
15 will be anywhere from 35 PCR-positive antibody-
16 positive units per PCR-positive antibody-negative
17 units, all the way up to 130. And it's probably
18 more like the 130 range because that probably is
19 more in line with the point estimate for HCV risk.
20 So the take-home message is, if we
21 screen antibody-positives most of those -- that will
22 be the largest contribution to our PCR yield.
23 I want to turn now to the other markers
24 very briefly, and summarize the REDS 1996 data on
25 viral marker prevalence in first-time donors for the
26 other markers. Here's our figures for HCV 2.0:

1 399. Here are our other figures for the other
2 virus: HIV at 15.3. So the lowest prevalence in
3 first-time donors, HTLV at 45.8 -- and these are 95
4 percent confidence intervals.
5 Surface antigen, somewhat comparable to
6 HCV; about half of HCV at 212. But again, we
7 believe that surface antigen positivity doesn't tell
8 us the whole story about HBsHBV infection since
9 people who acquire infection obviously don't keep
10 their surface antigen for life. They lost it; it's
11 a transient marker.
12 Here's the HIV prevalence numbers over
13 the years to show that we did see a drop in
14 prevalence between '92 and subsequent years. And
15 this has held true. We don't really have an
16 explanation for why this drop.
17 We know there were revised criteria put
18 into place in blood donor screening in 1992, but in
19 fact, most of these revised criteria were a bit more
20 liberal and did not require a lifetime deferral for
21 certain behaviors but had changed those to 12-month
22 deferrals. So I don't know why that would explain
23 it other than the fact that maybe blood centers
24 looked at their questions more carefully and maybe
25 the screening process was improved as a byproduct of
26 FDA coming out with its guidance document.

1 Now here's our viral marker incidence,
2 so new infections in '95/'96 in REDS per 100,000
3 person-years, once again we get the same sort of
4 relationships. HIV is low, HTLV is relatively low.
5 Surface antigen is higher but we did an
6 adjustment on surface antigen -- I don't have time
7 to go into it today -- but we do believe that
8 certain neutralized surface antigen positives, when
9 these donors don't have anti-core, actually
10 represent false positive surface antigen tests.
11 They don't affect the prevalence of
12 surface antigen very much but they do affect the
13 incidence because the numbers are smaller and that
14 effect is amplified. And so I'm not sure which of
15 these numbers are correct; whether it's five per
16 100,000 or 2.5 when we do our adjustments, which are
17 based on some assumptions.
18 And then for HCV we're at 4.0. So these
19 data are all summarized -- oh, I'm sorry, here's one
20 more slide on HIV incidence to show that while
21 prevalence decreased, incidence, while it looks like
22 it's varying a bit, these confidence intervals all
23 overlap, and 1996 is back up here at 1.5.
24 And so in fact, the incidence of HIV --
25 so those donors who we're not picking up by antibody
26 testing who might be transmitting infections to

1 other people, really has not changed since 1991.
2 It's been constant and it's been at a very low
3 level.
4 And here I've summarized all the
5 relative frequency data in this last slide and I'll
6 take you through the columns. First-time relative
7 prevalence using the '96 REDS data. If we take HIV
8 as a relative prevalence of one, then HTLV is three
9 times more common in first-time donors, surface
10 antigen is detected 14 times as commonly as HIV, and
11 HCV is detected 26 times as more commonly.
12 So it's that marker that has the biggest
13 yield in our screening of blood donors today; at
14 least the direct infectivity marker. Obviously,
15 anti-HBC would have the highest prevalence.
16 Now, this is the -- all donation data
17 from the Red Cross database and you see quite
18 similar relationships, although surface antigen
19 seems to be a bit lower in proportion; HCV again, is
20 very high.
21 And if you then take the incidence data,
22 the actual donors that we're worried about that
23 we're missing with current tests who might transmit
24 infection, again, if HIV is taken as the reference
25 at 1.0, HTLV is quite comparable.

1 Surface antigen, if we adjust and remove
2 the false positive cases, is probably only slightly
3 -- so hepatitis B transmission is probably only
4 slightly more common. However, if we don't remove
5 those cases we might project that it's much more
6 common. And HCV again, two-and-a-half times the
7 risk of HIV.
8 So that's the last slide. I think the
9 points I want to leave you with are that, when we
10 compare the viruses, HCV really is a good virus to
11 target as our first -- in our aim to improve the
12 safety of the blood supply it's probably the one
13 that carries the greatest risk currently, given our
14 screening procedures.
15 And at least if one takes into account
16 the potential for some percentage of those infected
17 recipients to develop chronic disease. Thank you.
18 CHAIRPERSON TABOR: Thank you, Dr.
19 Kleinman, for that interesting talk. The next
20 speaker is Dr. Peter Simmonds from the University of
21 Edinburgh.
22 DR. SIMMONDS: Good morning and thank
23 you very much for the invitation to this meeting.
24 I've been asked to talk about the protection of
25 hepatitis C in blood donors and detection of other
26 viruses, and so I'm going to start with hepatitis C

1 virus and just describe very briefly our experience
2 of introducing PCR-based screening for blood donors
3 in Scotland and Northern Ireland.
4 The background really, to our screening
5 has been the regulatory requirement that's imposed
6 on us to actually screen final pools for hepatitis C
7 RNA. And clearly, the costs of actually getting a
8 positive pool are substantial, and clearly, pre-
9 screening donations in mini-pools is fairly cost
10 effective in preventing that occurring.
11 So in fact, they're not really providing
12 the framework for our group to actually get the
13 mini-pool testing set up. And of course that has
14 knock-on benefits in terms of establishing of
15 framework for the screening for other viruses as
16 well.
17 Just to summarize our final pool
18 testing, we've been doing it for about two years and
19 have screened the equivalent of roughly 600,000
20 donations. And about a month before we started
21 mini-pool screening we actually got a final pool
22 positive, okay, which is roughly made out of 6,000
23 component donations. And obviously, that was quite
24 expensive.
25 Well, it would have been had we been
26 able to use U.K. plasma. Anyway, so we got a

1 positive unit and through the archive we managed to
2 identify the donor. And it was actually a donor who
3 was very close to the window period and was actually
4 only positive in one of the four, commercial
5 screening tests available, and that was mono-
6 reactive in RIBA-3. We don't know any more about
7 the donor. He has not donated again.
8 So with this framework established we've
9 got mini-pool testing set up and obviously, in the
10 future we acknowledge the need to screen for red
11 cell release, and possibly in the future, platelet
12 release, and obviously to introduce screening for
13 other viruses; and obviously HIV-1 is clearly a
14 candidate virus.
15 Okay, so mini-pool screening has been
16 going since April 1998, and we've got a mean
17 turnaround time of three to four days, which isn't
18 really good enough for platelets and it will be
19 difficult for red cell release as well at this
20 stage. So clearly that needs to be reduced.
21 Our initial testing is done in pools 96.
22 These include antibody-positive samples. When we
23 get the serology results these are excluded and we
24 retest. So far we've screen 150,000 donations. We
25 got about seven positive pools and these all
26 contained antibody-positive units. These in fact,

1 were the only antibody-positive units that were PCR
2 positive so far. So we've picked them all up in the
3 mini-pool system.
4 So far we have no antibody-negative PCR-
5 positive units, and that's roughly in line with
6 expectations. We think, are basing our strategies
7 on around about one in 200,000 -- perhaps one in
8 400,000 -- will actually be picked up this way. So
9 clearly we've not tested enough to find a positive
10 at this stage.
11 So what we are a bit anxious about are
12 these further development issues. Turnaround time
13 is clearly necessary. We would like to go towards
14 closed tube detection of PCR products to avoid
15 contamination. And at the moment we're just about
16 at the stage of validating parallel, HIV screening
17 for one at group O, and the new virus, N.
18 I'm going to turn on to other viruses,
19 and the second part of the talk is really just to
20 try and briefly summarize what the clinical issues
21 are involved in screening for some of the other,
22 more recently discovered viruses. And clearly there
23 are issues that are relevant that surround hepatitis
24 G or GBV-C.
25 And what I'm going to describe is just a
26 small study where we try and assess what the

1 significance of this virus will be. That's where we
2 wanted to work out the prevalence of viremia in the
3 donor population, and then to try and make some sort
4 of clinical assessment to the donors and to
5 establish whether there are any particular disease
6 associations in individuals who don't have hepatitis
7 C co-infection.
8 And then to do some incidence
9 calculations to see whether in fact, we could
10 actually screen for it anyway. And we screen 1,020
11 in pools of ten, split them down into component
12 donations. We identified 23 positive donors out of
13 1,020. These are all regular donors and it's
14 possible that the prevalence of viremia may be
15 higher in first-time donors.
16 And 19 came back and they had a mean age
17 of 32. We could establish no parental risk factors
18 for infection from interview. The ALT level was 20
19 and if anything, was lower than the ALT levels in
20 controlled donors. And they were quite carefully
21 clinically assessed, and in fact we could find
22 really nothing remarkable about them at all. All
23 the other liver function tests were normal, there
24 were no other disease associations that we could
25 see.

1 Unfortunately, we had no clinical
2 indications to do a liver biopsy and so we don't
3 really know what that would show. But obviously,
4 the suspicion would be that in fact, that might be
5 normal.
6 I think the relevance for any attempt at
7 PCR screening is the incidence calculation we were
8 able to make. These were regular donors; in fact,
9 in the archive we were able to retrieve samples from
10 previous donations collected right back to about
11 1984.
12 And what we did was to take a series of
13 prevalence calculations from '84, '86, and '89. And
14 in that way we could actually retrospectively work
15 out what the incidence. And when we did this we
16 were actually quite surprised to see that in fact,
17 there had been 17 seroconversions for PCR in a mean
18 of 9.7 years.
19 And so the incidence in this population
20 is about 200 per 100,000 donations. So it's much,
21 much higher than any other viral markers we've just
22 heard about, by possibly as much as a factor of 100
23 greater than hepatitis B and 50 times greater than
24 hepatitis C.
25 And the problem with that then is that
26 if you were going to screen for it, in mini-pools at

1 least, you'd have a real problem, because obviously
2 pools of 100 for example, would contain about -- ten
3 percent of these would actually contain a positive
4 unit if this was the incidence now.
5 And if you made pools of 500 you'd find
6 half of them were positive for hepatitis G, and
7 that's after excluding your positive donations
8 already. It's just the high incidence actually
9 stops you doing it in mini-pools.
10 Okay, so provisionally at least, and
11 obviously you know, we need to keep this under
12 review, there is no clinical indication to screen
13 for hepatitis G. Technically we can't do it with
14 the current setup because it's not feasible to do in
15 mini-pools.
16 And of course the final thing is, is
17 that even if we did it we'd actually have a major
18 reduction in the donor panel. We tried this and
19 obviously that has knock-on effects in terms of
20 blood supplies.
21 So the provisional decision at this
22 stage is not to introduce any hepatitis G screening,
23 and obviously we're keeping that position under
24 review.
25 I'm now going to talk very briefly about
26 the newly-discovered transfusion-transmitted virus.

1 And again, we're going to try and attempt to address
2 some of the issues that surround possible
3 transmission of TTV by blood transfusion.
4 This is really a summary of data
5 published by Nishizawa at the end of last year.
6 There's a new DNA virus. It's been partially
7 characterized. And it was actually initially
8 discovered by subtractive PCR from patients who had
9 non -- post-transfusion, non-A to non-C hepatitis.
10 They identified a 500 base pair clone
11 after three rounds of subtractive PCR. And the
12 sequence seemed to be coding but didn't correspond
13 to anything on the databases. There's been some
14 characterization work of this virus.
15 It seems to have a single-stranded DNA
16 genome which is quite unusual, and the only real
17 virus family which is comparable would be the
18 parvaviruses. The clone's been extended out to
19 about 3000-some hundred bases, and it's arranged
20 with a couple of open reading frames. We don't know
21 whether this is a full genome or not; probably not.
22 There is a large open reading frame
23 which is largely enriched and it's been suggested
24 this might be a structural protein that may form a
25 nucleic capsid.

1 So we embarked on a study looking at
2 relevance of this blood transfusion. It's quite
3 easy to pick up by PCR and so we wanted to really
4 look at the prevalence of active TTV infection of
5 blood donors.
6 And we've done a series of subsidiary
7 investigations looking at its role in fulminant
8 hepatitis, and also to see whether in fact, it's
9 transmissible by blood products. And you can get an
10 idea of that by screening hemophiliacs who have been
11 treated with non-viral, inactivated concentrates.
12 Just to give you the basic data, we
13 screened 1,000 donors. They're screened initially
14 in pools of ten and then split. And splitting them
15 we identified a frequency of nearly two percent per
16 1,000 donors, so that's quite high.
17 I'd like to just qualify this prevalence
18 figure. The virus itself is extremely heterogenous
19 in sequence, and certainly none of the primers that
20 are published to-date including our own, will
21 necessarily pick up all genetic variance of the
22 virus.
23 And in fact, it could well be that in
24 fact, the more we study the virus and gauge its
25 variability, the higher this prevalence figure is
26 actually going to get. Okay, certainly we know that

1 in Scottish blood donors there a much more divergent
2 variant than we've been able to pick up so far.
3 There's no association at this level
4 with hepatitis G, so there are no cases of co-
5 infection with TTV. There were an over-
6 representation of males in the blood donor
7 population. And in fact, the mean age was 53 which
8 is very unrepresentative of the blood donor
9 population and gives you a clue about its
10 epidemiology.
11 And at the moment we're getting the
12 archive samples out to look at the incidence of the
13 virus in the same way as hepatitis G.
14 We've since done some survey work in
15 other countries, and it's quite remarkable how the
16 prevalence varies in different parts of the world.
17 In Japan it's been described to be present in about
18 12 percent of donors, but if you actually look at
19 some of the data generated from tropical countries
20 you see a quite different pattern of epidemiology.
21 So for example, in African countries you
22 can see prevalences ranging from seven percent in
23 Sudan which we don't understand, right up to 83
24 percent of the adult population in Gambia, with
25 Zaire being roughly 50 percent.

1 We find the same very high prevalence in
2 Papua, New Guinea, and also some of the indigenous
3 peoples in Central and South America. And we've
4 done some work looking at the acquisition of TTV in
5 Zaire and we've been able to show that in a
6 population where the adult prevalence is around
7 about 50 percent it seems to be that you acquire TTV
8 in the first year of life.
9 So for example, in children born of a
10 cohort of about 150 women, we've found an instance
11 of 54 percent over the first year of life.
12 Interestingly, they're all negative at three months
13 but they became positive at 12 months, and we think
14 that in fact, there's an environmental source for
15 the infection.
16 Part of the reason for saying that is
17 that if a child acquires TTV it's not related to the
18 TTV status of the mother. So a child from a TTV-
19 negative mother is as equally likely to acquire TTV
20 as a child of a TTV-positive mother.
21 And so, you know, broadly I'd say the
22 epidemiology is more comparable to hepatitis A,
23 especially with this differential prevalence of
24 viremia in different countries.
25 Okay, there's widespread contamination
26 of blood products with TTV. These are figures from

1 pre-virus inactivation that we published before.
2 These are volunteer unpaid donors. We can see quite
3 frequent detection of TTV in at least Factor VIII
4 and Factor IX concentrates.
5 We also detected after virus
6 inactivation. So for example, amongst these
7 volunteer donors' products which were largely
8 solvent detergent treated, we still detect TTV quite
9 commonly with no evidence that in fact, this has
10 actually removed the virus from the concentrates.
11 On the other hand I think on these very
12 small figures, the concentrates manufactured
13 commercially seem to be lower than these. The
14 difference may be because these are largely heat-
15 treated whereas those are solvent detergent. And
16 this sort of makes sense in terms of the virus
17 possibly being non-enveloped and more similar to
18 parvavirus than to hepatitis C.
19 And the evidence at this stage will be
20 that it is transmissible by blood products, because
21 we do see high frequencies of infection in the
22 hemophiliacs. And I just want to qualify these
23 prevalence figures. These are based on primers that
24 will not pick up all genetic variants of TTV, so in
25 fact these figures may be higher than shown here.

1 But clearly, if you look at patients
2 with hemophilia A who were treated before 1986, so
3 they received non-inactivated concentrates, you can
4 see an increasing prevalence of viremia with the
5 disease severity and Factor VIII usage. Similarly
6 for Factor IX where the moderates to severe have a
7 greater than 50 percent prevalence of viremia.
8 This figure is the important one. These
9 are hemophilia A patients who have received only
10 virally-inactivated concentrates. And so far we've
11 only found one positive out of 19, but clearly we
12 need to increase that number to establish whether
13 any of the inactivation procedures are effective for
14 TTV.
15 Because clearly we know they're not
16 effective for B19, and we get regular transmission
17 of B19 in this particular hemophilia group.
18 So in summary, the regulatory position
19 as regards hepatitis C actually does give us this
20 sort of possibility of setting up a framework to get
21 PCR screening blood donors established. And if you
22 can do that then obviously there are a series of
23 interesting issues concerning what viruses you'd
24 actually want to screen for.
25 Obviously the greatest clinical benefit
26 will be for recipients of non-inactivated

1 components, so these are the red cell and platelet
2 components. So clearly that's going to be a major
3 strategy or priority in terms of any further
4 development and work.
5 And key in that is actually getting the
6 turnaround time down to about a day. But having
7 said that, B19 still transmits through blood
8 products and clearly non-enveloped virus may also
9 survive some of the inactivation procedures. So
10 again, PCR screen may be relevant for blood products
11 as well.
12 And obviously, the criteria that we need
13 to consider, obviously what the frequency actually
14 is, what the incidence is, whether the other methods
15 for screening are adequate. And obviously, there's
16 a contrast between hepatitis B and hepatitis C.
17 And then finally the disease
18 associations of the virus, and I think we've more or
19 less drawn a blank with hepatitis G. We may draw a
20 blank with TTV in the future.
21 And finally, I'd like to just
22 acknowledge the groups in the University of
23 Edinburgh and the Blood Transfusion Service who have
24 been responsible for this work. Thanks so much.
25 CHAIRPERSON TABOR: Thank you, Dr.
26 Simmonds, and we'll also look forward to hearing

1 more about your TTV work tomorrow at the Advisory
2 Committee Meeting.
3 The next speaker is Dr. Jens Bukh from
4 the National Institute of Allergy and Infectious
5 Diseases.
6 DR. BUKH: I'd like to thank FDA and the
7 organizers of this workshop for inviting me.
8 Hepatitis C virus is the sole member of
9 the genus hapacivirus within the flavivirus family.
10 Among the flavivirus the hepatitis C virus is most
11 closely related to a group of unclassified -- the
12 so-called GB agents -- in particular, to a virus
13 called GB virus B; a virus that causes acute
14 hepatitis in the experimentally infected Tamarins.
15 The single-stranded, positive sense, INA
16 genome contains a single, long, open reading frame
17 that encodes structural and non-structural proteins.
18 Characteristic is the extensive, genetic
19 heterogeneity at the nucleotide level and the
20 deduced amino acid level throughout this open
21 reading frame, but in particular in the two envelope
22 genes and also hypervariable region as Harvey also
23 mentioned, has been defined in the amino-terminal
24 end of E2.
25 Now, this genetic heterogeneity in the
26 open reading frame makes it next to impossible to

1 design primers and probes for sensitive diagnostic
2 assays throughout the open reading frame.
3 Fortunately however, the five prime non-coding
4 reading or untranslated region of about 341
5 nucleotides contains highly conserved domains.
6 This slide shows the genetic
7 heterogeneity of the 5 prime UTR of hepatitis C
8 among 90-plus HCV isolates from around the world.
9 The red bars indicate the percent of these isolates
10 which differs from the consensus sequence. And
11 green triangles indicate precisions with insertions
12 in certain isolates.
13 And the important message of this study
14 that we published back in 1992 is the existence of
15 these universally conserved sequences. For example,
16 this region of 63 nucleotides that are variant among
17 all these isolates that represented all of the six
18 major genotypes of hepatitis C virus. So these are
19 of course, unique for diagnostic assays.
20 I should mention also that in the 3
21 prime untranslated region that consists of the
22 short, variable sequence followed by a poly U-UC
23 region of variable composition and length, but at
24 the very 3 prime end there's a highly conserved
25 sequence that was actually first identified in 1995.

1 This sequence of about 98 nucleotides is
2 highly conserved among the genetic variance of HCV.
3 However, the secondary structure, especially at the
4 very 3 prime end, is very strong so this might cause
5 some problems for standardizing nucleic acid test
6 using this sequence.
7 Just to illustrate the importance of
8 selecting conserved primers or probes for diagnostic
9 assays, I'd like to show the data from this study
10 where we selected five different primer sets from at
11 the time, what we thought was conserved sequences
12 based on available sequence data.
13 One such primer pair was from within the
14 5 prime UTR; two other different primer sets had the
15 sense primers in the 5 prime UTR, and the anti-sense
16 primers in the capsid gene. And finally, a fourth
17 primer set had the primers within the conserved
18 helicase sequence in NS3.
19 We showed that these primers had
20 equivalence sensitivity on a matched control, and
21 when we tested equivalent amount of HCV RNA from 114
22 first-generation anti-HCV positive patients in a
23 nested RT PCR assay, we found that the primers from
24 NS3 only detected about one-third of the patients
25 that were detected with the primers from within the
26 5 prime UTR.

1 And we found that all of these 27
2 patients detected with the NS3 primers were of
3 genotype 1, whereas patients detected with the 5
4 prime UTR primers represented all of the six major
5 genotypes of HCV; again, illustrating the importance
6 of selecting conserved primers for diagnostic
7 assays.
8 Now, we originally characterized the
9 existence of six major genotypes by analysis of the
10 E1 gene from 51 HCV isolates from around the world:
11 genotype 1, 2, 3, 4, 5, and 6. Some of these major
12 genotypes had well-defined subtypes such as genotype
13 1A and 1B.
14 The same six major genotypes were
15 characterized by Dr. Simmonds by analysis of partial
16 NS5B sequences. And analysis of full-length
17 sequences have indeed, verified that HCV can be
18 classified into six major genetic groups.
19 This slides shows a phylogentic analysis
20 of the polyprotein in sequences that we recently
21 performed of representative isolates with genotype
22 1, 2, 3, 4, 5, and 6. It has been suggested that
23 the isolates that were published as genotypes 7, 8,
24 9, and 11 actually cluster with the genotype 6
25 isolates. And here is shown the full-length
26 sequence of genotype 11. The full-length sequence

1 of genotypes 7, 8, and 9 were only published last
2 month.
3 And also it's been suggested that the
4 isolates published as genotype 10 should cluster
5 together with the genotype 3 sequences, and an
6 analysis of full-length sequences certainly support
7 these suggestions.
8 So in essence, HCV can be classified
9 into six major genetic groups with a huge number of
10 sub-types that vary to varying degrees.
11 This slide shows the geographical
12 distribution of the six major genotypes of HCV.
13 Clearly, genotype 1 is the predominant genotype in
14 North and South America, in Europe, and also in most
15 areas of Asia.
16 Genotypes 2 and 3 are found throughout
17 these regions at a somewhat lower prevalence.
18 Genotype 4 is the predominant genotype in Egypt and
19 in Central Africa; although other genotypes have
20 been described in this region of the world.
21 And genotype 5 is the predominant
22 genotype in South Africa. Both of these genotypes
23 are only found sporadically outside of Africa. And
24 genotype 6 constitutes a significant proportion of
25 isolates in Southeast Asia and are only found
26 sporadically outside of this area.

1 A number of indirect methods have been
2 developed to determine the genotype of HCV. Dr.
3 Komoda and co-workers developed a type-specific PCR
4 for HCV genotyping in which a portion of the core
5 gene is amplified in a first round of PCR with
6 conserved primers. Then a nested round of PCR is
7 performed with conserved sense primers and type-
8 specific, anti-sense primers, and a genotype is
9 deduced from the size of the PCR amplicon.
10 This method is quite specific but the
11 shortcoming of course, is that this method can only
12 discriminate between a few of the described
13 genotypes.
14 Another method that was developed by Dr.
15 Simmonds and also by others is the restriction
16 fragment length polymorphism for HCV genotypes in
17 which typically a portion of the 5 prime UTR is
18 amplified with universal primers in a nested PCR and
19 then the amplicons are exposed to digestion with
20 specific restriction enzymes. And the genotype is
21 deduced from the specific pattern.
22 The shortcoming of this method is due to
23 the high degree of conservation in the 5 prime UTR.
24 Because of this conservation a number of the
25 recognized genotypes cannot be distinguished.

1 The LIPA assay which was developed by
2 Dr. Styver and Maertens at Inno Genetics -- this
3 assay is based -- consists of a strip with a
4 genotype-specific probes down to it. And then this
5 strip is reverse hybridized with amplicons of the 5
6 prime UTR.
7 The shortcoming of this method is the
8 same as those for IFLP and is due to the high degree
9 of conservation of the 5 prime UTR. So for example,
10 in Italy many genotypes that were classified as
11 genotype 2A with this method actually turned out to
12 be 2C. And there's other examples like that.
13 Finally, I should mention that there's
14 also serotyping methods on NS4 developed by Dr.
15 Simmonds and co-workers. This method can also
16 determine type-specific antibodies to the six major
17 genotypes of HCV.
18 Again, the most specific method of cross
19 sequence analysis, and it's generally recommended to
20 perform sequence analysis of core E1 or NS5B since
21 the most comprehensive reference data is available
22 for these regions. But in fact, genotype-specific
23 differences exist throughout the open reading frame.
24 And I just want to mention that of
25 course with the highly conserved sequences available
26 in the 5 prime UTR, it should be possible to design

1 diagnostic tests, both qualitative and quantitative,
2 that have equivalent sensitivity against the various
3 genotypes of HCV.
4 However, the commercial tests had had
5 problems in this regard. We have used both of these
6 tests in chimpanzees infected with various genotypes
7 of HCV and a number of other studies have looked at
8 this issue. The monitor tests which is -- the
9 first-generation monitor test which is still at the
10 market clearly underestimates the titers in patients
11 infected with genotypes other than genotype 1.
12 And I think this is being addressed in a
13 second-generation test that I believe will soon be
14 marketed. Also, this test underestimates the titers
15 in patients with high viral titers.
16 The first-generation bDNA test which is
17 now off the market, clearly also underestimated the
18 titers of certain genotypes. In the second
19 generation test this problem has presumably been
20 eliminated. It certainly seems like that.
21 However, the problem with this second-
22 generation test of course, is the low sensitivity.
23 I believe there's a third-generation test underway
24 to address this issue.
25 These genotype-specific differences of
26 course relate to all in-house RT-PCR assays as well:

1 qualitative or semi-quantitative. And this points
2 to the importance of using standardized controls as
3 the ones developed by Dr. Lelie in Europe where he -
4 - for certain genotypes.
5 And I also wanted to mention that we
6 have generated plasma pools of prototype strains of
7 HCV of genotype 1B, 2A, 2B, 3A, 4A, and 5A in
8 chimpanzees. This was study done in collaboration
9 with a number of scientists and also among those,
10 Steve Feinstone and the FDA.
11 And we've determined the genome titers
12 of these pools with available commercial testing,
13 also, and by in-house tests -- the tiers of which
14 are shown here. And we've determined the
15 infectivity titers of those pools by reverse
16 titration in chimpanzees, and they all had
17 infectivity titers ranging from 10^3 to 10^5
18 infectious titers -- doses per ml.
19 We also have a plasma pool, the 8s pool
20 that Dr. Harvey Alter developed, with known
21 infectious titer. And this is a genotype 1A pool.
22 It is our hope that these standardized pools could
23 be useful in standardizing diagnostic assays.
24 But also of course, these pools would be
25 important -- could be important for challenge in
26 future vaccine studies in chimpanzees.

1 I will also mention another very
2 important aspect of the genetic heterogeneity of HCV
3 is the fact that HCV circulates cross-species in a
4 single, infected individual. Whereas the
5 differences between isolates of some of the major
6 genotypes can be as high as 35 percent, and isolates
7 within a subtype vary typically by five to ten
8 percent. The differences among cross-species is
9 generally less than two percent.
10 We recently determined the complete open
11 reading frame sequence from 18 clones deduced from
12 the acute phase sample that Harvey Alter also
13 mentioned earlier. And we found that cross-species
14 -- that some of these sequences varied by about two
15 percent over the entire genome.
16 We found the changes were found too,
17 after genome, although there was most changes in the
18 hypervariable -- in the envelope genes, in
19 particular in the hypervariable region. But in
20 fact, the cross-species could be observed throughout
21 the genome.
22 We also went on and studied a sample
23 taken 20 years later in this patient. We determined
24 the consensus sequence of the complete open reading
25 frame. And this slide shows the mutation rate in
26 this patient during 20 years in the individual gene

1 regions, expressed the substitutions times 10^{-3} per
2 site per year.
3 Nuclear-type mutation rates are shown in
4 blue and amino acid mutation rates are shown in red.
5 The highest note that I've divided the E2 gene into
6 the hypervariable region, which is the amino
7 terminal 30 amino acids of E2 and the remainder of
8 E2 are 333 amino acids.
9 The highest mutation rates were found in
10 E2, P7, and NS4A, but there was a particularly high
11 mutation rate in the hypervariable region and most
12 of the changes in this region resulted in amino acid
13 changes.
14 And of course, this region is the region
15 that has been found to undergo sequential changes in
16 infected individuals. And also this region has been
17 found to contain potential neutralization epitopes.
18 So as Harvey Alter indicated, this region is thought
19 to be of great importance for the persistence of
20 HCV.
21 Now, one of the problems with studying
22 the cross species in humans or in experimentally-
23 infected chimpanzees, is that from the outset these
24 patients are infected with cross-species. So it's
25 different to see which mutations are new mutations

1 or which mutations are just selection of pre-
2 existing variants.
3 Last year we developed an infectious
4 clones of genotype 1A and genotype 1B, and the
5 infectivity of these clones was tested by
6 transfection of chimpanzee liver with INA
7 transcripts. And the advantage of studying HCV
8 evolution in such infected chimpanzees is that these
9 chimpanzees presumably are infected with the
10 monoclonal virus population.
11 We transfected two chimpanzees with the
12 infectious clone of genotype 1A. And this slide
13 shows the course of the infection of one of these
14 chimpanzees. This chimpanzee had an acute resulting
15 infection with viremia from week-1 to 23 post-
16 inoculation. We could not detect the virus in serum
17 samples from week 24 through week 52 post-
18 inoculation. This site shows data to week-40.
19 The viral titer detected with the
20 monitor test and also with in-house shown in red
21 dots, and with the in-house RT-PCR shown in blue
22 columns -- that viral titer increased from about 10^2
23 of week-1 post-inoculation to peak viral titers of
24 10^5 to 10^6.
25 The chimpanzee developed acute hepatitis
26 with elevated liver enzyme values and also, although

1 not shown in this slide, necroinflammatory changes
2 in liver biopsies. The second-generation ELISA
3 tests became positive around week-15 post-
4 inoculation, and again the virus was cleared in this
5 chimpanzee.
6 The other chimpanzee however, although
7 this chimp was infected with a monoclonal virus,
8 developed chronic, persistent infection. This chimp
9 had viremia from week-1 post-inoculation through
10 week-70 post-inoculation. This slide shows data
11 through week-52 post-inoculation.
12 Again, the viral titer increased from
13 about 10^2 at week-1 up to peak viral titers of 10^5
14 to 10^6. The chimpanzee developed acute hepatitis
15 with elevated liver enzyme values and inflammatory
16 changes in liver biopsies.
17 This chimpanzee became positive for
18 anti-HCV in the second-generation ELISA test at
19 week-13, and also we detected two antibodies in this
20 chimpanzee with an experimental ELISA test performed
21 at Abbott. And the E2 antibody titers increased
22 over time.
23 The viral titers following appearance of
24 antibodies and acute viral hepatitis decreased by
25 one to two logs and reached its lowest level at
26 about week-24 post-inoculation with titers between

1 1000 and 10,000 copies. Again, the viral titers
2 rebounded somewhat and was about 10^5 after one
3 year's follow-up.
4 Now, we've studied the sequence in this
5 chimpanzee over time. We determined the complete
6 open reading frame sequence at seven different
7 timeframes during the first year of follow-up. At
8 week-2 post-inoculation the complete -- the
9 nucleotide and the deduced amino acid sequence of
10 the complete open reading frame was identical to
11 that of the infectious clone.
12 However, at week-8, 12, and 20 post-
13 inoculation there was a single nucleotide change
14 that resulted in an amino acid change, and this
15 slide only shows amino acid changes in the NS3
16 protease domain. This mutation could actually be
17 detected already at week-5 post-inoculation.
18 At week-31 and week-40 there was six
19 additional amino acid changes: two in P7, one
20 additional change in P in NS3, two changes in NS5A,
21 and a single change in NS5B. And at week-52 there
22 was a single, additional change in NS3.
23 Note that there was absolutely no
24 changes in E1 or E2 proteins during the first year
25 of follow-up. And clonal analysis at week-31 of
26 multiple clones showed no evidence of cross-species

1 in this chimpanzee. And in particular, all of the
2 clones that we analyzed had identical, hypervariable
3 region sequence both at the nucleotide and at the
4 deduced amino acid level.
5 So a main difference from the mutation
6 rates that we saw in this chimp after one year of
7 follow-up and the patient H that we followed for 20
8 years, was a total lack of amino acid changes in
9 this envelop proteins, suggesting that clearly this
10 virus was able to persist in this chimpanzee without
11 evolution in this region.
12 Maybe also suggesting that a number of
13 factors could influence the -- could result in the
14 high persistence rates of HCV. Of course we have to
15 remember that these studies were performed in
16 chimpanzees and there could potentially be important
17 differences in humans and in chimpanzees.
18 And the data that I've presented here
19 today, the actual data, was performed in the
20 Hepatitis Virus Section at NIH. Thank you.
21 CHAIRPERSON TABOR: Thank you very much,
22 Dr. Bukh. The next speaker is Dr. Betty Robertson
23 from the Centers for Disease Control and Prevention.
24 DR. ROBERTSON: Thank you. First of
25 all, I'd like to thank the organizers for inviting

1 me to give this talk. If I could have the first
2 slide please; the remote.
3 The title of my talk today is the
4 Application of PCR to the Molecular Epidemiology of
5 HCV. And as mentioned earlier, Harvey used the
6 elephant analogy. I'd like to use the iceberg
7 analogy to look at HCV infections in which about
8 five to maybe 20 percent of infections are
9 symptomatic -- either acute or chronic -- but 80 to
10 95 percent of them are asymptomatic, whether they're
11 acute or chronic.
12 And as in previous studies which have
13 looked at HCV infections within the United States --
14 and I'm focusing on HCV infections within the U.S. -
15 - most of the studies have looked at these cases
16 here. And these are summarized on this next slide.
17 There have been three or four studies
18 which have looked at chronic liver disease patients.
19 Most of these individuals are from tertiary care
20 units. And what we see is in most of the studies
21 there's been approximate ratio of subtypes 1A and 1B
22 being equivalent in these studies.
23 The one study in which there was a
24 different ratio of sub-type 1A to 1B was from the
25 Zein Study in 1996. These three studies here used
26 either LIPA or a mixture of LIPA, RFLP, and sequence

1 to assign a sub-type. The Zein Study here used
2 sequencing of the NS5B region.
3 We have focused on a population of
4 patients from the NHANES III collection. This is
5 the National Health and Nutrition Examination
6 Survey, and the study population was selected by a
7 stratified, multi-stage, probability cluster design,
8 and the participants were representative of various
9 ethnic groups within the U.S. population. They were
10 selected to represent the total civilian, non-
11 institutionalized population within the United
12 States.
13 The methods that were used to do the
14 analysis for this particular collection were the
15 avid HCV assay, the HCV-2 assay, followed by matrix
16 confirmation. RNA detection was an in-house,
17 nested, 5 prime UTR amplification. Sub-type
18 determination was done by NS5B RT-PCR followed by
19 sequencing.
20 And the overall sero-prevalence using
21 antibody assays was 1.8 percent within the United
22 States. And within the various ethnic groups that
23 were represented in this study, whites had a sero-
24 prevalence of 1.5 percent, blacks, 3.2, and Mexican-
25 Americans 2.1 percent.

1 If we look at the sero-prevalence by age
2 we see that there is increasing sero-prevalence with
3 age with the majority of the individuals -- majority
4 of the anti-HCV positive being between the ages of
5 30 and 49, as shown here on this slide. Also shown
6 are the different ratios of anti-HCV positives in
7 the different ethnic groups.
8 If we look by race and ethnic group and
9 gender in this particular population for sero-
10 prevalence, what we find is that males overall have
11 a higher sero-prevalence than females, and in
12 particular, black males had the highest sero-
13 prevalence with over four percent serum anti-HCV
14 positive within that particular group.
15 We then looked at the PCR-positive
16 samples within this population. The total number of
17 samples that were antibody positive were 378. Of
18 these, 364 were available for PCR testing. We then
19 used the 5 prime UTR region for detection and found
20 283 of these positive. This calculated out to a
21 73.9 percent of the antibody-positive samples were
22 RNA-positive.
23 And then when we looked at the NS5B
24 region we were able to successfully amplify 97
25 percent of the 5 prime UTR samples. And these will
26 be the ones that I'm going to talk about now.

As we look at the proportion of antibody-positive that were RNA-positive, in general 60 to 70 percent of the antibody-positive samples were RNA positive. The one difference was found in black males in which they had about a 95 percent of the antibody-positive samples were also RNA-positive.

This is the overall data that we found from this population of anti-HCV positive individuals. The ratio of 1A to 1B is slightly different with about 52 percent being 1A and 27 percent being 1B. The remaining sub-types that were present in this population include 2A, 2B, 3A, and a few number of 4A and 6A.

This looks at the sub-type distribution in the various ethnic groups. And what is striking when we looked at this analysis is that whites and Mexican-Americans had roughly the same ratio of 1A to 1B as we found in the overall population. However, if we looked at the blacks the proportion of sub-type 1B that is represented in this particular ethnic group was much higher than in whites and Mexican-Americans.

When we looked at sub-types as distributed by age we saw no specific sub-type at any particular age group, and each sub-type was

1 generally spread over the general curve of anti-HCV
2 positives -- with the exception perhaps, in the
3 greater than 70 age group in which there were a
4 higher number of 1B infections.
5 The conclusions from these studies were
6 that the overall anti-HCV sero-prevalence in the
7 United States is 1.8 percent, with blacks having the
8 highest sero-prevalence. Secondly, the majority of
9 the infected population is between 30 and 49 years
10 of age. In general, 75 percent of anti-HCV-positive
11 individuals are RNA positives; however, 90 percent
12 of the antibody-positive black males -- that should
13 be 95; typographical error -- of antibody-positive
14 black males are RNA positive.
15 Within the general U.S. population sub-
16 type 1A predominates over 1B. And lastly, the
17 proportion of sub-type 1B infections is higher among
18 blacks compared to other ethnic groups.
19 And lastly, I want to acknowledge the
20 individuals who are responsible for the data I
21 presented in this talk. Omana Nainan, FengXiang
22 Gao, Emory Meeks performed a lot of the laboratory
23 analysis; Pat Coleman, Linda Moyer, Helen Margolis,
24 Miriam Alter involved in analysis in the
25 epidemiology of it; and Deanna Kruzon-Moran and

1 Jerry McQuillan who were involved in the analysis
2 and conception of the studies. Thank you.
3 CHAIRPERSON TABOR: Thank you very much,
4 Dr. Robertson. We'll now have a 15-minute break.
5 After the break we have time for discussion and
6 questions followed by industry presentations, and I
7 think the industry presentations will provide some
8 of the most interesting technological information of
9 the meeting.
10 I understand that some of the speakers
11 in the industry portion are interested in using a
12 multimedia format, and we do have equipment
13 available. So if any of you are planning to do that
14 please see me now.
15 The coffee and other food is available
16 in the cafeteria which is on this floor. The floor
17 is arranged in a somewhat circular format so you can
18 get to the cafeteria by going either left or right
19 when you leave the conference room. We'll reconvene
20 in 15 minutes. Thank you.
21 (Whereupon, the foregoing matter went
22 off the record at 10:20 a.m. and went
23 back on the record at 10:40 a.m.)
24 CHAIRPERSON TABOR: It's time to
25 reconvene the workshop. Could I ask all of the
26 speakers from this morning's session to sit at the

1 tables and we'll have a period of questions from the
2 audience. Would anyone asking questions please use
3 the microphones that are -- there's one at the front
4 of the audience.
5 Perhaps I can start off the questions by
6 asking Dr. Robertson why she thinks the percentage
7 of black males with HCV infection, with anti-HCV and
8 HCV RNA detectable is as high as 95 percent; which
9 is quite a bit higher than the general population.
10 DR. ROBERTSON: I don't have an
11 immediate answer for that, unless Miriam Alter might
12 have an idea. It's an interesting observation at
13 this point.
14 CHAIRPERSON TABOR: Could we ask Dr.
15 Miriam Alter? Do you have any explanation for that?
16 It's just an observation then, at present?
17 DR. MIRIAM ALTER: It's also interesting
18 that they happen to have -- they're more likely to
19 have 1B than 1A, but we haven't done any cross-
20 analysis to look at RNA by genotype within that sub-
21 group to see if that's the reason or if that --
22 well, it wouldn't be a reason, but if there's some
23 correlation between that as well in that sub-group.
24 But I don't know.
25 CHAIRPERSON TABOR: As a long-term,
26 public health issue this might mean that several

1 decades from now there could be a more serious
2 problem with severe liver disease in the black males
3 with anti-HCV than in others? Is that a
4 possibility?
5 DR. MIRIAM ALTER: If in fact, this is
6 related to progression, which we don't know.
7 CHAIRPERSON TABOR: Thank you. Are
8 there any questions or discussion from the audience?
9 Please identify yourself when you begin.
10 MR. BRASS: I'm Cliff Brass, Schering
11 Plough. I just wanted to make one additional
12 comment which relates to this and I think it will be
13 an important public health issue. There are several
14 small studies now soon to be presented which suggest
15 that the response rate to typical therapy in
16 African-American patients is much lower than the
17 caucasian population.
18 I wonder if there's any other
19 epidemiologic evidence that may give us a reason for
20 that, and just suggest again that this is going to
21 be an important issue.
22 DR. MIRIAM ALTER: If it's related to
23 genotype then it would be consistent with what we
24 see with other patients with genotype 1 and 1B,
25 particularly. So that -- do you know whether or not
26 they were more likely to have 1B as well?

1 MR. BRASS: Well, in the studies that
2 I'm familiar with, both populations predominantly
3 had 1, and I'm not sure of the ratio 1A to 1B. I'm
4 not sure if the studies are big enough to really
5 ferret that part of it out.
6 DR. MIRIAM ALTER: Right, but that could
7 be the reason behind their much lower response as
8 well; in that they are more likely to have 1B.
9 MR. BRASS: Although I think most of the
10 data I'm aware of right now suggests that, probably
11 certainly for combination therapy and probably for
12 monotherapy, the 1As and the 1Bs have a similar
13 response rate.
14 MR. JACKSON: Brooks Jackson, Johns
15 Hopkins. A question for Harvey Alter or Dr.
16 Robinson. Of the persistent HCV antibody-positive
17 donors, about ten to 25 percent are PCR-negative, do
18 you think that's -- really reflects containment of
19 the virus or just failure of the PCR assays to
20 detect point mutations in the primer binding sites
21 and that sort of thing?
22 CHAIRPERSON TABOR: I don't see Harvey
23 Alter. Dr. Robertson, would you like to answer
24 that?
25 DR. ROBERTSON: I guess -- I don't know
26 whether it's an inability for detection based upon

1 point mutations in the primer binding site,
2 especially when you're using the 5 prime UTR. The
3 sites are fairly conserved.
4 You know, it might be possibly a
5 reflection of the viremia level. That's always a
6 possibility.
7 CHAIRPERSON TABOR: Dr. Miriam Alter.
8 We're going to have to get rid of the one dead
9 microphone that's there.
10 DR. MIRIAM ALTER: There are some
11 supporting evidence that would suggest these could
12 in fact, represent resolved infections in that
13 you're much more likely to find RNA negativity in
14 people who also have persistently normal enzymes.
15 Whereas those with elevated enzymes obviously
16 largely have abnormal ALTs and so it would suggest
17 that in fact, it could be real.
18 And I know at least in Harvey's data
19 this was a repeat -- these individuals were
20 repeatedly sampled over at least a year and in some
21 cases, more. So he -- even though he's not in the
22 room, we speak for each other all the time -- I
23 think he feels fairly confident that these truly
24 represent individuals who have resolved their
25 infection.

1 And if I'm not mistaken there are biopsy
2 data showing that these individuals have normal
3 livers. And I think that data is probably available
4 from Europe as well.
5 MR. TICEHURST: John Ticehurst from FDA
6 and Johns Hopkins. Back again, dealing with this
7 observation about the ratio of RNA to antibody
8 positivity in certain groups, I wonder if you could
9 look at it from a sort of flip side; that maybe
10 there's some differences -- that's pertinent to the
11 subject of the meeting -- if there are differences
12 in antibody response or persistence in certain
13 groups that would be a reason for that ratio to be
14 different.
15 Have you -- you look puzzled, Betty Jo.
16 Do you understand what I'm -- what I'm saying is, is
17 it possible that there are, for example in the
18 African-American males, that they would have a --
19 there would be a reason for fewer of them who are
20 truly HCV-infected to be antibody-negative, either
21 relating to chronic infection or a window period?
22 The only way to look at that, you'd have
23 to look at a subset of your antibody-negative people
24 for PCR positivity.
25 DR. ROBERTSON: We have not looked at
26 the antibody-negative population; that's true.

1 Based upon what I showed we've preselected for
2 antibody-positive.
3 DR. SEEFF: Leonard Seeff from -- I'm
4 not sure where I'm from.
5 CHAIRPERSON TABOR: Now from NIH.
6 DR. SEEFF: NIH and the VA. With
7 respect to the racial difference, we have some
8 samples I think, that you know about Ed, going back
9 to 1948. A very large sample that we happened to
10 screen for hepatitis C and there was a
11 fantastically, strikingly different frequency of HCV
12 sero-reactivity between whites and blacks.
13 It's about 25 times higher in blacks
14 than it was among whites. And actually, it's
15 interesting. These were stored sera; they had not
16 been collected in the way we would like to have it
17 collected to make sure that nothing is lost. But a
18 large proportion of the entire groups -- some 65
19 percent -- were still PCR-positive in saved sera
20 over a 50-year period.
21 And when breaking according to the
22 racial grouping, about 80 percent of the ones who
23 were black were positive by PCR and a much lower
24 percentage of the whites. And follow-up to see
25 what's happened to them, we have a lower frequency
26 of mortality than we would have expected, but the

1 mortality was higher among the black group than the
2 white group.
3 So there's a real issue I think, about
4 the difference in the frequency of sera positivity
5 and outcome, and now the issue of response to
6 treatment, which seems to be less -- the results
7 seem to be less good among blacks than among whites
8 -- is an area that really deserves a lot of
9 attention.
10 CHAIRPERSON TABOR: Thank you.
11 DR. LELIE: Nico Lelie from CLB in
12 Amsterdam. I've a question to Steve Kleinman about
13 the use of the RIBA-3 -- the introduction of the
14 RIBA-3 and the reliability for confirmation. We did
15 sort of validation study a couple of years ago with
16 the RIBA-3 test and we found that especially double
17 bands could very well be false-positive. And so you
18 have a chance for about a ten percent to find an
19 indeterminate result.
20 It's also a chance of about one percent
21 to have a double reactivity that in fact, is non-
22 specific. And I have a feeling that this
23 contribution of non-specific RIBA-positive, double
24 reactivities also are included in the numbers or the
25 slight increase in prevalence of HCV infections in
26 your risk analysis.

1 So maybe could you comment on what
2 happens with the counseling of donors that have
3 those double band reactivities in the RIBA? Or
4 maybe Susan Stramer, what is the experience in the
5 United States?
6 DR. KLEINMAN: Yes, that's a good
7 comment, Nico. We haven't had a chance to look at
8 the data carefully enough to break out the two bands
9 from the three bands within the REDS experience.
10 And actually, this increase in
11 prevalence in the 3.0 tested is something that we
12 just really analyzed quite recently for this
13 conference. So I think a lot more work needs to be
14 done. I think there are several, potential
15 explanations.
16 One other possibility that's revealed by
17 the studies is that we presented last year through
18 the REDS group at ABB that there is an additional --
19 you can find some samples that are 3.0 positive,
20 RIBA-3 positive that are missed by EIA-2, but they
21 were all PCR-negative.
22 So whether we're picking up false
23 positivity or resolved infection is unclear. Now I
24 know that -- Sue can maybe comment on this -- but
25 the Red Cross has done studies on 2-band positives

1 and taken them to PCR, and I don't recall the exact
2 figures but was it 45 percent or --
3 CHAIRPERSON TABOR: Sue, could you --
4 DR. KLEINMAN: Yes. Maybe I'll let Sue
5 address that and maybe she can address -- as far as
6 I know the counseling messages are not different
7 depending upon whether people have two bands or
8 three bands at this point.
9 DR. STRAMER: No, the counseling
10 messages, Steve, you're correct; they're the same.
11 But I support what Nico said and probably the
12 increase of -- it's about less than ten percent of
13 confirmed positives converting from RIBA-2 to
14 unlicensed RIBA-3. And under our research protocol
15 for using the unlicensed test part of our research
16 goal is to look at the 2-band positive and determine
17 what percentage of those are true positives.
18 I don't have any follow-up data for
19 those 2-band positives but what we do know is that
20 44 percent of the 2-band positives are in fact, RNA-
21 positive; whereas 90 percent or greater of the 3-
22 band positives are RNA-positive. So there's
23 definitely a shift in the numbers of viremic
24 donations that are associated with both of those.
25 CHAIRPERSON TABOR: Thank you. I think
26 we should move on to the industry presentations.

1 We've had the luxury of extending some of the talks
2 of this morning a few minutes because Dr. Gretch was
3 prevented from attending today at the last minute.
4 However, I think during the industry
5 presentations we should try as much as possible to
6 stay within the timeframe. We'll begin with a
7 presentation by Dr. Conrad from National Genetics
8 Institute.
9 DR. CONRAD: I guess it's still good
10 morning. I'm going to present the methodology that
11 we've used to several million donations in the
12 plasma industry. My colleagues from Alpha and
13 Baxter will present the clinical data derived from
14 this methodology.
15 Essentially what we're doing is a sample
16 pooling regime using a matrix, and in consideration
17 of what matrix -- what size, what shape, what color
18 of matrix to use -- the issues are sensitivity, time
19 for identification of a donor where we eventually
20 hope to extend this into the whole blood industry,
21 and if you can identify donors reliably, and the
22 quality control exists to make sure that that
23 happens reliably.
24 So under IND we began investigating
25 hepatitis C HIV on these donors using a pooling
26 algorithm dependent upon automatic pipetting devices

1 computers that was very data management intense.
2 I'm going to describe the one that we decided to
3 use.
4 Basically you could use a variety of
5 different matrices: 2-dimensional pyramids and 3-
6 dimensionals. I think some of the other speakers
7 from industry will talk about the 2-dimensional and
8 pyramid methodologies. We opted not to use it for
9 reasons that we had trouble with it, and arrived at
10 a 3-dimensional matrix.
11 And essentially what a 3-dimensional
12 matrix is, it's -- the pipetting device makes a
13 cube. It's really not a cube but to understand it
14 for humans it's basically a cube. What the
15 pipetting device does is it takes 512 samples -- and
16 it doesn't have to be 512 samples; we validated the
17 system for up to 512 samples it can be any
18 symmetrical cube smaller than an 8 X 8 X 8 is what
19 we validated, which is -- 8 X 8 X 8 happens to equal
20 512.
21 It puts those samples into row, layers,
22 and columns. What happens then simply, is you test
23 the entire cube. If the entire cube is negative
24 then the components within it are negative. If the
25 cube is positive you test 8 rows, 8 layers -- the

1 yellow -- and 8 columns. The intersection of those
2 row, layers, and columns is the positive donor.
3 It's fairly straightforward when you say
4 it like this. It was complicated to get it to work.
5 Basically, the device that we chose is
6 called the TECAN Genesis machine. It's been two
7 years of hard work getting these machines up to
8 snuff where we've modified the software I think
9 probably 60 times to have it working as well as it
10 is now.
11 As of the 10th of September we have
12 tested 3,500,000 donations with this system. In the
13 clinical trial components of this discussed by Alpha
14 and Baxter you'll get some ideas of the accuracy,
15 the functionality, and it may be even a bit of the
16 expense involved.
17 Some very important terms that you'll
18 hear from my colleagues from those two companies.
19 Basically, the master pool is the term we use for
20 the 512 cube. It's called a master pool. It's
21 automatically made in duplicates so we have some
22 resolution if there's any issues with positivity.
23 It's tested four times in our system in
24 a multiplex mode. In order to ensure that we could
25 detect all different strains of hepatitis C equally
26 we have to use two primers. We do everything in

1 duplicate. We think redundancy is one of the most
2 important quality control steps that you can employ.
3 The row, layers, and columns we call
4 them primary pools. There's 64 samples in a primary
5 pool. And then the individual sample we call the
6 individual sample. It's very important to note, by
7 the time we call an individual "positive", we've
8 actually done 12 tests. So there's some concern
9 about PCR and false positivities.
10 At National Genetics we separate
11 everything into different buildings, we have all
12 this fancy contamination control. But there's
13 nothing like 12 repeats to help, and I'm sure people
14 like Dr. Stramer who are very experienced in, you
15 know, what repeat testing can bring, can tell you
16 that 12 iterations of a test brings an awful lot of
17 security, and I think that's what you'll see from
18 the Baxter and Alpha data that's presented.
19 The system is not tremendously prone for
20 false positives, and we learned an awful lot about
21 people who were truly positive though some of the
22 other tests didn't convince us of in these
23 processes.
24 Basically what any one given run looks
25 like is, it's all the samples. There's 60 samples
26 per membrane; they're transferred onto a membrane.

1 These are positive controls, these are positive
2 samples with the dark bands. They're done with one
3 primer set and then with an exactly identical second
4 primary set. Then they're hybridized with an
5 internal control. Any negative can only be called a
6 negative if the internal control shows and the
7 sample is not showing.
8 Basically -- so you would see is, in a
9 multiplex run you would see that there would be two
10 different repeats of the sample -- this is how we
11 test a master pool -- two with a second primer set.
12 You can see for this particular sample the second
13 primer set didn't hybridize as well and that has to
14 do with the heterogeneity of the hepatitis C virus,
15 even in the 5 prime, non-coating region.
16 We wanted to show that the internal
17 control -- we also evaluated what we called the
18 split mode which was a non-multiplex mode -- this
19 was at the urging of some of the fine folks from the
20 FDA -- to compare the internal controls and the non-
21 internal controls.
22 You can actually see that there is an
23 inhibition of the internal control competing with
24 the native HCV in cases where they are certain
25 heterogeneities in the sequence.

1 So a positive looks like this, a
2 negative looks like this, but note the negative has
3 to have all four of the internal controls showing.
4 And this is essentially what the computer would read
5 and then give us the positive or negatives, feed
6 that back into the TECAN file, implicate the
7 positive or negative donor. We would then pull out
8 the positive donor sample and test it.
9 Just briefly, it's important to know
10 that in order for the HIV and HCV portions of this
11 test we performed ICH-3 guideline limit tests to
12 determine the sensitivity. Sadly, there really are
13 no standards for HIV or HCV; this is what we did for
14 both of them. However, we did use the standards
15 from the EuroHep from Nico Lelie's group, from CDC
16 from Ming-ying Yu of FDA.
17 We used as many of the quality panelists
18 that we could get our hands on, and from Dr.
19 Saldanha -- sorry to leave you out there -- at
20 NIBSC, to ensure that some of the numbers that we're
21 giving are in agreement, at least closely in
22 agreement with the numbers that you'll see from
23 those panels.
24 For hepatitis C we have a sensitivity, a
25 mean sensitivity around 13 copies per ml using these
26 sensitive methods. For HIV where we needed to

1 compete rigorously with p24 -- and you'll see some
2 data that the pooled PCR system is actually better
3 and certainly equal to p24 testing. And Chuck
4 Frisbie from Alpha will show that to you.
5 We had to increase the sensitivity by
6 centrifuging more of the original master pool so we
7 brought it down to nine copies. And that was in
8 order for it to compete effectively with p24.
9 Genotypes, there's been a lot of talk
10 and some of the founders of the information of
11 genotype, Drs. Bukh and Simmonds, can tell you that
12 this is a big issue.
13 What we did is, we cloned all the major
14 sub-types, put them into transcription vectors,
15 transcribed them, quantitated the RNA and high copy
16 level, diluted it, and then detected it with the
17 multi-primer system that we're talking about and
18 found that the p-values or the Rho values were
19 highly significant, indicating that the system that
20 we're employing now detects all the major genotypes
21 and their sub-types evenly.
22 That was a bear. But we used those
23 highly conserved regions in the 5 prime region that
24 Dr. Bukh showed earlier. We called them our CON
25 primers, or consensus primers, as well as some of
26 the other primers in order to do this.

1 Results from the clinical studies
2 performed in collaboration with Alpha and Baxter
3 will be presented today; represents roughly 700,000
4 donations that we did during this finickal window
5 when we followed the donors up, from about 100,000
6 donors. So it's 700,000 donations from
7 approximately 100,000 donors. They'll talk to you
8 about that.
9 What I've tried to do here is describe
10 the methodology, make you familiar with the terms.
11 Now you're going to hear from the results of the
12 studies. I just wanted to mention, we've done pilot
13 studies in hepatitis B and we are finding higher
14 incidences of hepatitis B than we originally
15 suspected.
16 And it is similar to what Dr. Kleinman
17 said; that there is a tremendous amount of hepatitis
18 B that is not being detected with the surface
19 antigen or core antibody tests. And that data will
20 come forward later. We'll stick with HCV and HIV in
21 those clinical results.
22 Also, prepare yourself to hear
23 comparisons with the p24 antigen tests, and this is
24 going to be the comparison of pooled PCR in the 512
25 pool as compared to HIV testing with p24 in the
26 individual. And that's an important distinction.

1 This isn't PCR in the individual sample against PCR
2 in the -- p24 in the individual. It's pooled PCR
3 versus the individual.
4 You'll also hear some of the
5 implications and the importance of ALT. Does ALT
6 precede antibody or not precede it; some of those
7 issues. We'll really expand on window periods and
8 the length of window periods. This is the first
9 time we've really been able to prospectively look at
10 window periods by using nucleic acid testing.
11 I think that you'll see a little
12 interesting data on the different antibody kits
13 because now we have a standard to compare them, and
14 that's the gold standard issue. Is nucleic acid
15 testing really the gold standard in that it's the
16 hallmark of the virus; without the nucleic acids
17 there there's not an infectious virus.
18 There's been questions whether or not
19 the detection of nucleic acids is really -- you
20 know, the fact that some people don't have nucleic
21 acids but have antibodies; what's the origin of
22 that? And we really do believe that that's someone
23 who's resolved the infection. Because we've tried
24 with many different primers, many different nucleic
25 acid tests, and those patients seem not to have HCV.

1 So that's what we'll see in the
2 subsequent two talks. And I will then turn it over
3 to Chuck Frisbie from Alpha for the first part of
4 that presentation.
5 MR. FRISBIE: I'm Chuck Frisbie and for
6 the last two-and-a-half years I've been helping
7 Alpha to implement PCR testing of plasma pools in
8 collaboration with National Genetics Institute. And
9 what I'm going to present today is some of the data
10 we've obtained in our clinical trial and also post-
11 clinical trial HCV testing.
12 This is a brief description of our
13 clinical design. Eligible subjects had to be for
14 HCV, antibody-negative, PCR-positive. And once
15 enrolled these subjects would be followed for six
16 months or until sero conversion.
17 For HIV it was similar except for the
18 fact that to qualify for enrollment you could either
19 be p24 antigen-positive and/or PCR-positive. And
20 you'll see later where there are -- actually HIV
21 donor who was enrolled was enrolled based only an
22 the initial PCR-positive result and the absence of
23 p24 antigen.
24 As far as participation we had 33 study
25 sites. From those sites we collected and tested
26 342,729 donations and again, over a 4-month period.

1 And in that number we found 22 individuals who were
2 qualified to be enrolled in the HCV study. Of
3 those, 13 were enrolled and nine were not.
4 For the HIV study we had found four
5 individuals who were qualified and two of those were
6 enrolled and two were not. However, all the data
7 obtained was considered in our evaluation.
8 This slide gives some numbers regarding
9 the number of donations that were interdicted by PCR
10 that would not have been found to be a reject based
11 on any other testing. For example, with HCV they
12 were negative for antibody and they were not
13 elevated for ALT. We had 75 donations that fell
14 into that category.
15 In the post -- that's over a 4-month
16 period, again. In the post-clinical trial we added
17 more sites to the study and it lasted -- so far the
18 data includes 12 months, approximately, and we have
19 373 additional donations that have been interdicted
20 based in that same category, for a total of 448
21 since we've implemented PCR testing. And again,
22 this is through testing of the 3-dimensional plasma
23 pool of 512.
24 HIV, reflecting the smaller incidence,
25 we found six donations that were not detected either
26 by p24 antigen testing or antibody testing. And

1 those were also interdicted that would not have been
2 otherwise.
3 This overhead shows data collected
4 during the clinical trial. For HCV donors it
5 demonstrates sero conversion periods. The chart on
6 the left here shows negativity. The green bars are
7 PCR and antibody testing; the gray are only antibody
8 testing.
9 And on the right side, the blue bars
10 depict PCR-only positivity and the red caps show
11 sero conversion. And in some of these instances
12 donors who were not enrolled were not followed to
13 sero conversion. In one instance we had an
14 individual who, when sero converting, HCV was not
15 picked up by -- or, HCV RNA was not picked up by PCR
16 in the pool.
17 The two donors best demonstrate the sero
18 conversion range. This donor here, number 3, shows
19 approximately 93 days of PCR-positive donations in
20 the absence of antibody, and also shows a negative
21 PCR result and antibody result of course,
22 immediately prior to the PCR detection.
23 This individual here is similar; however
24 that period of time is only 28 days before that
25 individual sero-converted.

1 These are the four HIV subjects; again,
2 similar. The blue bar again, is PCR positivity in
3 the absence of any other maker and the yellow
4 depicts PCR and p24 antigen positivity. And the red
5 cap again shows sero conversion. Here we had a
6 range of eight to 20 days.
7 And I want to emphasize, that period
8 represents the period from the last sero-negative
9 result. So they could have sero-converted anywhere
10 here, or this individual could have sero-converted
11 anywhere within this range. So we are counting from
12 the point of the last test that was antibody-
13 negative from the first PCR.
14 We observed an interesting phenomena
15 through the course of HCV testing. This slide
16 represents HCV antibody reactivity in percent
17 ranging -- and the red line depicts when PCR pool
18 testing was implemented.
19 As you can see, the green band shows the
20 actual reactive rate; the blue band is a rolling
21 average before and after PCR implementation. And
22 you can see a significant drop in the reactivity
23 rate of HCV antibody, suggesting perhaps the donor
24 population is being cleared by the PCR tests before
25 the sero conversion.

1 Now, as Dr. Conrad had mentioned, we
2 have done some studies of -- comparison head-to-head
3 studies with p24 antigen testing against HIV PCR in
4 a pool of 512 donations. When we tested the samples
5 with p24 we used both the culture and the added
6 license test, and if either of them produced a
7 positive result then they were counted as positive.
8 This contingency table shows that 61 of
9 these samples were found positive by both PCR and
10 the antigen tests. None of the samples that were
11 positive by p24 were negative by PCR. And 32 of
12 the samples that were negative by p24 were positive
13 by PCR.
14 So we show at least equivalency to the
15 p24 tests when testing PCR in a pool of 512 against
16 p24 in single donation.
17 So to sum up, we are concluding that PCR
18 pool testing decreases the viral load in the
19 manufacturing pool; that PCR pool testing also
20 provides an opportunity for the infected donor to
21 seek early treatment; and HIV PCR is at least as
22 effective as p24 antigen testing in screening out
23 window period source plasma donors.
24 Thank you.
25 CHAIRPERSON TABOR: The next speaker is
26 Dr. Cushing from Baxter.

1 DR. CUSHING: I'd like to thank everyone
2 for inviting us here today to talk about the data
3 from the clinical trial.
4 This study was conducted in two phases.
5 The first phase was the screening phase and the
6 study duration was approximately three months. We
7 began it on December of '97 and ended in March. The
8 plasma donor qualifications for this study to be PCR
9 tested were to be EIA-negative and also, unlike
10 Alpha's study, to be p24-negative.
11 We had 46 plasma centers participating
12 in this study, and the number of plasma donations
13 PCR tested were approximately 345,000 donations.
14 And the number of plasma donors tested was
15 approximately 42,800. And the number of master
16 pools tested was approximately 675, and we had a
17 mean donation per donor in this study of eight.
18 Phase II of the study was called the
19 sero conversion follow-up phase. And the duration
20 of this phase was six months as a maximum or sero
21 conversion. And out of the 46 centers we had ten
22 centers that had eligible donors.
23 And eligible donors for this phase were
24 found to be HCV or HIV PCR-positive and antibody
25 negative. In the case of HIV it would also be p24-
26 negative. Donors that were enrolled in this phase

1 were tested by PCR and antibody testing on a weekly
2 basis.
3 One of the things that we did in this
4 was to add blinded samples to our master pools. We
5 had 50 control samples that were both positive and
6 negative samples for HIV and HCV. And of these 50
7 samples, all 50 of them were correctly identified by
8 PCR.
9 We had zero HIV PCR donors who were
10 eligible for enrollment in our Phase II study. We
11 had 22 HCV PCR donors eligible for enrollment in the
12 Phase II study. And of these eligible donors, five
13 of them declined to enroll. And other four sero
14 converted prior to enrollment in the study.
15 We had six donors who did enroll in the
16 study and sero converted within six months. And we
17 had two eligible donors who enrolled in the study
18 and did not sero convert in the 6-month period but
19 they were found consistently to be PCR-positive.
20 And I'll speak more about those donors later.
21 Also, I told you that we had 22 donors
22 who were eligible to enroll. Five of these we found
23 to be false positives or eligible donors whose
24 initial positive HCV PCR test result could not be
25 confirmed upon repeat testing.

1 And we feel that the probably root cause
2 for this was contamination during pooling on the
3 TECAN -- and we think that three of the cases were
4 due to that. And we think the other two were due to
5 contamination at the donor center during sampling.
6 The corrective action that was taken was
7 to upgrade the TECAN pooling software -- and Dr.
8 Conrad talked about that. We've also made -- and
9 it's not conformation, it should be confirmation --
10 procedural changes. Initially we sent in the sample
11 for confirmation that had been on the TECAN and now
12 we sent in a backup sample, so we think that this
13 will control for the contamination due to pooling on
14 the TECAN.
15 And we've also implemented a new
16 sampling methodology. We were using the cut-and-
17 drip method; now all of the centers are using a
18 closed method with vacuum tubes.
19 From our study we did have ten HCV PCR
20 sero converting donors. And we found that the range
21 of time from the first positive HCV test -- PCR test
22 -- to sero conversion -- and again, this isn't
23 infection but this is the first PCR-positive test --
24 was anywhere between 30 and 115 days.
25 And we found that the range of time from
26 the first HCV PCR test to deferable ALT levels

1 defined as two times the high normal, or an ALT of
2 greater than 76, was anywhere between 30 and 101
3 days.
4 And HCV-positive donors who exhibited
5 deferable ALT levels prior to sero conversion was
6 three out of our ten. We had two out of the ten
7 donors who sero converted -- I don't know, actually
8 we had two of the ten donors who never did exhibit
9 elevated ALT levels but they did sero convert.
10 And this is a graph that demonstrates
11 the relationship between sero conversion and
12 elevated ALT levels. And you can see that there is
13 a rough correlation but it isn't -- you can't use it
14 exclusively to predict. And of course the two
15 individuals who never did have elevated ALT levels
16 are not even represented on this graph.
17 This is a graph that -- it's a little
18 difficult to tell but it looks at what the PCR or
19 the viral load was of five of our donors where we
20 knew when their first PCR-positive test was and time
21 to sero convert.
22 We did a quantitative PCR analysis on
23 these donors and what we found is that all of them
24 started with a fairly low level. Now this is a
25 logarithmic scale so even this fairly low level
26 could be 10,000 or more. And fairly rapidly the

1 levels increased, and within three days you can see
2 some of them going way up.
3 Now, some of these donors' levels, viral
4 load, stayed elevated the entire time to sero
5 conversion; whereas others -- this pink one was kind
6 of all over the place. And the donor represented by
7 green, his levels dropped way down. So it does seem
8 to be extremely variable.
9 Of our ten sero converting donors we
10 found a male/female ratio of ten males and zero
11 females. And our mean female -- or rather, our mean
12 male to female donor ratio was 68 to 32. And we
13 found no correlation with the number of prior
14 donations and HCV infection as defined by the PCR
15 analysis.
16 You can see out of our ten donors that
17 we only had one donor who was a first-time donor.
18 We had two donors who had donated between one and
19 five times; one donor who had donated between six
20 and ten times; one donated between 11 and 20 times;
21 one between 21 and 40 times; one donated between 41
22 and 60 times; and we had three donors who had
23 donated between 61 and 80 times.
24 Okay, now these are the findings that we
25 have for the HCV PCR-positive, non-sero converting
26 donors that I talked about before. Here the

1 male/female ratio was zero males and there were two
2 females.
3 The very first time they came to these
4 centers their PCR tests result were positive. So we
5 have no idea when they first became infected. The
6 duration of HCV PCR-positive tests was greater than
7 176 days -- which was the length of our trial.
8 Their viral loads by quantitative PCR
9 during this time ranged anywhere between 140,000 and
10 4.5 million copies per ml. The duration of HCV
11 branch chain DNA-positive tests correlated with the
12 PCR-positive test and was also positive for greater
13 than 176 days.
14 Now the duration of normal ALT levels
15 was greater than 176 days, so at no time did they
16 ever exhibit a deferable ALT level. And the
17 duration of negative antibody tests result using the
18 Abbott HCV EIA-2.0 test was negative the entire
19 time.
20 We then went back and performed the
21 ortho HCV EIA-3.0 test, and here we found that the
22 results of this antibody test was positive, and it
23 was positive from the very beginning of their PCR-
24 positive tests.
25 I mean, I don't think that we can say
26 anything really, from this about the validity of the

1 two tests because we didn't set out to check that,
2 and so we certainly have not looked at the reverse
3 to see if that's true. But certainly, given this
4 system I think what we can say is that there was
5 nothing that we routinely would have done that would
6 have picked up this infection in these two
7 individuals.
8 Also, in the course of performing the
9 HCV PCR test on our sero converting donors we found
10 that there was a transient negative PCR result
11 following an HCV PCR-positive result of three out of
12 78 PCR tests. And we think that possibly the cause
13 for this is the formation of an EIA undetected
14 antibody that mediates viral clearance.
15 We did do quantitative PCR analysis in
16 this case, and it did come out as undetectable. And
17 the quantitative PCR values preceding or immediately
18 following the negative ones were extremely low.
19 So in summary, what we've done is
20 demonstrate the ability of PCR testing of pooled
21 plasma samples to detect HCV-infected donors; we
22 validated the process of pooling donor samples,
23 testing the pooled samples by PCR and linking
24 donations positive by PCR testing back to the donor;
25 and we have collected data on the sero conversion of

1 donors who are positive by PCR testing and non-
2 reactive by HCV antibody testing.
3 And I'd just like to thank all of my
4 colleagues at Baxter who were involved in this, and
5 the colleagues at NGI and ICRC, our contract
6 research organization. Thank you.
7 CHAIRPERSON TABOR: Thank you. The next
8 speaker is Dr. Dragon from Roche.
9 DR. DRAGON: Good morning. I'm going to
10 present today and describe, the Roche Molecular
11 Systems products for nucleic acid testing for HCV
12 and plasma donations. I'm going to be speaking
13 about current reagents that are either about to be
14 on the market or are in some countries, perhaps
15 available already. I will not be talking about
16 future, single unit screening at this time.
17 To give you a brief history of our
18 collaboration with Bayer Corporation, we each filed
19 a joint IND in July of 1997 to begin the work
20 looking at pooled specimens. We then had Bayer
21 begin testing in September of 1997, and just this
22 month we have actually filed amendments for our IND
23 to upgrade the tests from the Version 1 reagents to
24 the Version 2 reagents.
25 We are calling our blood screening
26 products AmpliScreen as to contrast them to the

1 diagnostic products -- the Amplicor products -- and
2 I'll describe some of these differences.
3 We have two platforms that we are
4 supporting. You will see data from Bayer on the
5 microwell plate format where we are working with
6 pools of 96 with reflex testing and pools of 8 X 12
7 followed by single resolution.
8 In addition, we are beginning
9 collaborations where we will be working on our
10 automated COBAS Amplicor platform where we will be
11 working with pools of 24 with subsequent, secondary
12 resolution of pools of 4 X 6 and single resolution
13 after that.
14 The most significant improvements
15 between the Version 1 and the Version 2 tests are
16 twofold. One is the ability to equivalently amplify
17 all the known genotypes of HCV.
18 We have kept our primers constant;
19 however we have put co-solvents into the master mix.
20 There's been a slight master mix formulation change
21 which has resulted in this higher efficiency of
22 amplification for all the genotypes.
23 Secondarily, we have an improved
24 sensitivity due to procedural changes in our
25 specimen preparation procedure, including a larger
26 input volume.

1 Briefly, to just look at the summation
2 here, the most important things to see are that in
3 the first-generation kit, the microwell plate kit,
4 we did have an internal control but it only
5 controlled amplification and detection. It was not
6 included in the specimen preparation.
7 In the new methodology we now actually
8 have extracted internal controls, either with the
9 microwell plate or the COBAS Amplicor system. In
10 addition, we used to start with 100 microliters of
11 specimen. We now have gone to a one ml input sample
12 volume. We used to resuspend in one ml; we are now
13 resuspending in 200 microliters.
14 The most important difference as I said,
15 is a procedural difference. We're using what we now
16 call the multiprep specimen processing where we'll
17 take a one ml specimen -- in this case from the
18 pools -- and we do a one hour spin at 23,600 times
19 g. Following that spin we will draw off 900
20 microliters of the supernatant, leaving 100
21 microliters still in the tube.
22 We will then add the quanidinium lysis
23 buffer spiked with the QS or IC control; mix,
24 incubate so we have viral lysis going on at this
25 point in the presence of the internal control.
26 We'll then follow with a isopropanol precipitation,

1 pellet the virus, do a wash in 70 percent ethanol,
2 resuspend in the specimen diluent, and then proceed
3 with amplification and detection.
4 I'd like to share with you some of the
5 results that we have from in-house on the non-
6 clinical performance characteristics of this test.
7 It will compare both microwell plate and the COBAS
8 Amplicor work we've done.
9 The first test was working with the WHO
10 International Standard. And we prepared using the
11 standard which started out at about 50,000
12 international units per ml. We diluted this into a
13 negative HCV plasma and we brought it down to either
14 200, 150, 25, 15, 12, or 10 IUs per ml of HCV.
15 We then did 24 independent specimen
16 preparation amplification and detections to look at
17 sensitivity levels and reproducibility.
18 And what you will see is that with the
19 microwell plate tests as we've described, we had 100
20 percent hit rate at 25 IUs per ml. Interestingly
21 you'll see that we have only 23 of 23 indicated
22 here. In that particular reaction we had one
23 invalid result which was an IC-negative result. So
24 again, the internal control acts as a flag if things
25 are working or not in the mixture.

1 For the COBAS we've actually been able
2 to achieve again 100 percent at 25 IUs per ml and
3 greater than 95 percent sensitivity at 15 IUs per
4 ml.
5 A second international standard that we
6 have looked at is the NIBSC working reagent, and in
7 this case we're looking at -- we've been able to get
8 down to 100 percent to 70 genome equivalents per ml,
9 and at 60 we're still at greater than 95 percent.
10 With initial results that we have looked
11 we feel that one Roche copy is equivalent to
12 approximately 1.4 IUs per ml. We're looking at
13 about a 4-fold difference between what the NIBSC
14 standard is and what the IU is, which I think is
15 similar to what Dr. Saldanha has seen and reported
16 in the past.
17 Genotypes are very important. We've
18 looked at genotypes from two perspectives. We've
19 looked at them from actually real clinical specimens
20 that have been documented to be a variety of
21 genotypes and then do serial dilutions with them
22 looking for analytical sensitivity.
23 We too, also have cloned copies of the
24 different genotype sequences at the 5 prime
25 untranslated region and have done limit detection
26 for various input copies numbers of genomes. And

1 you can see that we are five copies per PCR where
2 it's still the 100 percent hit rate.
3 In conclusion, I've just presented to
4 you some of the non-clinical performance data that
5 we are starting to generate within Roche for the
6 Version 2.0 kit. My colleague from Bayer will
7 present some of the clinical studies that have gone
8 on.
9 The important things to note is that we
10 are looking at now with the Version 2.0 kit, a
11 sensitivity of less than 50 international units per
12 ml which is well below the 100 IUs that was
13 recommended by the Agency to be able to use for
14 plasma screening.
15 We have picked up the remaining of the
16 genotypes that we did miss in the first-generation
17 test. We also still have our own sterilization
18 capabilities working in there.
19 Finally, we have performed interfering
20 studies -- interfering substance studies with a
21 variety, and we have no known inhibitors. We have
22 also validated both EDTA and ACD as the collection
23 tubes of method.
24 Thank you.
25 CHAIRPERSON TABOR: Thank you. The next
26 speaker is Barbara Masecar from Bayer.

1 MS. MASECAR: Good morning and I
2 appreciate this opportunity to provide you with an
3 update of the current status of nucleic acid testing
4 for HCV in plasma donors being performed by Bayer
5 Corporation at our facilities in Raleigh and
6 Clayton, North Carolina.
7 Briefly a timeline. This replicates
8 very closely the slide that Betty showed. We
9 jointly filed an IND in July of 1997. Testing was
10 initiated at our PCR laboratory in Raleigh in
11 December of 19 -- I'm sorry, September of 1997.
12 We began testing with the version 1.1B
13 test kit using the Amplicor Version 1 reagent with a
14 modified specimen preparation to give us added
15 sensitivity. And that has been in-place in use
16 since September 1997 to the present.
17 And the initial validation work done to
18 support the IND filing in July was based primarily
19 on the NIBSC working run control.
20 As far as the test system and work flow
21 that we use at our laboratory -- this has been
22 presented at other meetings -- but our samples are
23 received, batched and decapped. Our batches are in
24 multiples of 96 and an 8 X 12 array. We use the
25 Hamilton AT plus to create the mini-pools of 96.

1 If that is positive we go to the reflex
2 testing of the rows and columns, and then the
3 individual positive is identified by the intersect.
4 And this is a very basic graphic representation of
5 the rack if we test the column samples and the row
6 samples.
7 In this example column 7 is positive,
8 row F is positive. The only sample shared in those
9 two mini-pools is number 67 so we've identified our
10 positive.
11 We are anticipating very soon the
12 transition to the Roche Version 2.0 test. We have
13 filed our amendment this week, both Roche and Bayer,
14 and we will transition to Version 2.0 next month.
15 There will be essentially no change in the test
16 system or the work flow. We still maintain our
17 mini-pools of 96.
18 The version 2.0 does incorporate an
19 ultracentrification step. This is the multiprep
20 method that Dr. Dragon referred to. We don't have
21 this in here really to affect the hepatitis C
22 sensitivity necessarily, but it really is in
23 anticipation of adding HIV testing next year.
24 And now that we have the WHO
25 International Standard available the validation work

1 for Version 2 was based on that standard. And
2 that's what I'd like to present.
3 The validation of Version 2.0 that was
4 filed in our amendment was performed according to
5 ICH guidelines. We concentrated in two major areas:
6 the limit of detection by limiting dilution using
7 the WHO standards, and then after that was performed
8 standardizing our own in-house control in
9 international units -- which is a positive plasma
10 unit that we have, high titered.
11 And then after we determined what the
12 limit of detection was for the assay and we had our
13 in-house controls calibrated, we used a less dilute,
14 in-house control to input into the total system to
15 include the creation of the mini-pools to verify
16 that operationally our assay would meet the
17 validation requirements.
18 The limit of detection was performed
19 simply by the creating of dilution panel to bracket
20 the detection limit of the assay. And we performed
21 this with three operators with not less than 16
22 tests per dilution.
23 And the graph on the next slide shows
24 the data represented. At 50 international units we
25 had 96 percent positive -- which is represented by
26 this datapoint. At 25 international units we had 90

1 percent positive, and so we just did a simple linear
2 interpolation. So we determined that at 95 percent
3 test positivity, Version 2 using the multiprep, our
4 limited detection is 46 international units per ml.
5 Then we did the same set of experiments
6 with the in-house controls. This in-house material
7 had been previously quantitated several times with
8 the Chiron bDNA assay. And we diluted the in-house
9 control to get the 95 percent test positivity rate
10 and then assigned that the 46 international units
11 per ml level.
12 The operational validation for our total
13 test system, we took our in-house control and from
14 the stock of that we diluted that to approximately
15 4400 to 4600 international units per ml. And that
16 would represent the detection limit times the 96
17 dilution factor that we see in creation of our mini-
18 pools.
19 The total test system was employed --
20 real-life situation, real techs, real pipetters,
21 real lab -- and we found that in 144 tests we were
22 positive 137 times, which gave us a 95.1 percent
23 positive, which agrees quite well with our initial
24 validation.
25 A limited number of these that were
26 positive were carried through to the row and column

1 test and they were correctly identified in a blinded
2 fashion in every case and they were all positive.
3 Briefly, as Dr. Dragon mentioned, Roche
4 determined the limited detection for Version 2.0 in
5 a similar manner. They were positive 100 percent of
6 the time at 25 international units, and their 95
7 percent test positivity rate was between 20 and 25
8 international units.
9 Regarding the correlation of unitage, in
10 our Bayer validation studies using our in-house
11 control that itself was directly quantitated with
12 Chiron bDNA, we found that one international unit
13 was equivalent to approximately two genome
14 equivalents.
15 And as mentioned by Dr. Dragon, in Roche
16 validation studies, one international unit was
17 equivalent to approximately 4.5 genome equivalence.
18 However, the Roche Version 2 Amplicor monitor assay
19 was used as an intermediate step here, so that could
20 affect the difference. And also we're talking a
21 single lot of Version 2 that was used.
22 Regarding the overall performance of the
23 Version 2.0 assay, to-date there has been excellent
24 agreement between independent validation studies
25 performed at the Roche and Bayer laboratories.

1 Our in-house control has been
2 calibrated. We will run a 100 IU control with each
3 test. And the performance of this control taken
4 with the validated detection limit of Version 2
5 meets and exceeds CPMP recommendation for test
6 sensitivity, and also what FDA has stated.
7 Brief clinical study update: we are
8 ongoing with our clinical study; it has been ongoing
9 since testing began last September. It supported
10 the amendment. All initially positive clinical
11 study samples that were discovered with Version 1.1B
12 were polled from ultra-low, freezer storage, diluted
13 1:96 and retested, and all were found positive with
14 version 2.0.
15 We anticipate continuation of our
16 clinical study for one year or less from the time of
17 transition. We have waited to do the repository
18 samples until we are fully transitioned to Version
19 2.0, so that will be a major goal.
20 And we anticipate that data accumulated
21 to-date with the Version 1.1B will be submitted in
22 support of Version 2.0 kit licensure.
23 Future directions: we expect to
24 implement HIV PCR testing in the first quarter of
25 1999. We already have the specimen prep in place so
26 this will be a shared sample prep with HCV. And

1 then implementation of a third target as yet not yet
2 named, in the fourth quarter of 1999.
3 In conclusion I'd like to acknowledge
4 some people that provided data for this talk: Rita
5 Sun and her group at Roche Molecular Systems who
6 performed the validation work; Todd Gierman and
7 Michael Gray at Bayer Corporation for the
8 validations that were performed at Bayer. Thank
9 you.
10 CHAIRPERSON TABOR: Thank you. The next
11 speaker will be Dr. Watson from Centeon.
12 DR. WATSON: Good morning. Thank you
13 for the invitation to present our results, however
14 preliminary they are. When contacted about speaking
15 today I was asked to concentrate on our results and
16 not our methodology. If anybody wants to know about
17 the methodology that was presented by Dr. Weimer
18 that Blood Safety Meeting in February. I will go
19 into a little bit of it just so you can see the
20 differences in the systems.
21 So this is our initial report. We
22 concentrated on the results that we have and the
23 very preliminary clinical results from our sub-
24 study.
25 Our IND is basically the same as just
26 about everybody else. We want to see if pool

1 testing can identify potentially more infectious
2 units and remove them from production.
3 Our IND started -- not started -- we
4 received authorization to proceed in February this
5 year. We started collecting samples under the IND
6 in April and we started our testing in May. So what
7 I'm going to present in May is a report on the first
8 90 days' results.
9 We have a deferral policy. Any donor
10 who is PCR-positive will be deferred, both in the
11 corporate registry and the national registry. And
12 we have a look-back/look-forward policy which I'll
13 explain. I'll go into some of these numbers that we
14 actually have units that we've interdicted under
15 that policy.
16 Originally we started with a 3-month
17 look-back. We've had to expand that to six months.
18 And we also look forward because it takes time to do
19 the PCR test. So from the time the PCR test, that
20 unit result is available, all the other units that
21 were given subsequent to that are also removed.
22 Our system tests for three viruses. All
23 samples have to be -- all units have to be serology-
24 negative. They have to be p24 negative. They have
25 to be ALT negative. If a unit is ALT-positive we

1 will not test that unit but we will test all the
2 other units from that donor that were ALT-negative.
3 The virus load in this is not test
4 sensitivity. This is the concentration in genome
5 equivalence in a unit that needs to be present for
6 us to find a positive PCR result 95 percent of the
7 time. For HBV we used EuroHep; for HCV we used
8 Pelispy; and for HIV we used the preliminary working
9 standard.
10 Our pool size is 1,200 to begin with;
11 our maxi-pool. We then have an intermediate pool of
12 120, then we work back to 12, and then we go to the
13 individual donor. Twelve works well for us because
14 that's our logistics system.
15 Okay, here's our results: 600,000
16 samples; five HBV-positive donors; 36 HCV; and one
17 HIV. The HIV was interesting. It was his 13th
18 donation since November, but unfortunately we
19 haven't been able to find him.
20 By the way, these numbers include both
21 donor applicant and qualified donor because we test
22 everything. Now, we have not had an opportunity --
23 what you're getting here is raw data. We have not
24 been able to sit down and analyze anything yet. Our
25 IND is for one year. There's no 300,000 cutoff or
26 particular timeframe less than a year; we decided to

1 go with one year so we're going to have a lot more
2 data to analyze.
3 Average number of units -- actually, in
4 reviewing this this morning I realized that this
5 slide is wrong. That's the average number of units
6 identified during the period of time for look-
7 forward and look-back.
8 If we look at a 60-day timeframe and we
9 say that that 60-day timeframe where we would have
10 the units within the company, that would be -- at a
11 minimum, that would be 40 percent. So you're
12 looking at 260 units in a 3-month timeframe that
13 we've stopped from going to production from donors
14 that were PCR-positive. Our donors do come back
15 multiple times.
16 We have clinical testing -- we call it a
17 sub-study -- eligibility, PCR-positive. What we're
18 going to do is, we're going to have bring them back
19 once a month, we're going to do a PCR test, we're
20 going to do all the relevant serology testing, and
21 we're going to continue that for a maximum of six
22 months, or until sero conversion -- or, if they're
23 rather interesting, maybe we'll extend it on them.
24 We're just in the beginning of that,
25 since we just started our testing. By the time you

1 notify the donor and try and locate him there is
2 some time that goes along.
3 And the last slide shows what we've done
4 as far as enrollment and what we've been able to
5 test. So far we've only enrolled one HBV donor, and
6 hot off the result press yesterday morning came
7 another PCR-positive. We do not have the serology
8 result yet. My guess is, we'll get that later this
9 week.
10 Three HCV donors have enrolled. We've
11 tested two of them. For their initial sample both
12 were PCR-positive. They both continue to be
13 antibody-negative. We have a third one that
14 recently enrolled. We just received the sample
15 earlier this week, and we're now beginning to get
16 the second samples on the other two donors.
17 And the HIV, we're looking but we can't
18 find him. And that's the results that we have.
19 Thank you very much.
20 CHAIRPERSON TABOR: Thank you. Our next
21 speaker is Dr. Giachetti from Gen-Probe.
22 DR. GIACHETTI: Okay, first I'd like to
23 thank the organizers for inviting me to give this
24 presentation. I will talk today about Gen-Probe's
25 high throughput assay for the single strand of
26 detection of HIV and HCV RNA. My presentation will

1 focus mostly on the methodology we'll use as well as
2 results with HCV (unintelligible) as well as
3 analytical sensitivity.
4 These objectives are to have an
5 analytical sensitivity for both targets, HIV and
6 HCV, of 100 copies per ml. Here to demonstrate
7 detection of infection before sero conversion,
8 detection of all non-subtypes of HCV and HIV, able
9 to incorporate an internal control and in the future
10 other targets, analytical specificity higher than
11 99.5 percent, show non-cross reactivity with other
12 blood borne pathogens, and have throughput and
13 amenable to automation.
14 Assay protocol is simplified here. Our
15 technology uses an integrated approach where sample
16 processing, amplification, and detection are all
17 performed in the same tube. This simplified test
18 steps reduce the chance for contamination, and one
19 operator is able to process 200 samples in six
20 hours.
21 First step of the assay sample
22 preparation, we use target capture and magnetic
23 tactical separation. First step is to lyse the
24 virus. We use 500 microliters of a specimen.
25 During the lysis the plasma or serum is mixed with

1 the lysis buffer which contains captured probes as
2 well as micro particles and internal control.
3 During this step the viral RNA
4 hybridized to a captured probe that is a chimeric
5 sequence that will be complimentary to the viral RNA
6 as well as contained in polidity tail, and these
7 polidity tails will attached to a polidity tail that
8 is attached to the macro particle.
9 We use magnetic racks to be able then to
10 wash away the plasma and all potential inhibitory
11 substances. These render the samples ready to
12 amplify in the same tube.
13 Next step is the amplification. We use
14 transcription mediated amplifications. This is an
15 isothermal reaction, utilizes two enzymes -- reverse
16 transcriptase as well as T7 polymerase -- can be
17 used for RNA or DNA targets, and produces an RNA
18 amplicon. The reaction is exponential for using
19 more than one billion-fold amplification in less
20 than one hour.
21 For detection you use the hydrization
22 protection assay. This assay utilizes acridinium
23 ester probes. The reaction consists in three steps.
24 First step is a hydrolization in solution where the
25 level probe hydrolyzed to the viral RNA.

1 Second step is a selection where the
2 level on the unhydrolyzed probe is hydrolyzed as
3 shown here, where the label on the probe that has
4 been hydrolyzed to the amplicon is protected. The
5 third step is detection where the level on the
6 protected hydrolyzed probe is detected by
7 chemiluminescence.

8 In order for us to be able to
9 incorporate an internal control in each sample and
10 to be able to detect an internal control without the
11 need to separate a reaction into different vessels,
12 we use the two kinetic analysis.

13 This is an application of the
14 hydrolyzation protection assay that utilizes
15 acridinium ester level probes with the
16 (unintelligible) kinetics of light off. We have
17 different type of probes: probes with very high
18 kinetics of light off that we call pleasure probes,
19 that hydrolyze to our internal control; and probe
20 with the slow kinetics of light off, which we call
21 lower probes, that hydrolyze to our targets.

22 So each reaction would have two results:
23 one is for the internal control that validates a
24 reaction in each specific tube; and the second
25 result for each sample is the presence or absence of
26 a target.

1 Next I will show data about analytical
2 sensitivity and clinical sensitivity for HCV
3 detection. We determined our analytical sensitivity
4 preparing dilutional panels which were calibrated
5 using the international standard provided by Dr.
6 Saldanha using a conversion factor of 2.3 to
7 transfer genomic equivalence per ml.
8 With the different operators, different
9 days, several lots of reagents, it all totaled about
10 120 replicas of each level. And our sensitivity is
11 100 percent at 100 genomic equivalence per ml, 92
12 percent at 30 genomic equivalence per ml, and we go
13 down to 12 percent at one genomic equivalence per
14 ml.
15 We also participated in several studies.
16 This is a study organized by John Saldanha this
17 year. When we compared our detection limit using
18 different reference standards, here if we use the
19 WHO reference standard we have detection of 100
20 percent at 10 international units per ml, and 93
21 percent on three international units per ml.
22 Part of this study compared also the
23 CBER FDA standard, the Paul Ehrlich standard, and
24 the Pelispy standard. The CBER standard as well as
25 the Paul Ehrlich Institute, the standard runs very
26 close. We have 100 percent detection at 100 genomic

1 equivalence per ml, 70 percent detection at ten, 40
2 percent at three, and we go 12 percent at one.
3 These correspond very well with the
4 previous data that I showed with our in-house
5 panels. Pelispy also would have good sensitivity
6 showing 100 percent at 360 genomic equivalence per
7 ml, 95 percent at 76 genomic equivalence per ml.
8 To explore the application of these
9 assays not only to single dot units but also to
10 pools -- and this is part of the collaboration
11 (unintelligible) -- we tested several sero
12 conversion panels that were obtained from BBI, Nabi,
13 as well as Bioclinical Partners.
14 And we tested the different panel
15 members at undiluted samples or in a pool diluted
16 fashion. What we found here is that the days of
17 detection before sero conversion is very similar in
18 all the cases.
19 I should mention, and here shown by
20 (unintelligible), that unfortunately most of these
21 panels we were RNA-positive in the first bleed of
22 the panels. So if there were differences between
23 the undiluted/full diluted samples we are not able
24 to detect it.
25 Here is more panels, the
26 (unintelligible) panels. And this is the only panel

1 out of 20 or 21 that we tested that show a
2 difference between the undiluted sample and the full
3 diluted sample. In this case was 35 days before
4 sero conversion and here was 28 days.
5 Here are data from the Nabi panels. We
6 have a broad difference in detection limits going
7 from 60 days down to zero.
8 Conclusions. I will not show
9 specificity data which show the specificity of the
10 reaction. We haven't found any cross reactivity
11 with other infectious agents or autoimmune
12 conditions of heart or liver diseases. We haven't
13 found any interfering substances so far. It works
14 very well with different kinds of anticoagulants or
15 serum or a percent of problematic samples.
16 The sample processing method removes
17 potential interfering substances. It's very
18 efficient. And we found internal control very
19 useful for confirmation of amplification performance
20 in each sample.
21 Sensitivity conclusions are here. We
22 have 100 percent detection at 100 genomic
23 equivalence per ml, and 50 percent detection at four
24 genomic equivalence per ml. We were able to
25 demonstrate HCV RNA detection on average, 39 days
26 before sero conversion. I haven't shown, but we

1 have demonstrated also very sensitive detection of
2 all genotypes from 1 to 5. We haven't tested 6;
3 that's why it's not there.
4 Finally, I'd like to acknowledge that
5 this project has been funded in part with Federal
6 funds from the National Institute of Heart, Lung,
7 and Blood, and also in the future, Karem Corporation
8 will share in our efforts. Thank you.
9 CHAIRPERSON TABOR: Thank you. The next
10 speaker will be Dr. Stramer from the American Red
11 Cross.
12 DR. STRAMER: Thank you very much.
13 Initially I was told that I could have Dave Gretch's
14 15 minutes so I had intended to use it, but I'll
15 just speak faster now that everyone else has seemed
16 to have used it.
17 I was asked to present data on
18 information we've collected at the American Red
19 Cross thus far using pooled GAT testing, and also
20 talk about our future implementation plans. I will
21 first go through the data that we have collected
22 initially in collaborative studies with Andy Conrad
23 and Rich Smith at National Genetics.
24 These are some of the parameters that
25 one must consider when looking at pooled GAT
26 testing, and I will go highlight some of these

1 studies that we have done, again, in collaboration
2 with NGI.
3 We will be collecting samples -- these
4 are whole blood samples now, in contrast to the
5 previous speakers. We talked about plasma only for
6 further manufacture. In the whole blood sector we
7 obviously have to deal with the issues of red cells
8 and platelets as well as plasma.
9 We will be collecting samples in a
10 closed tube container to minimize transfers --
11 minimize contamination from transfers. This is a
12 plastic tube that may be frozen. It is a gel
13 separator. It's called a plasma preparation tube,
14 manufactured by Becton Dickinson.
15 It is spray-coated with EDTA and as
16 you've heard references by speakers in the past,
17 both EDTA and ACD are probably acceptable
18 anticoagulants. Serum is not an acceptable sample
19 for GAT testing; at least in our hand.
20 Looking at stability in two phases --
21 because again, we collect whole blood -- we've had
22 to do a whole blood component to our stability
23 studies and a plasma component to our stability
24 studies. Since most of the samples are antibody-
25 positive, one must distinguish between stability in

1 antibody-negative and antibody-positives because
2 frequently you will see differences.
3 These studies were carried out at two
4 different temperatures to understand what
5 temperature we have to do shipping at following
6 collection. This is a whole blood, pre-sero
7 conversion series, and you can see over the entire
8 course of 72 hours we didn't really see any major
9 decreases in data.
10 The blue color here represents the lower
11 temperatures: 6 to 10 degrees which is the upper
12 end of our shipping validation versus elevated
13 temperatures, ambient temperatures, 25 to 30
14 degrees. We will ship at 6 to 10 degrees because it
15 does afford us higher recovery and better stability
16 as has been reported in the literature for both HIV
17 and HCV RNA.
18 These studies again, are HCV. We have
19 not yet completed our HIV studies.
20 Looking at the same series post-sero
21 conversion -- or in the case of antibody-positives -
22 - you basically see the same phenomenon. At the
23 lower temperature, 6 to 10 degrees, you see higher
24 recovery but you really don't see a difference in
25 rates of decline.

1 You do have lower recovery in perhaps a
2 more significant -- well, they're both significantly
3 different in this case, but less recovery at least,
4 in the higher temperatures. So again, we will be
5 going with 6 to 10 degrees or shipments on ice.
6 Following separation in the PPT, which
7 the gel separator separates the cellular components
8 from the plasma above the plug following
9 centrifugation, these are the results of our
10 combined plasma stability studies looking at
11 combinations of whole blood at 6 to 10 degrees,
12 whole blood at room temperature, plasma at low
13 temperatures, ambient temperatures, low temperatures
14 and high temperatures.
15 But again, as has been reproduced in all
16 of our studies, we see the highest recovery, best
17 stability long-term when we use the combinations of
18 the low temperatures for both whole blood and
19 plasma.
20 And from the results of our study we
21 will be recommending a 3-day whole blood ice
22 shipment followed by a 7-day plasma time on ice with
23 no greater than 24 hours at room temperature for the
24 entire process of collection, shipment, pooling and
25 testing -- including resolution testing.

1 Converting now to sensitivity data,
2 again, these are data that we collected with NGI
3 looking at window period reductions, if you look at
4 antibody positives collected from this serial plasma
5 donor. So this again, is the antibody-positive
6 curve and this is the RNA-positive curve.

7 If you look at the time difference
8 between the first antibody-positive -- here's the
9 antibody cutoff, here's the first antibody-positive.
10 So the first PCR-positive here, you can see that
11 there's a window period closure of 26 days.

12 In our studies with NGI we use pools of
13 500, so using a cutoff of their pooled PCR tests
14 here you can see even with a pooled PCR test we do
15 get significant window period reduction even using
16 pools as large as 50 donations.

17 Here's another series in which you can
18 see the same effect. Here there's a 40-day window
19 period reduction between the first antibody-positive
20 and the first GAT-positive. And you can see here
21 high viral load. So the important features to take
22 home from HCV RNA sensitivity studies is long window
23 period and high viral load.

24 If you look at a combination of all the
25 plasma series we looked at in our studies with NGI,
26 which were 19, this shows you the different phases

1 during sero conversion. Time here indicated on the
2 X-axis, the cutoff for the PCR and the viral load
3 during different phases of sero conversion.
4 So here's the first phase, pre-antibody
5 positivity. So this is the RNA-only samples. And
6 the mean copy number here was greater than five
7 million copies per ml. So really pool size is
8 really not a factor of important consideration when
9 you're doing HCV because again, viral titers are so
10 high.
11 Looking at some specificity studies we
12 did using crossover matrices, we did a study of
13 20,000 unlinked donations in which we removed the
14 sero reactive samples. From our studies looking at
15 these agents -- HIV was not included -- what I want
16 to highlight here are really three findings.
17 We did have, for HBV DNA and HCV RNA,
18 two unresolved pools of this study. And in
19 combination, if you look at these unresolved pool
20 results it would have held up release of 8.1 percent
21 of blood products. So that was an unacceptable
22 outcome of the study.
23 But interestingly enough we did find one
24 HCV-positive which was a unique genotype to the
25 spiked samples that we included in the study. The

1 study did include spiked samples of genotype 1A and
2 our finding was a genotype 3A.
3 It was sero negative by all tests; both
4 the EIAs and the HCV RIBA-3, our strip immuno assay.
5 I mentioned it was genotype 1A -- where's our
6 spiking sample -- was 1A. And because this study
7 was done in serum we believed we had a low viral
8 recovery because the samples were handled in serum
9 with long, ambient temperature storage times.
10 I mentioned that all the spikes were
11 correctly identified. I just wanted to acknowledge
12 that even though we used a very complicated pooling
13 system which I'll briefly discuss, NGI did an
14 excellent job of resolving, identifying to the
15 correct location, all positive spikes.
16 Interestingly enough, relative to our
17 HBV DNA viral input, we've recovered all DNA. As we
18 know DNA is a lot more stable than RNA, but in the
19 cases of HCV RNA we only could recover 23 percent of
20 our viral inputs; again, because we did the study in
21 serum.
22 We used a 2-dimensional matrix which has
23 been discussed at least twice -- one for each
24 dimension. And as we talked about, by pooling in an
25 X-direction and a Y-direction testing the results in
26 pools, if you have a positive pool in this dimension

1 and this dimension, the only unique donation
2 represented by those two reactive pools would be in
3 the inner section.
4 We used this approach in a 2-tiered
5 fashion such that four reactive pools were required
6 to identify two reactive sub-pools to identify one
7 reactive donation. Be that as it may, the two
8 yellow boxes here represent our spiked sample.
9 Interestingly enough -- I just said you
10 would need four pools to identify donation. Here we
11 had these two columns as positive -- that's A5 and
12 A6; we had two rows reactive, B1 and B6, which
13 pointed to the two sub-pools which implicated the
14 one spiked sample.
15 However, we also had an additional
16 reactive pool at B8. The inner section did not
17 contain a reactive donation so by the definitions
18 that I and others have told you, you would assume
19 that this would have been an aberrant result. But
20 because of the way we designed the study we were
21 required then, to test all of the sub-pools.
22 We found another sub-pool reactive that
23 corresponded to this one-fifth reactive row I showed
24 you. But his column was falsely negative. I also
25 mentioned to you that you need two primary pools to
26 implicate a single donation. So we identified

1 finally -- this was the sister positive to this
2 sample to identify the unique donation that was at
3 genotype 3A.
4 So here, this positive was masked
5 because it was in the same column with another
6 positive, and here we had another false negative
7 result. The reason that these two false negative
8 results could have occurred is because again, the
9 sample study was done in serum and perhaps we did
10 have RNA degradation.
11 Moving forward, what we will be doing
12 is, we not only would like to do the pooling but we
13 will be doing the testing. So we really need to
14 implement a kit that we can test on-site. Again,
15 because we're dealing with plasma red cells and
16 platelets, turnaround time for us is extremely
17 critical.
18 So what we are now endeavoring in is a
19 joint IND with Gen-Probe as the technology was just
20 described by Christina Giachetti. We will be doing
21 pipetting and testing, we will control CGMP which
22 gives us the flexibility of using backup
23 laboratories. We will be evolving to decrease pool
24 sizes.
25 I mentioned that we started at pool
26 sizes of about 500. Now we will be using pool sizes

1 of 128 and in order to decrease turnaround time and
2 be able to release red cells and platelets at the
3 same time we release plasma, we probably will have
4 to evolve to smaller pool sizes.
5 We are using a multiplex test, the Gen-
6 Probe test. It's semi-automated; the features have
7 been described to you. One advantage of doing this
8 type of testing -- if we can have the turnaround
9 time impact the release of all products -- that is,
10 cellular and plasma products -- you've seen the data
11 that perhaps testing that we do today such as p24
12 antigen and ALT, could be removed since they would
13 not have efficacy in those cases.
14 So in our joint IND with Gen-Probe, what
15 our goal is as others have described, is to evaluate
16 the efficacy, feasibility, and performance
17 characteristic of the multiplex, transcription,
18 mediated amplification assay, and to meet the
19 European requirements for GAT-tested plasma for
20 further manufacture by the July 1, 1999 CPMP
21 deadline.
22 But what's critical to us in the whole
23 blood industry is that we must initiate testing in a
24 way that does not compromise the availability of
25 blood, but generates information and support of the

1 eventual GAT-based control of labile products as
2 well as plasma-derived products.
3 I mentioned that we're doing a 2-part
4 IND. This requires IRB approval; our clinical data
5 to be collected and provided to FDA validating our
6 specific intended use. We will evaluate and plan to
7 meet all the international standards that are
8 required; that is, the CPMP 100 international unit
9 per ml standard.
10 We plan to also meet the PEI standard
11 and the FDA standard that has been tentatively set
12 at 100 copies per ml.
13 When we're dealing with whole blood
14 there are factors here that are much more complex
15 than dealing in the plasma arena, and it's
16 complicated from the point of donor all the way
17 through the patient. And if you don't think each of
18 these areas are affected one should think again
19 because they certainly are. And I will highlight
20 some of those.
21 Due to the complexity of our system
22 we're managing our entire project very centrally
23 with a centralized team that makes all management
24 decisions. We will operate off a single set of
25 procedures; one laboratory. We will have a

1 centralized information flow because we're using
2 code 128 whole blood numbers.
3 We will plan to implement at all
4 locations, all allogenic donors. We will use as a
5 specific sample type the plasma preparation tube.
6 This will go to the specific lab for a Gen-Probe
7 test. Other tubes will go for serology. We will be
8 removing the HIV1 and HCV serologic samples so that
9 they don't contaminate pools.
10 I will end with this slide, our first
11 phase will be under IND as an evaluative phase. We
12 will use conservative policies to assess logistics;
13 that is, false positive rates, turnaround time. It
14 will include one million donations.
15 We will then hopefully move to a second
16 phase in which we can make some better management
17 decisions involving red cells, and instead of
18 reacting to a single donation as we plan to do now
19 after the resolution of pools, we then would be able
20 to respond to a single, reactive pool. And again,
21 then we would proceed to including the entire
22 system.
23 Thank you.
24 CHAIRPERSON TABOR: Thank you. The next
25 speaker is Dr. Zerlauth from Immuno.

1 DR. ZERLAUTH: Thank you for the
2 opportunity to share with you a few of our results
3 that we have obtained by using PCR in a routine
4 screening scenario. I do not have any IND-related
5 data yet because we have not yet filed an IND but we
6 are in the process of compiling one and should be
7 submitted very soon; probably this month.
8 I would like to go briefly through our
9 IQ-PCR system, the PCR assay system that we have in
10 place. We have the classical extraction on
11 guanidinium isothiocyanate and phenol
12 chloroform/ethanol precipitation.
13 We have a single round PCR; it's not
14 nested. It's single round. We are using RTTH. We
15 are using hot start and we are using fluorescent-
16 labeled primers, and we are using an internal
17 control which I am going to show you in a second.
18 We have size-specific separation of our
19 amplicons and polyacludamide gels. And we are
20 detecting those size-separated amplicons based on
21 their fluorescence, by a laser-induced fluorescent
22 equipment.
23 Our internal standards which we have
24 called internal quality markers, are almost
25 identical to the target we are interested in. We
26 just have modified the lengths of the amplicons to

1 be created. We have added a few bases -- a few mean
2 5 to 11 bases -- that we have added to the original
3 sequence.
4 We then have transcribed it into RNA for
5 HIV and HCV, and we used plasmids for the HBV. So
6 we have not altered the primer binding sites, only
7 the lengths of the target sequence. We now add
8 those constructs into each vial so the internal
9 control goes along with each vial to be treated.
10 We have co-processing with the virus:
11 co-construction, co-reverse transcription required,
12 co-amplification, and co-detection. Which leads to
13 the must that one band can be seen as a result if
14 the whole process worked correctly. In case of a
15 positive sample with virus present, we have to see
16 two bands.
17 So in case we don't see any bands we
18 know that process had some failure and that means go
19 back to square one.
20 In reality that looks like this. In
21 lane A corresponding to this scan graft here, you
22 see one peak only. This is our amplified material,
23 fluorescent for that one fluorescence primer that we
24 have added. We have five -- only three here shown -
25 - but we have five bands of lane markers of known

1 size, so we can exactly calculate the lengths of the
2 amplicon which is given us by the computer.
3 And this is 153.59 and the expected
4 length is 154 for HIV in our case. This would be a
5 positive PCR but a negative result.
6 On the contrary in lane C, we do have
7 two peaks. This is the positive, the wild type peak
8 for HIV, and we can easily distinguish, as you can
9 see here, the lengths, the different lengths of
10 these two amplicons.
11 In the middle, corresponding to lane B,
12 we have no amplified material indicating that
13 something either went wrong or as it is in this
14 case, we have control -- completely negative control
15 which goes along of course, with every test.
16 Using this method we, as many other
17 speakers this morning have shown, went along to show
18 our sensitivity. This is a scoring graph. The
19 percentage of samples being found correctly positive
20 in a serial dilution of an HCV preparation.
21 These are nominal values, genomic
22 equivalence per ml, and you can see that the 95
23 percent cutoff level is in the range of 150 genomic
24 equivalence per ml. In our hands the calculations
25 to the WHO standard is 1:4.

1 So this is about 40, 45 international
2 units per milliliter sensitivity, case sensitivity;
3 which corresponds fairly nicely to the Centeon data,
4 leading to something 10^4 viruses per milliliter
5 needed in the original sample to be detected in our
6 system.
7 Just to show you that the nominal virus
8 load here is not an invention of ourselves, we have
9 calibrated our scoring graph with the NIBSC working
10 standardizing from John Saldanha.
11 We have made dilution corresponding to
12 1,110 genomic equivalence per milliliter, and
13 repeating that 26 times under different conditions
14 over the length of about five months we succeeded in
15 showing that we have to expect scoring with that
16 material that has been quantitated by a number of
17 colleagues around the world.
18 Now I would like to show you some of our
19 data that we have obtained by using this PCR in the
20 frame of quality control. We had in mind to use
21 this PCR a little bit different from what the
22 outline now shows that has been given by the FDA.
23 And here I would like to show you first
24 of all the data we have obtained in the last, about
25 three years, testing more than six million

1 donations. And this is just a summary of one recent
2 observation period.
3 But first of all I would like to go a
4 little bit more into detail just to give you an idea
5 of how this procedure is done. Looking at qualified
6 donors that undergo all the conventional testing we
7 bring in the donor that qualifies in this particular
8 donation, into an inventory hold of three months.
9 Each sample is sitting there for three months.
10 After three months, prior to being used,
11 we are making a pilot sample pool which is an
12 additional vial that goes along with each donation.
13 We do not cut pigtails or cut off the corner of a
14 bag, but rather have samples. It's a closed system.
15 We have an additional sample to each bag.
16 We are doing PCR and if the PCR of this
17 pilot sample pool is negative these samples in this
18 pool are released for pooling. A real pool is
19 formed; pool out of all these donations. And we are
20 doing another PCR, which is the test of record
21 required by the European authorities.
22 If this is negative we release that for
23 production; if not we would destroy that pool. If,
24 in the pre-screening period, the sample pilot pool
25 turns out to be positive in that period I'm showing

1 to you, we had no possibility to go down to the
2 single donor.
3 This was done by intention because we
4 are quality control, not a medical diagnosis
5 laboratory. So we made all -- well, we didn't
6 create the possibility to go down to the single
7 donor. That was kind of blinded for us, just not to
8 come in touch with clinical laboratories.
9 So we cannot identify the donor by
10 itself, but we have several steps as we made the
11 pools to verify the different samples show
12 positivity. And we have not identified the
13 individual donor, and of course not at sero
14 conversion, but we know that the testing was all
15 right.
16 In a period of about one year covering
17 most of 1997 we have tested roughly 1.8 million
18 donations and we have 36 -- I'm saying here,
19 donations because I don't have the donor on-hand, so
20 to speak. But it was 36 cases that turned out to be
21 PCR-positive.
22 And please note this is even after a 3-
23 month inventory hold; a period which was used to
24 clean out all post-donation reports, look-back
25 positives, and such.

1 We have also found two donations
2 positive for HBV and we have found none to be
3 positive for HIV. And contrary to production pools
4 made from the real donations we found all negatives
5 so far. We never, ever had any positive production
6 pool that we have submitted to the authorities.
7 Now let me turn to the new donor
8 screening that goes concurrent with classical market
9 testing. We have set up this system and it's about
10 to be installed -- not yet running but very soon --
11 in order to comply with both worlds: with the
12 European requirements that we have covered in the
13 first phase quite well, and also to obey the rules
14 given out by the FDA, just to be more closely to the
15 donor when we are going to test.
16 Now, this I have explained to you. The
17 difference is now that the qualifying donor will
18 undergo again, the conventional testing, and those
19 donors that qualify according to standard rules, we
20 will also make a pilot sample pool. But we do not
21 put the samples into an inventory hold for three
22 months but rather do the testing immediately.
23 If this pool is negative all these
24 samples go into an inventory hold of a length that
25 I'm going to talk about in a second. And then we
26 follow the standard procedure: after inventory hold

1 we would release it for pooling, do the test of
2 record to cover European requirements, and release
3 negative production pools, and we would destroy
4 positive production pools.

5 If such a pilot sample pool turns out to
6 be positive we now can detect or identify the donor
7 and the donation. Of course, the donation will be
8 rejected, the donor identified, and now we can start
9 to go onto a look-back. Which means having those
10 samples on hand we can now call the samples from the
11 look-back storage and do individual PCR.

12 And the lengths of this inventory hold
13 period of time is one of the targets that we are
14 addressing. We would like to know what is the
15 optimal inventory hold to cover this -- or most of
16 the window donations, and not to have the plasma
17 sitting around for much too long, which is a costly
18 process as you can imagine.

19 Now, consider at the time point A you
20 have a cohort of donors and some of them or one of
21 them might be in the diagnostic window. And PCR
22 does have a diagnostic window as well. Just as a
23 serological diagnostic window, PCR will not detect
24 an infected person at day-1 of infection. It takes
25 a couple of days as we have seen, until enough virus
26 has been produced to be seen.

1 So if a person comes in right after
2 infection we would not see it even on type PCR. So
3 we have to make sure that this person comes back a
4 second time after a given period of time, which of
5 course is dependent on the window period of the
6 virus.
7 Let's say this is 30 days. After 30
8 days each window donation, or each window period
9 donor should have sero converted -- or PCR
10 converted, not sero converted -- and we will be able
11 to detect. And now you can imagine it will take a
12 couple of days more because if it's today and we
13 need 30 days, the donor won't come back in 30 days.
14 We are adding so-called observation
15 period for the time to start of 30 additional days,
16 and we calculated from the return rate of our repeat
17 donors that we will have 90 percent of donors being
18 here at the -- will come up within two months for a
19 second donation.
20 And by this approach we expect that the
21 efficiency of a 60-day inventory hold is helping to
22 reduce the likelihood of having overlooked a window
23 donation by 90 percent. That's what we have to
24 build into our -- or, what we want to build into our
25 IND.
26 Thank you for your attention.

1 CHAIRPERSON TABOR: Thank you. The next
2 speaker is Dr. Flanders from Abbott.
3 DR. FLANDERS: Thank you. I want to
4 present some information, internal development
5 information on our HCV assay that runs on the LCx
6 instrument.
7 First of all just to describe the
8 overall process, this is using already existing
9 platform -- the LCx Analyzer. We do a sample
10 preparation which I'll describe very briefly, for
11 extraction of the RNA, amplified normally and then
12 automated -- detected in an automated way on the LCx
13 Analyzer.
14 The sample prep procedure that was used
15 for the HCV assay as well as HIV and HBV assays that
16 we've developed assays for internally, and uses the
17 Chiagen column procedure modified to include a
18 vacuum manifold to provide for ease-of-use and also
19 to provide for multiple sample volume capability.
20 Every assay, each assay includes or each
21 run includes of course, a standard negative control
22 and positive controls which do go through the sample
23 prep procedure as well as each individual sample
24 includes, as we've heard on several of the assays,
25 an internal control which has the same primers as
26 the target.

1 And then there's a non-sense sequence in
2 the amplicon that's inserted, and we can detect both
3 the target and the internal control simultaneously
4 in the single sample on the LCx -- primarily as an
5 indication that the PCR reaction performed as
6 expected. Again, the internal control does go
7 through the sample prep with the sample.
8 Now, I will describe just some internal
9 data for the performance of the assay. This assay
10 is not currently involved in IND studies. To
11 understand the sensitivity and what has been lacking
12 over the course of development of these various
13 assays but now we do have some standards that we can
14 compare to and to keep everything apples and apples,
15 I want to describe the sensitivity of the assay with
16 the WHO standard.
17 We prepared a sample from the original
18 stock to about 5,000 IUs per ml, and then serially
19 diluted that standard over 1,000-fold. We processed
20 each of the diluted samples multiple reps of each of
21 the samples through sample prep using either a one
22 ml input sample of the diluted sample, or .2 mls of
23 the diluted sample.
24 The one ml was originally designed such
25 that from one ml of input sample the final extracted
26 RNA could be split into three separate assays: one

1 for HCV, one for HIV, and one for HBV. So we don't
2 use the entire content; we use about a third of
3 that. And then test each of the process samples
4 with the LCx system.
5 Using the standard with a one ml sample
6 prep which is a little more sensitive than the .2,
7 we can detect with a cutoff signal of about 50, we
8 can detect about ten copies or 10 IUs per ml of the
9 standard; with a .2 ml it's about 40 IUs per ml.
10 And this is consistent with the
11 difference in the sensitivity between a one ml
12 sample prep and a .2 ml sample prep. So I think
13 similar to some of the data that we've seen for some
14 of the other systems.
15 In order to understand whether or not
16 this sensitivity which is in fact, in a serial
17 dilution and is a single donor, understand whether
18 or not pooling of the same standard would have an
19 impact on sensitivity, we tested and created 20
20 separate pools. We used a pool size of 64
21 specimens, so about 1200 individual, negative
22 plasmas.
23 We used a Hamilton Microlab pipetting
24 station for creation of the pools. Each pool was
25 spiked with the WHO standard to reflect if one of

1 the specimens was at 5,000 IUs per ml consistent
2 with the Paul Ehrlich recommendations.
3 This results in a final pool that should
4 have about 78 IUs per ml in the final pool. We
5 processed each of the pool samples through the
6 sample preparation; again, using either a one ml or
7 a .2 ml input sample volume and then tested each in
8 the LCx system.
9 Before I go to that data, this is just
10 to show that prior to spiking the pooled samples
11 with the standard, they were also tested without the
12 spike and they were all negative for HCV. This is
13 the internal control signal which is positive on all
14 the samples, and this is the HCV signal which is of
15 course, negative.
16 In the case of the one ml sample, the
17 internal control again as I said, was positive for
18 all the pools -- positive after spiking as well so
19 they were all positive for the internal control --
20 and the HCV signal was positive with an average
21 signal of 80.6.
22 And at the .2 ml the internal control is
23 positive. Out of the 20 pools, 17 were positive.
24 There were three that were slightly below the cutoff
25 of 50. The average signal here was 62.5. This is
26 very consistent then with the dilution curve that I

1 showed previously. It's indicating that the pooling
2 isn't impacted -- does not impact the sensitivity of
3 the assay in this study.
4 We've also tested, as many have
5 described, the various -- that looks out of focus to
6 me, but anyway, these are all the different sub-
7 types of HCV. We've tested it with true isolates of
8 the various sub-types as well as transcripts that
9 have been quantitated independently of all the
10 various sub-types to the same level.
11 We do detect all the sub-types, A1
12 through 6, equivalently, and if we do this in a
13 quantitative way we've shown with a quantitative HCV
14 assay that we quantitate the various subtypes across
15 the dynamic range from about 1,000 copies up to 10^7
16 copies equivalently as well.
17 Of course, there's a wide separation
18 between the negative population and a normal,
19 positive population, partly reflected by the fact
20 that the amount of virus in most positive samples is
21 quite high. Which has also been reflected and we've
22 seen before, in the sero conversion panels.
23 I just have two here; we've tested a lot
24 more than this. But just representative of what we
25 see in most of the panels that are generally
26 available; that is, that the RNA is already positive

1 on the very first bleed that's available, and prior
2 to antibody sero conversion.
3 The assay on all the panels that are
4 commercially available have been tested has been
5 positive on the first bleed available except one --
6 and again, this is from Bioclinical Partners. This
7 one actually does go through both a sero conversion
8 or sero conversion ALT conversion, as well as an RNA
9 PCR GAT conversion.
10 But again, very rapid increase. This is
11 day-8 I believe, and day-11; within a 3-day period
12 it's already a very high viral load and very high
13 signal for HCV.
14 And actually I think that's it. We are
15 not in an IND study currently with this assay, but
16 that reflects the HCV assay and its performance
17 internally. Thank you.
18 CHAIRPERSON TABOR: The last speaker in
19 this session is Dr. Psallidopoulos from Nabi.
20 DR. PSALLIDOPOULOS: Thank you, the
21 organizers, for inviting me here. Thank you for
22 attending this very short, brief introduction to
23 Nabi's PCR test system.
24 Our primary goal is to devise a plasma
25 test system to screen out units that are going to
26 the Nabi immunotherapeutics in order to limit the

1 virus load, to define maximum increase this safety
2 of Nabi products, and these test should be of a high
3 level of screening.
4 Very quickly, introduce you to the
5 design, our methodology, and give you some of our
6 results and our future directions.
7 We have a physical separation of the PCR
8 processes through two different buildings using
9 different laboratories, to get the PCR and then to
10 get at work areas within the PCR laboratories. We
11 have a unidirectional work flow, we have dedicated
12 equipment and consumer boats and non-consumer boats
13 in each work area or laboratory. And we generally
14 follow the good PCR laboratory practices.
15 Our plasma pooling is what has already
16 been described by Dr. Conrad from NGI. It's a 3-
17 dimension matrix using the 512 pooling sample and
18 using robotics to do the pooling. Our testing
19 algorithm is already described.
20 Basically if the master pool is negative
21 then all the 512 units are released. Otherwise we
22 test the primary pools to identify individual unit
23 which is then tested and verified in an independent
24 laboratory like NGI.
25 The nucleic acid extraction again, is
26 the standard ultracentrifugation, with the addition

1 of an external PCR control to give us a feeling of
2 the sense of the quality of the RNA extraction.
3 We're using the RNA study guanidinium method to
4 extract the RNA.
5 Basically, one-fifth of the RNA sample
6 is duplicated tested for the HCV and the same thing
7 for the PAW109. The amplification process is the
8 single test that combines RT-PCR for the first round
9 of amplification, followed by nested amplification
10 using rTth, and the primers that are described by
11 the FDA PCR-2 protocol.
12 The detection method is the
13 ethidiumbromide gel, standard procedure, using the
14 followed recommendation system and keep records of
15 both pre- and electronic forms.
16 In the past we have participated in the
17 well viral quality control proficiency study which
18 were given half ml samples and we actually used an
19 old protocol of extraction, diluted down into two-
20 and-a-half mls and then extract the entire two-and-
21 a-half mls.
22 And this is the performance of our assay
23 on the replicates. We have achieved somewhere
24 around 40 copies of genomic equivalence, the 50
25 percent hit rate.

1 We also use Pelispy, which is obtained
2 from the Dutch Red Cross, Nico Lelie -- it's a run
3 control. And this is the results of the last, about
4 36 runs, and you can see that the performance of our
5 assay is about seven genomic equivalence, a 50
6 percent hit rate.
7 So having established this assay,
8 current screening studies is that we screen
9 something like 5,000 units so far; that they went
10 into clinical lots, and we haven't found anything
11 positive yet. We are preparing to submit an IND and
12 we are performing the assay validation.
13 Our future plans is to transfer this
14 assay to the clinical labs and introduce new testing
15 -- a prime test for other viruses as needed, and
16 also currently we're trying to use the genetic
17 analyzer to detect the amplicons.
18 That would give us enhanced sensitivity,
19 which is an issue for the next point; to use this in
20 other Nabi products. As I said in my introduction
21 one of the primary causes of using the PCR test is
22 to eliminate the virus load to define maximum.
23 Nabi uses other products like civacir
24 which is made up from pools of HIV-positive plasma
25 units. This is quite the reverse. In the first

1 time we want to eliminate any positive units; here
2 we actually get all the positive units.
3 This have to be fractionated so we use
4 various virus elimination and virus removal
5 protocols and the PCR is important to make sure that
6 these pools are negative before they enter the plant
7 so the sensitivity and specificity for the assay has
8 to be improved.
9 This is where we stand at the present.
10 Thank you.
11 CHAIRPERSON TABOR: Thank you. We'll
12 now break for lunch. I'm afraid the only nearby
13 place to eat is the cafeteria in the building. Most
14 of the other sandwich places on adjacent streets are
15 too far to go. We'll reconvene in about an hour,
16 depending on the efficiency of the cafeteria lines.
17 Thank you.
18 (Whereupon, a brief luncheon recess was
19 taken at 12:47 p.m.)

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

2 (1:51 p.m.)

3 CHAIRPERSON TABOR: We're ready to begin
4 the session on Regulatory Perspectives on Assay
5 Validation. The first speaker is Dr. Indira Hewlett
6 from the Food and Drug Administration.

7 DR. HEWLETT: Thank you, Ed, and good
8 afternoon everyone. This afternoon I'll be
9 discussing some of the major regulatory issues in
10 regard to assay validation from an FDA perspective.

11 I think we can all agree at this point
12 that nucleic acid testing may be the most sensitive
13 method currently available for early viral
14 detection. And implementation of nucleic acid
15 testing would result in reduced viral burden in
16 blood and plasma.

17 The blood and plasma industry has
18 proposed testing plasma pools for nucleic acid, and
19 this is partly because pool testing may be most
20 practical at the present time.

21 Plasma pool testing is currently
22 occurring in at least three different scenarios.
23 The first involves the use of an in-house test
24 developed by a blood product manufacturer. The
25 second approach is to use a commercial tested. And
26 a third option is to contract out the testing to a

1 testing service that is competent in performing this
2 type of testing.
3 Regardless of the testing scenario,
4 FDA's view is that assays used to test plasma pools
5 rather than single donations would still be
6 considered to be donor screening assays and
7 therefore subject to validation and license
8 requirements.
9 This would ensure manufacturing
10 consistency and define the performance
11 characteristics of the assay by clinical studies.
12 As with other blood screening assays, donor
13 notification and product management strategies will
14 have to be in place at the time of implementation.
15 I'll now get into the issues, specific
16 issues in regard to validation. Assay validation to
17 be stated briefly, consists of development of the
18 appropriate quality control procedures to assure
19 manufacturing consistency under GMP of the
20 components of the test method or the test kit, and
21 the accumulation of relevant laboratory and clinical
22 data to support the intended use of the product and
23 the manufacturer's claims.
24 I should mention that the points I'm
25 going to discuss rather briefly today are actually
26 discussed in great detail in a draft guidance

1 document for industry in the manufacturer and
2 clinical evaluation of in vitro tests to detect
3 nucleic acid sequences of HIV1.
4 This document, although it addresses
5 issues pertinent to HIV1 is also expected -- the
6 criteria listed in this document are also expected
7 to apply to tests that are being developed for other
8 viruses that are screened for in blood.
9 I should also mention that this document
10 is currently available at a CBER Web site, so people
11 that are interested should be able to access it.
12 And it's currently been published for comment, so
13 we're taking comments about the criteria at this
14 time.
15 The second document, which is the draft
16 Federal Register Notice on FDA's proposed approach
17 in regulating nucleic acid tests for plasma pools is
18 still under review within the FDA. And I will not
19 be discussing this particular document since it's
20 been presented at a blood products advisory
21 committee meeting in the past.
22 Some general criteria apply to all in
23 vitro tests and they are listed on this slide.
24 Specifically, there should be some rationale for the
25 design and format of the test: for example, the
26 type of specimen that is being tested, the controls,

1 calibrators, the cutoff. These are examples of
2 design that impact the design and the format of the
3 assay.
4 The stability of specimens under various
5 processing conditions such as collection, transport,
6 and storage should be determined. The components
7 and reagents used in the test method or kit should
8 be subject to appropriate quality control methods
9 and their stability determined as well.
10 The performance of the assay should be
11 defined in terms of reproducibility, analytic and
12 clinical sensitivity and specificity, and any
13 instruments including software, that is used to
14 perform the test method or to calculate results
15 should be validated for their function.
16 Most importantly, statistical methods
17 should be used to validate results from laboratory
18 and clinical studies.
19 The design of the assay -- I'm actually
20 going to get into each of the points I had on my
21 previous slide and I'll discuss the key points in
22 regard to each of those criteria.
23 The design of the assay should take into
24 account several features, most critical of which are
25 selecting the appropriate primer and probe sequences
26 and the target region for nucleic acid assays. For

1 example, one may wish to base the selection on the
2 degree of conservation of the particular region so
3 that multiple sub-types may be detected.
4 Inclusion of appropriate controls is
5 necessary to ensure that the reset results are
6 valid. These controls would include internal and
7 external controls such as controls for
8 ultracentrifugation if such a step is involved in
9 sample processing, for extraction, and positive
10 controls of course, that would define the validity
11 of the run.
12 Additional controls that are used in
13 nucleic acid amplification methods are controls for
14 false positive reactions that might arise from
15 contamination as you heard this morning, during the
16 pooling process, or cross contamination during the
17 PCR amplification process.
18 Finally, if an assay has a quantitative
19 format, calibrators and quantitation standards
20 should be designed to have an acceptable range and
21 limit.
22 Specimen stability has an impact on
23 assay performance -- and I think you heard a little
24 bit about the stability studies this morning;
25 they're actually quite impressive.

1 The conditions of stability need to be
2 defined. These conditions include collection,
3 transport, and storage of the original specimen; the
4 specimen undergoing extraction by a specific
5 protocol -- as well as the stability of extracted
6 material on storage.
7 For such studies, FDA is willing to
8 accept the use of well characterized specimens, well
9 uncharacterized spiked materials, but the use of
10 clinical specimens, naturally occurring clinical
11 specimens, is highly recommended.
12 The analytic sensitivity of the pool
13 test may be determined by testing dilutional panels
14 of known positive clinical specimens. Testing of
15 sero conversion panels and low titer RNA specimens
16 are also useful in evaluating analytic sensitivity.
17 FDA's current proposed limit is 100
18 copies per ml for the full test. This proposed
19 limit may be modified in the future depending on the
20 actual experience in regard to performance of pool
21 tests in the field.
22 And we've decided to start with this
23 limit because as you'll hear from some of the
24 studies that Dr. Yu will discuss later, that this
25 100 copy limit is actually achievable by many of the
26 tests that are currently in use. In all cases,

1 tests should be run in parallel with a comparator or
2 reference assay.
3 All tests have to be evaluated for their
4 performance on clinical samples to determine their
5 specificity and sensitivity. Specificity is
6 established by testing random blood donor specimens
7 with follow-up testing to resolve reactive results.
8 Sensitivity is established by testing
9 known positive specimens. Sensitivity studies
10 should include testing of genetic sub-types and
11 specimens from persons at different stages of
12 disease and possibly different risk groups. And
13 this morning we heard about differential detection
14 rates among ethnic and racial groups, so those are
15 some things to consider at assay validation of the
16 HCV tests, in particular.
17 Specimens that may be expected to cause
18 interference in the assay by producing false
19 positive or false negative results should also be
20 tested to determine their impact on analytic
21 specificity of the test.
22 And finally, the reproducibility of the
23 assay should be determined by testing at multiple
24 sites, a given panel or a given number of specimens
25 that have viral copies -- that have deferring viral
26 copies -- including samples that have low copy

1 numbers, to determine how reproducible the assay is
2 across the linear range.
3 Some issues are of special concern in
4 regard to pool testing. They are pool size -- we'll
5 start with pool size which we know has a substantial
6 impact on the sensitivity of the test. And the
7 derivation of the pool size should be based on the
8 ability to achieve acceptable limits of sensitivity
9 in the final assay.
10 Another aspect is the analytic
11 sensitivity of the pool tests. And both of these
12 parameters are extremely important, particularly in
13 light of the need to demonstrate equivalence or
14 enhanced sensitivity of testing pools to currently
15 licensed methods.
16 An issue that should also be addressed
17 but may not be as much as big an issue as we had
18 thought originally, is the generation of
19 interference due to matrix effects that might result
20 from pooling of specimens which may cause
21 interference in the assay.
22 And I think we wish to continue to see
23 data addressing matrix effects because there are
24 different pooling regimens and different pooling
25 schemes, so there's going to be a continued need to

1 see if in fact, matrix effects are an issue with
2 pool testing.
3 There should be mechanisms for logging
4 and tracking the specimens and quality assurance of
5 systems that are used to pool and test pool
6 specimens, as well as to trace back positive results
7 to the original donation and the donor.
8 And this of course will include
9 instrumentation and software validation since
10 instrumentation is expected to play a major role in
11 the implementation of pool testing.
12 So in summary, implementation of nucleic
13 acid testing of plasma pools should further reduce
14 the risk from window period donations. FDA still
15 continues to view pool testing as an interim step
16 towards single donation testing due to technology
17 development.
18 Assay validation should occur under the
19 IND PLA mechanism which provides adequate control of
20 manufacturing procedures and procedures for tracking
21 inventory and resolution of reactive results.
22 The use of reference reagents
23 established here at CBER and elsewhere should be
24 helpful in determining assay performance and
25 laboratory proficiency, as well as in lot release

1 testing of the pool test method or test kits that
2 are used to test pools.
3 Laboratory testing and clinical trials
4 will need to take place to establish sensitivity,
5 specificity, and reproducibility. And finally,
6 appropriate procedures for donor and product
7 management are expected to be in place at the time
8 of implementation.
9 Thank you for your attention.
10 CHAIRPERSON TABOR: Thank you. The next
11 speaker will be Dr. Nubling from Paul Ehrlich
12 Institute.
13 DR. NUBLING: First of all I also want
14 to thank for an invitation to this interesting
15 meeting. I will start with a short overview about
16 the current situation in Germany concerning NAT
17 testing and then we'll switch to the validation
18 issue.
19 It's meanwhile, nearly three years ago
20 that the first blood banks introduced NAT screening
21 on a voluntary basis in Germany, and as you can
22 imagine, this implementation into the routine
23 screening program pushed discussions quite strongly
24 if such NAT testing, first is feasible in general,
25 and also if it makes sense concerning increasing of
26 viral safety.

1 And just one year ago there was a
2 meeting of the Paul Ehrlich Institute where all
3 blood banks from Germany participated and the
4 results obtained in the meantime were given at this
5 meeting.
6 And this is just a summary of the
7 results concerning HCV NAT testing. Up to this time
8 there were five blood banks which had introduced NAT
9 testing for HCV on this voluntary basis.
10 And as you can see, quite different
11 numbers of donations had been tested until end of
12 last year, and also the pool size chosen for testing
13 was quite different between these different blood
14 banks.
15 Nevertheless, more than one-and-a-half
16 million donations were tested in total by HCV NAT
17 and the results for entity-positive but antibody-
18 negative donations were 13 among this number.
19 This results in incidence among German
20 blood donors for NAT-positive, antibody-negative
21 donations, approximately 1 in 120,000. And is much
22 higher than for example, for HIV or HBV. This may
23 explain why the diagnostic window phase -- we have
24 discussed this early in the morning -- and another
25 important feature is the titer during the window

1 phase which is much higher for HCV compared to HIV
2 or HBV.
3 These facts were a reason for the
4 decision of Paul Ehrlich Institute to introduce HCV
5 NAT from the first of April of next year. A
6 sensitivity limit for the single donation was given
7 with 5,000 initial units per ml, and that's the main
8 topic of my talk today, validation is required of
9 the methods and the documents of the validation are
10 order to be given to the Paul Ehrlich Institute
11 until end of this year.
12 First the question, what's the reason
13 for this sensitivity limit on the single donation
14 phases? We've performed quantitative analysis of
15 quite many sero conversion panels. Here are seven
16 sero conversion panels and make conductive PCR.
17 And as you can see, here's the
18 logarithmic scale of copy numbers per ml. All PCR-
19 positive donations among these sero conversion
20 panels would be picked up by a method which is able
21 to detect 5,000 international units permitted under
22 single donation basis.
23 And what you have also seen this morning
24 already is between the last PCR-negative donation
25 and the first PCR-positive donation there's a quite
26 steep increase in titer. So that we expect quite

1 few samples which are under their 5,000
2 international units limit.
3 This is also confirmed by quantitation
4 of single donations which have already been
5 identified by nucleic acid screening. Here's a
6 limit again, and we quantified different genotypes,
7 different single donations, and all of them are
8 quite higher than the 5,000 international units
9 limit, and the sensitivity limit used for the
10 identification of these donations was even lower
11 than the 5,000 international units.
12 So that's the background for our
13 decision. Now to the validation. In principle we
14 differentiate between in-house tests and commercial
15 tests, and in-house tests may be used by the blood
16 banks if they are validated.
17 For in-house tests the features and the
18 modifications of the tests have to be validated by
19 the user. For commercial tests we accept the
20 validation performed by the manufacturer.
21 Independent, if the commercial tests are complete
22 tests starting with extraction and ending with
23 detection of the amplification products, or if there
24 are only parts of the NAT procedure -- for example,
25 only extraction kits.

1 Modifications of these commercial tests
2 of course, have again, to be validated either by the
3 user who introduced the modification, or by the
4 manufacturer if he recommends this modification.
5 Documents which were relevant for our
6 interpretation of validation requirements are mainly
7 the ICH documents which have been mentioned also in
8 the morning here. There are several other documents
9 available which are quite useful when a PCR system
10 is established, but the requirements validation are
11 based on these ICH notes for guidance.
12 HCV NAT in blood bank setting is
13 considered as a limit test and these five points are
14 the main points which should be validated, starting
15 with specificity.
16 Specificity means the identify of the
17 amplification products and I think it's obvious that
18 it's strongly dependent on the choice of primers,
19 probes, assay stringency. And before establishing a
20 PCR NAT system, a databank comparison of primers
21 with target sequences can avoid false positive
22 results.
23 During validation the amplification
24 product has to be characterized either by size
25 restriction, hybridization, or sequence. And for
26 validation of specificity we require at least 100

1 samples; either negative samples if single donation
2 PCR is performed or 100 negative pools if pool PCR
3 is performed. Both is possible, of course.
4 Sensitivity, I think it's the most
5 important point during validation. In principle
6 there are different definitions of detection limits.
7 Some people speak about the detection limit if 100
8 percent hit rate is obtained; 95 percent hit rate we
9 have heard today; also others defined detection
10 limits with 50 percent hit rate.
11 When the statement or the requirement
12 for 5,000 international units, a single donation was
13 made, it was meant in the way that it should be
14 detected in more than 95 percent. This detection
15 limit should be guaranteed for the most prevalent
16 virus types in Europe or in Germany. These are
17 mainly virus type 1, but also virus type 3.
18 And of course, the detection limit
19 should be controlled on a routine basis by a
20 positive controller.
21 Now it's the question, how should the
22 detection limit be determined? We recommend to
23 perform 3 half log10 dilution series of the WHO
24 standards, HCV RNA, or a reference preparation which
25 is calibrated against this gold standard, and to

1 perform per dilution point, 8 replicates so that 24
2 values are obtained for each dilution point.
3 Then PROBIT analysis is performed and
4 from the curve, the 95 percent value and 50 percent
5 value and even 100 percent value can be obtained.
6 We encourage also, persons of blood
7 centers to calibrate their own in-house reference
8 preparations in order to create run controls, and
9 for calibration of this in-house reference
10 preparations we recommend the same procedure in
11 parallel with WHO standards.
12 There are difference reference
13 preparations already available. The most important
14 of course, is WHO standards, but it's available only
15 in limited amounts, for good reasons.
16 From CLB, BBI we have also meanwhile,
17 created a hydrolyzed preparation which has been
18 mentioned also in the morning already, and also
19 other organizations -- also NIBSC has a working
20 reagent. This working reagents are all accepted as
21 calibrated material as soon as they are calibrated.
22 Next point concerning sensitivity is the
23 avoidance of false negative results. We require
24 inhibition control for a pool size of bigger than 50
25 donations. Also internal control or a parallel
26 spiking control to be performed with an assay.

1 The next is genotype sensitivity. It
2 can be obtained before establishment of the PCR
3 system by databank comparison of the primer
4 sequences with the sequences of the available
5 genotypes. And of course, it has to be checked with
6 genotype samples; for example with panels available
7 already.
8 Precision means laboratory internal
9 variation. It is dependent on variation of persons,
10 equipment, and also should be investigated on
11 different days. We require that for in-house PCR
12 reagents have to be defined with a shelf life and
13 also quality control of new batches of primers,
14 enzymes, dNTPs, etc., has to be performed.
15 Reproducibility means precision between
16 different labs. Here we recommend to use control
17 panels and to participate in collaborative studies
18 in order to estimate the results in comparison to
19 other laboratories.
20 Robustness of an assay can be obtained
21 by appropriate training of personnel, by creating
22 meaningful, standard operating procedures.
23 Avoidance of contamination should be validated by
24 using also high titer standards during the
25 validation phase, alternating with negative samples
26 in order to detect potential carryover.

1 And of course in the routine setting the
2 robustness is controlled also by appropriate
3 controls for sensitivity, inhibition, and
4 contamination. If a (unintelligible) should occur,
5 one should have in mind that this can occur. A
6 second amplification -- at least access to a second
7 amplification system should be available in order to
8 have no shortage on blood products.
9 This validation criteria I think are
10 essential for reliability of methods, especially for
11 in-house methods. And in Germany there are many
12 blood banks which are creating their own in-house
13 systems and it's a major challenge for them but I
14 think it's really essential.
15 And it's of course, also a pre-requisite
16 for approval of tests which will be performed in
17 future in Germany, meaning that commercial tests at
18 least then have to give authorization documents to
19 our institutes. And I think our requirements are a
20 compromise between what can be done and what should
21 be done in the optimum case.
22 Thank you.
23 CHAIRPERSON TABOR: The next speaker is
24 Dr. Rautmann from the European Pharmacopoeia
25 Commission.

1 DR. RAUTMANN: First I would like to
2 thank the organizer for inviting me to this meeting.
3 And when I started preparing my talk for this
4 presentation I had some discussion. I was also
5 asked to actually present to you how the system
6 operates, who the institution involves, and what is
7 the legal background behind it.
8 I must admit that on the other side of
9 the ocean it's not always understood by all the
10 people and the system is quite complex.
11 Therefore, my talk today will be divided
12 in three parts. I will briefly mention the
13 institution involved in the implementation of NAT,
14 the EU regulation, and what are the impact actually,
15 on the implementation of NAT.
16 So actually this first slide is showing
17 you how actually things are organized on the
18 national level to guarantee quality, safety, and
19 efficacy of medicinal products, and how was it
20 switched from national level to the European
21 framework.
22 To make the discussion a little bit
23 easier I have put on the bottom of the different
24 parts, the place where the institutions are located,
25 which is Brussels in Belgium, London in the U.K.,
26 and Strasbourg in France.

1 Since the first of January '93 actually,
2 community legislation provides for a free trade of
3 goods within the European Union, which also implies
4 for medicinal products. Therefore, the European
5 Union has created a few tools.
6 Among those by community regulation,
7 there has been the creation of the European Medicine
8 Evaluation Agency in London which is in charge of
9 making the evaluation of medicinal product proposed
10 to be put on the market in Europe through the
11 centralized procedure.
12 Which means counting Europe there are
13 two ways. Either you have the centralized procedure
14 -- and this is compulsory for products of list A,
15 which are medicinal products involving a
16 biotechnologic process.
17 And in this case the dossier is
18 evaluated by the EMEA in London, which makes a
19 recommendation and this recommendation is forwarded
20 to the Commission and it's actually the Commission
21 which then agrees and provides the centralized
22 marketing authorization.
23 This is on the part of the licensing.
24 On the part now, of post-marketing activity,
25 actually the European Union has made a contract with
26 the Council of Europe in Strasbourg asking the

1 European Department for the Quality of Medicine,
2 Division 4, to create and coordinate a network of
3 the official medicine control laboratories of each
4 of the member states.
5 And therefore Division 4 is in charge of
6 coordinating this network, and this is by contract
7 between the two institutions.
8 EDQM has also in charge in one of the
9 divisions, the European Pharmacopoeia. Briefly,
10 this slide is showing you the organization of the
11 European Department for Quality of Medicine, which
12 as I said, located in Strasbourg, and is department
13 from the Council of Europe.
14 EDQM is organized in four divisions: 1,
15 2, 3, 4. I am working in Division 4. As I said,
16 within EDQM Division 4 is actually the European
17 Pharmacopoeia Commission which, in conjunction with
18 this group of experts is actually drafting and
19 adopting the monograph of the European
20 Pharmacopoeia.
21 We have also Division 2 which is what we
22 call the Publication Unit. This unit is responsible
23 for publishing the Pharmacopoeia and other
24 publications -- Pharma Rupa and proceedings of
25 several meetings we're organizing.

1 Well, then we have Division 3 which
2 actually is the laboratory, and this laboratory is
3 split into two sections: one is the chemical
4 section and the other one is the biological section.
5 And actually, the laboratory will serve
6 both the European Pharmacopoeia Commission but is
7 also supposed to serve activity within Division 4
8 which are on one side, the biological
9 standardization program. And in this program we are
10 either establishing biological reference preparation
11 which will be used in the European Pharmacopoeia
12 monograph.
13 And the second activity of Division 4
14 is, as I said before, coordinating the official
15 medicine control laboratory network.
16 Okay, let me now switch to community
17 regulation. This includes two types of documents:
18 either the legally binding documents under the form
19 of directives or regulations, or on the other side
20 you have also non-binding documents which are called
21 guidelines, or sometimes Note for Guidance.
22 The reason why community has decided to
23 use also guidelines is to allow certain element of
24 flexibility and not provide a too-strong legal
25 constraint on evolution of technology. And
26 therefore, it's always possible for manufacturer to

1 deviate from what is actually recommended in a
2 guideline, provided the data that manufacturer is
3 having is supporting that he was right in deviating
4 from the guideline.
5 In addition to this we have also
6 monograph of a European Pharmacopoeia, which like
7 directives, are binding documents. For the topic of
8 today there are several documents or legal texts
9 which are of importance.
10 We have "Council Directive 89/381",
11 which is as I said, a binding document. In the
12 European Pharmacopoeia we have "NAT General Method",
13 and we have the monograph on "Human Plasma for
14 Fractionation". Those three texts are biding
15 documents.
16 Then we have guidelines, and in
17 guidelines we have the "CPMP/BWP/269/95", which is a
18 guideline on medicinal products derived from plasma.
19 We have the second guideline which is "390/97",
20 which is the guideline recommended in the
21 implementation of NAT as of July 1st, '99.
22 And the last guideline here which was a
23 guideline which was developed by an adult working
24 group within the official medicine control authority
25 network. And in this guidelines and this expert

1 group which designed this guideline, we had Dr.
2 Nubling and Dr. Saldanha who are present today.
3 If we rapidly go through the first
4 document which is the "Directive 389/381", the three
5 articles which are important on the topic of
6 implementation of NAT -- actually the guideline in
7 Article 3.1 asks that member states takes the
8 necessary measure to prevent the transmission of
9 infectious diseases.
10 And the text is referring that the
11 Directive 75318, as well as the European monograph,
12 should be applied when a member state is applying
13 those measures.
14 In Article 4.1 it's stated that member
15 states have a responsibility of ensuring that the
16 process as well as the purification steps used to
17 produce medicinal products derived from human blood
18 or plasma are properly validated; that they contain
19 batch-to-batch consistency; and that as state-of-
20 the-art technology permits, the process guarantees
21 the absence of viral contamination.
22 The last article, 4.3, actually is the
23 one providing for control authority batch release,
24 which means that the person responsible for
25 marketing a product on the member states within the
26 European Union, if the law of the member states

1 provides for, then this person must submit to the
2 competent authority a sample of each batch of the
3 product which will be released on the market.
4 Which means that each batch will be
5 retested by the competent authority. And in this
6 article it's written that actually a batch will be
7 retested only once, which implies mutual recognition
8 within the OMCL of different member states of the
9 European Union that once a batch has been tested and
10 released for one of the market, one of the OMCL
11 within the European Union, no other OMCL can retest
12 the batch for the purpose of batch release.
13 I'll now go to the CPMP/BWP -- actually,
14 this series of letters in front of a guideline means
15 that the guideline has been developed by the
16 biotechnology working party of the EMEA in London,
17 proposed to the CPMP, adopted by the CPMP, and then
18 a guideline is actually proposed to the Commission
19 and becomes an official guideline for the European
20 Union.
21 As I said, implementation date of this
22 guideline is July 1st, 1999. The guideline states
23 that plasma pool must be tested for HCV by NAT; that
24 only plasma pool shown to be non-reactive can be
25 used for the manufacturing process.

1 It applies also to excipient, which
2 means that actually the date in which an excipient
3 is either entered in the final product or used to
4 manufacture an intermediate, will be the date
5 compared to the implementation date of July 1st,
6 1999.
7 This has some impact also, for
8 manufacturer in the vaccine field or for some
9 biotech product in which we have quite a lot of
10 albumin for instance, inside. The guideline says
11 also that the test method must be validated and that
12 each of the assays must use a run control calibrated
13 against the WHO international standard, and that
14 this run control must be equivalent to 100
15 international units per ml.
16 This guideline applies to manufacturer
17 but as I said, within the process of official
18 control authority batch release this guideline will
19 also apply to official control medicine laboratory.
20 Therefore, within the network we created
21 another working party having experts from the
22 different OMCL who are actually going to perform
23 those assays within the OMCL at the occasion of
24 batch release. And we discussed about the
25 implication of the implementation of this guideline.

1 When we had this discussion one of the
2 items which came up was actually validation and how
3 the different OMCLs were going to validate their in-
4 house or their method in the OMCL.
5 The other point that was raised during
6 those discussions were, some OMCL were already using
7 NAT for quite a long time; others were just
8 developing the methodology. Therefore, two types of
9 studies were organized within the OMCL network.
10 One study is the so-called self-
11 assessment study in which a set of samples were
12 distributed to the different OMCLs and tested. The
13 results were then sent back to EDQM who analyzed the
14 data, coded the data, and provided data to all OMCL.
15 This is the self-assessment study which is supposed
16 to help OMCL to improve the performance when
17 performing NAT.
18 The second study which will be organized
19 at the end of this year is proficiency testing
20 study, again, within the OMCL. A panel of sample
21 will be distributed to the OMCL and they will test
22 it blindly in a way as it was done by the OC study.
23 So I'm just briefly mentioning this NAT
24 validation guideline which actually now is a
25 proposal. It was released the beginning of this
26 week for public inquiry, which means that we have

1 all possibility to access on this guideline. And
2 please if you have comments, send those comments
3 back to EDQM, but not later than the first January,
4 1999.
5 As I said, this guideline was drafted by
6 experts within the OMCL network. I intended to
7 briefly go through specificity and detection limits
8 but since Dr. Nubling has covered a lot of it and a
9 lot was already described by Dr. Nubling is actually
10 within this guideline, it would be merely a waste of
11 time. Yes, you have seen this more or less in the
12 slide of Micha already.
13 Concerning the detection limits,
14 actually it was also stated this morning that NAT
15 can be considered as a limit test, and therefore if
16 one looks to the ICH guideline about limit tests,
17 the ICH guideline will commence the use of a
18 detection limit.
19 But for practical purposes the expert in
20 the group proposed to use the positive cutoff point
21 as defined in the European Pharmacopoeia Monograph,
22 which means that the positive cutoff point is
23 defined as the, more or less the 95 percent hit
24 rate.
25 In the guideline there is also given a
26 way on how actually to determine this positive

1 cutoff. And just in addition to what was just said
2 before by Dr. Nubling, the guidelines foresee that
3 actually the 24 test results from each dilution may
4 be obtained by three different ways: either by
5 making three independent tests on three days, by
6 using eight replicates for each dilution;
7 alternatively, four independent tests with four
8 replicates for each dilution; or six independent
9 tests and four replicates. All together, having
10 always 24 test results for each dilution.

11 So what I would say in conclusion is
12 that actually the implementation dates for the
13 European Union is July 1st, 1999. I tried to show
14 you that actual legal provision is provided at
15 community level, either in the CPMP Note for
16 Guidance, or in the European Pharmacopoeia.

17 But official control of these batch
18 release is still at the national level, which means
19 this operates under the principle of subsidarity,
20 and in the directive it is also foreseen that this
21 operates with mutual recognition.

22 And this is a very important point in
23 which we at EDQM -- for which we develop a lot of
24 activity because what we must is foster the mutual
25 recognition of test results in one cell, and the
26 other in cell within the European Union.

1 So I thank you for your attention.

2 CHAIRPERSON TABOR: Thank you, Dr.
3 Rautmann. I'd like to say that I think it's very
4 interesting to hear the comments of Dr. Rautmann and
5 Dr. Nubling about the European approach to this
6 problem; something we're going to have to keep in
7 mind here in the United States as we approach the
8 problem from a regulatory perspective.

9 The next speaker is Dr. Saldanha from
10 the NIBSC.

11 DR. SALDANHA: Thank you. I'd like to
12 thank the organizers for inviting me to talk at this
13 meeting, and I'm going to talk really, about the
14 development of working reagents and the first
15 international standard for NAT testing of HCV.

16 And I think we heard this morning about
17 the use of these reagents and I'll try and give you
18 a very brief overview on the development and the
19 characterization of these reagents.

20 So I will start off by briefly stating I
21 think the obvious, which is why do we need standards
22 in the first place? I think we all accept when NAT
23 testing was first introduced that there was a
24 tremendous variation of the sensitivity and
25 specificity of these assays, making it very

1 difficult to compare data from different
2 laboratories.
3 As Guy Rautmann said in the previous
4 talk, that in Europe there's going to be mutual
5 recognition of results, and to be able to do this
6 you have to have confidence in results from
7 different laboratories.
8 Another major problem that we face,
9 especially with the manufacturers, is the discrepant
10 results between the manufacturers and the official
11 control laboratory which could cause problems with
12 the release of final products.
13 And finally, before we can introduce any
14 sort of routine NAT screening such as the CPMP
15 guidelines, we need to have standards in place.
16 I'll take you very briefly through the
17 CPMP guideline which in fact, Guy Rautmann mentioned
18 earlier on, which is the introduction of NAT testing
19 for hepatitis C virus RNA. And there are two main
20 points to this guideline.
21 The first one is that each run of the
22 validated assays should include a suitable working
23 reagent, or a run control. And the level of RNA in
24 this run control should be equivalent to 100
25 international units.

1 And a non-reactive pool in this system
2 is defined as a pool which is found non-reactive
3 using an assay which can detect this run control.
4 And what's going to happen is, from the
5 first of July, 1999, only batches derived from
6 plasma pools tested and found non-reactive will be
7 released by the marketing authorization holder.
8 So the first reagent that we developed
9 at NIBSC was a working reagent, and this working
10 reagent was based on the results of a collaborative
11 study that was run in 1994. And the reagent is a
12 1:1000 dilution of a positive donation, which is a
13 genotype 3 diluted in human cryosupernatant.
14 The RNA content is approximately 4,000
15 genome equivalence. This has been determined by the
16 branch DNA assay and this is equivalent to 1,000
17 international units. And I'll go into this
18 derivation later on.
19 To-date we've made three batches of the
20 working reagents -- about 2,000 to 3,000 vials of
21 each. And these have been sent out to laboratories
22 since August 1995. And we request the laboratories
23 to assay the reagent NAT, 1:10 and 1:100 dilutions
24 and return the results to NIBSC so they can be
25 analyzed.

1 And what I'd like to say is the 1:10
2 dilution is equivalent, approximately, to 100
3 international units. So if laboratories can pick
4 this up regularly then they can comply with the CPMP
5 guideline.
6 So the first report that came out
7 analyzing the data between '95 and '97 shows that of
8 the 30 laboratories returning results, the NAT
9 reagent was not in fact, as you'd expect, detected
10 by all assays. There were only from 50 to 92
11 percent of assays, depending on the type of assay.
12 The same with the 1:10 dilution which
13 was from 20 to 80 percent, and the 1:100 was rarely
14 detected. And the way this data was analyzed was to
15 collect all the data from the laboratories and pool
16 them to get these final figures. Which I think in a
17 way is misleading because if you look at the data on
18 an individual basis the results look a bit better.
19 So I'll go very briefly through the
20 results of three laboratories. These bubble charts
21 for the 1:10 dilution -- that's the LABCODE and the
22 type of assay which is an in-house. The size of the
23 bubble represents the number of assays done, which
24 is written at the side. That's the time and
25 percentage of assays.

1 Now, if you look at individual labs you
2 can find that some laboratories can detect that
3 dilution of the reagent in all assays. We have
4 other laboratories which actually show an
5 improvement with time, and I think in this assay
6 this laboratory switched from a first generation
7 amplicor to a modified amplicor using a different
8 extraction method for the RNA. And I think it's
9 obvious that there's an improvement in sensitivity.
10 And other assays really just crash and
11 really, they can't detect the 1:10 frequently.
12 So the second report which looked at 19
13 laboratories which returned results over the last
14 year, and in this analysis we only looked at
15 laboratories which submitted more than 12 assay
16 results.
17 Because again, one of the problems with
18 this sort of self-assessment study I guess, is that
19 not all laboratories return results regularly, so
20 it's very difficult to get a complete picture of the
21 overall specificity -- sensitivity of the assays.
22 So we looked at 11 laboratories which
23 submitted 12 or more assay results for the last
24 year, and I'll show you very briefly again the
25 bubble charts for some of these. Again, I think you
26 can see that 100 international units can be detected

1 very consistently by laboratories. This is over a
2 period from April last year to April this year.
3 Occasionally you find laboratories which
4 miss an assay, and there are some laboratories in
5 the third bubble chart which have problems
6 consistently detecting the study up to the standard.
7 Now as we heard this morning there are -
8 - sorry. For the working reagent -- to briefly
9 conclude these results -- show that it's useful for
10 monitoring the performance of each assay run, so
11 it's a run control.
12 And it can demonstrate an occasional
13 failure of the assay, and it can also show whether
14 the assay is improving over a period of time or
15 whether you need to change the assay. So I think
16 it's quite important to use some sort of run control
17 in routine assays.
18 Now we know I think, that there are
19 several working reagents available at the moment.
20 There's the CLB Pelispy; there's a CBER reagent; a
21 Paul Ehrlich reagent, and the NIBSC working reagent.
22 And I think again, you're aware that people define
23 the unit -- the RNA in these reagents in different
24 units.
25 So we have anything from copy numbers
26 per ml to genome equivalence per ml, to PCR-

1 detectable units per ml. And I think you're also
2 aware that we now have an international standard.
3 Following several meetings over the last
4 two or three years we decided to prepare an
5 international standard for the WHO. And this
6 standard was accepted by the Expert Committee for
7 Biological Standardization in October last year.
8 It's a batch of 2,000 vials which
9 contain a lyophilized preparation of genotype 1
10 donation diluted in cryosupernatant. And the RNA
11 content of this sample is expressed in international
12 units. And this is a purely artificial unit. And
13 each vial contains 50,000 units because the
14 concentration is 10^5 per ml and there's half a ml in
15 each vial.
16 And I've done some preliminary
17 calculations. I think you probably got the idea this
18 morning that the calibration of the international
19 unit with the genome equivalence varies, anything
20 from two to four.
21 And I've done a preliminary
22 characterization at NIBSC in which the concentration
23 of the international standard was determined very
24 kindly by Chiron using the branch DNA assay as 5 X
25 10^5 genome equivalence per ml. And this was an
26 average of two assays.

1 And the assigned concentration of the
2 international standard is 10^5 IU per ml, so that
3 works out at one international unit being equivalent
4 to five genome equivalence.
5 I've done a similar calculation for the
6 concentration of the NIBSC working reagent 96586,
7 where again the undiluted donation was titered by
8 the Chiron branch DNA assay to give a titer of about
9 4×10^6 genome equivalence per ml. And the working
10 reagent itself is a 1:1000 dilution of that
11 material.
12 And the next thing that we did was
13 parallel assays using the international standard and
14 the working reagent. And the difference in titer
15 between these two reagents was two logs, roughly,
16 and that approximately gives one international unit
17 is four genome equivalence.
18 I realize these data are preliminary, so
19 in fact what we've done this year -- and in fact the
20 study has just been completed and I think we saw
21 data from one of the labs this morning -- is to set
22 up a collaborative study to calibrate several
23 working reagents.
24 The NIBSC working reagent, which is
25 about 4,000 genome equivalence; the Paul Ehrlich
26 working reagent which is 10^5 genome equivalence per

1 ml -- and this is lyophilized; a reagent from the
2 ISS from Rome, which is about 2,000 international
3 units; the CBER panel 1 which is about 1,000 genome
4 equivalence per ml; and the CLB Pelispy run control
5 which is 3,600 genome equivalence per ml.
6 And these have all been calibrated
7 against the international standard using parallel
8 assays. I'm afraid I haven't finished the analysis
9 of the results yet but I should be able to have
10 those available by the end of the year. And by that
11 time we should have all these reagents calibrated in
12 international units against the international
13 standard.
14 And finally, the last study that we are
15 planning to do is to calibrate different genotypes
16 against the international standard, because I think
17 at a couple of meetings, especially the last meeting
18 in Amsterdam, there were concerns expressed about
19 the feasibility of calibrating different genotypes
20 against the international standard, which is the
21 genotype 1 virus.
22 So we're proposing to use this study to
23 determine the RNA content of different genotype
24 samples and then to determine the efficiency of the
25 different assays for different genotypes, and then

1 to calibrate these RNA contents against the
2 international standard.
3 And so far we've collected five
4 genotypes; we're still looking out for the 6th, and
5 this study I hope, will get underway by the end of
6 the year.
7 And I think I'd like to conclude with
8 probably saying that working reagents and standards
9 are important to validate individual assay runs, and
10 they're especially essential for the introduction of
11 routine NAT testing.
12 And I think we still have to bear in
13 mind, especially that nucleic acids -- the presence
14 of nucleic acid doesn't really necessary indicate
15 infectivity. Thank you.
16 CHAIRPERSON TABOR: Thank you. The next
17 speaker is Dr. Lelie from the Netherlands Blood
18 Transfusion Service.
19 DR. LELIE: Yes, thank you for allowing
20 me to show you, give you some idea what we are doing
21 on the CLB. And to do this I always start with the
22 first slide which reminds me of what we are doing.
23 We have available, dilutional standards
24 that are available in large amounts. From those
25 dilutional standards, plasma standards, we produce

1 panels -- proficiency panels. And we did that in
2 the past with the EuroHep studies and the VQC study.
3 And in 1999 we also do this as part of a
4 European concerted action of the European Society of
5 Clinical Virology, and we also try to harmonize the
6 activities that are done by Mrs. Dex or Ellen Alan
7 of Australia, that also does similar studies in the
8 Australian and Asian Pacific region.
9 From the data that are submitted by the
10 laboratories that are proficient, we then can
11 characterize the standards and we also have an
12 instrument to validate the assays in the field.
13 And then this is an ongoing system that
14 should be done every year or so. So that it then
15 enables us to produce similar panels, actually
16 proficiency panels or reference panels that can be
17 used for validation of the assays.
18 So we now are doing a multi-center
19 validation study with reference panels in Europe,
20 and the creation of the standards and legalization
21 of assays also is the instrument for defining levels
22 of run controls that should be used in the different
23 test options.
24 Just I give you an example of the HCV
25 proficiency study of 1997. There, 81 laboratories
26 in the world submitted about 140 datasets. And we

1 found that the undiluted samples -- so from positive
2 and negative control samples -- that there were 84
3 percent of the labs that submitted correct results.
4 And we had two dilution series -- the
5 EuroHep and the VQC dilution series -- and we found
6 that 71 and 73 percent of those labs submitted
7 correct results. So in total, two-thirds of the
8 laboratories that participated produced results
9 without errors.
10 Then we can look at the proficient
11 laboratories, so we then only take into account the
12 labs that passed the inbuilt proficiency test, and
13 for instance look at the regular Amplicor kit that
14 is used by 27 labs. And you see that the 100
15 percent detection limit of the normal or of the 1.0
16 Version of this test has a detection limit of 200 --
17 it is about 1,000 international units per ml.
18 You can, so draw through for instance,
19 the Amplicor results, an ideal line which is in fact
20 the PROBIT analysis. You can use these sort of
21 statistics to determine a 95 percent detection
22 endpoint or the 50 percent endpoint, but you can
23 also use the data -- if all laboratory test
24 dilutions are standards in parallel you can, from
25 the shift in parallel lines, calculate the relative
26 potency.

1 And we did this to calibrate the EuroHep
2 standard against the VQC standard, and the VQC
3 standard isn't available in the larger supply and
4 this is important for the future. You can see that
5 if you combine all the 94 datasets of proficient
6 labs you see a relative potency of a factor 2.3.
7 And if we do test runs in parallel with the branch
8 DNA at CLB we find similar results; also 2.3.
9 We cannot simply use the branch DNA
10 assay because we have seen that there are half-log
11 differences between lots of the branch DNA assay.
12 So you really should express the results based on
13 the standard to which you have in the past, assigned
14 a value.
15 So we talked about the level, the CLB
16 run control that is now produced and should be
17 further characterized, is equivalent to 270 genome
18 equivalence per ml. And these data are just derived
19 from the WHO evaluation study in which 22 labs with
20 good results provided about 80 test runs where the
21 VQC standard and WHO standard were tested in
22 parallel, and from the average results -- which are
23 huge variation of course -- but the average result
24 in that study was that one international unit is
25 equivalent to 2.7 genome equivalence.

1 This level was -- if you look back then,
2 to the proficiency study in 1997, we found that the
3 dilution equivalent to this detection or this
4 concentration, was not achieved -- or this detection
5 limit was not achieved by roughly two-thirds of the
6 laboratories that were proficient.
7 So one-third of the laboratories at
8 least in 1997, was not able to comply with the EMEA
9 regulation. And also if we look at the WHO study
10 then also you look at a 95 percent hit rate cutoff
11 point in that study, 60 percent of the laboratories
12 were able to achieve their limits. So again, 40
13 percent was not.
14 And of course there are new methods now
15 available such as the NucliSens extractor that is
16 under validation of CLB; where you have sort of
17 plastic cartridge where you can use silica-based
18 extraction with a number of steps, and the silica
19 particles are then trapped in with the filter and we
20 end up with a 50 microliter L weight of RNA.
21 When we look at the manual, NucliSens
22 extraction kit and the extractor we have seen in
23 dilutions of the panels that we have that the two
24 methods were not statistically significantly
25 different so that they were roughly the same.

1 Here you see the detection limits found
2 with this NucliSens extractor. Here you see the
3 total number of results -- the 100 percent detection
4 limit was here about 100. And this of course, is
5 the NucliSens extraction method in combination with
6 the 1.0 Version of the Amplicor kit. And we now
7 move to the 2.0 Version, so we -- I come back on
8 this later.
9 So if you then calculate as we talked
10 about, that the 95 percent hit rate cutoff points or
11 the 50 percent hit rate cutoff points and we look at
12 the regular Amplicor in which in fact, a five
13 microliter plasma equivalent is amplified, you see
14 that the -- where we then modify this method by
15 using the NucliSens extraction system, that you will
16 increase the plasma equivalent which is amplified
17 from five microliter to one ml.
18 And doing this you see that there's
19 almost 100-fold increase in sensitivity to a level
20 of about 32 to 60 genome equivalence at a 95 percent
21 detection limit.
22 Now, if Gerold will help me with showing
23 a few overheads. Well, I told you that we are
24 coordinating a sort of multi-center validation study
25 for not only the OMCL network but for all that are
26 interested in looking at sensitivity.

1 For instance, the Qiagen robot or the
2 NucliSens extractor, in conjunction with the Roche
3 COBAS Amplicor system or the Roche COBAS system as
4 it is, or the Gen-Probe TMA, and of course also in-
5 house methods.
6 We use a panel that is called the
7 Pelicheck panel where we have these dilutions with
8 include dilutions which is the 100 international
9 unit level or the 100 genome equivalence per ml
10 level, etc. And you see here the data that we have
11 found as a sort of QC test at CLB where we did four
12 test runs, and you see that the 100 percent
13 detection limit here was about 11.
14 We also have a similar sort of panel
15 which is based on the EuroHep type 3 standard.
16 Again you see here the genome equivalence levels and
17 the international unit levels, and also here you see
18 that is quite sensitive.
19 So the idea is that this panel can be
20 used by multiple sites. So for instance, tests are
21 done on this panel in some of the larger test
22 centers in Switzerland, Germany, and England and in
23 Finland. And it's possible to either test it eight
24 times or 24 times by using these panels which
25 contain multiple aliquots per dilution.

1 The results are an effort coordinated by
2 Theo Kuypers at our laboratory which works together
3 with Oregonal Teknika, where this validation is a
4 standard with the other items which should be looked
5 at, like specificity and robustness, and where
6 either the type 3 or the type 1 panels are tested in
7 parallel, or even the WHO standard in parallel with
8 the genotype 1 standard dilution panels.
9 So this gives you an instrument for
10 doing validations in your in-house system, making
11 use of commercial test components.
12 And doing this then, we will at the same
13 time, validate a number of run controls of which we
14 then know what will be the detection rates on those
15 dilutions in the different commercial methods that I
16 used.
17 Thank you.
18 CHAIRPERSON TABOR: Thank you. The next
19 speaker will be Dr. Yu from the Food and Drug
20 Administration.
21 DR. YU: I will talk about the
22 standardization -- CBER perspective.
23 Nucleic acid test NAT methods used to
24 test plasma pools are considered by CBER to be donor
25 screens. Current proposed limit by CBER is 100
26 copies per ml for testing mini-pools. So we need

1 analytical standards to evaluate sensitivities
2 because of diverse NAT methods and also varied pool
3 size. They're as small as 20 mini-pool units or
4 1200 mini-pool units.
5 Currently we have two standards. The
6 first one has already been available since 1995.
7 This particular standard, second one, is currently
8 being evaluated and I will talk about both standards
9 right now.
10 As an interim measure the low titer --
11 you know, the low titer HCV RNA-positive standards,
12 this is really an IG lot that is really spiked with
13 the HCV RNA. But as an interim measure on December
14 27th, 1994, CBER instituted laboratory testing of
15 HCV RNA by PCR on all lots of immune globulin
16 products that have not undergone validated, viral
17 inactivation removal steps during manufacture.
18 So this was formulated then, and then as
19 I said, it's 16 percent IgG concentration spiked
20 with mixed genotypes; in fact contains all six
21 genotypes of HCV.
22 We filled 2,000 vials, three ml per
23 vial, and store currently at 80 degrees Celsius.
24 And we use this as a really, a low level positive
25 control for laboratory testing. We also provided to
26 manufacturer and other testing laboratories.

1 And determining a consensus level by
2 collaborative studies, right now we haven't really
3 finished the calculation but it's about 100 copies
4 per ml. But because it's low, very high protein
5 concentration it's really not suitable as a standard
6 for plasma pool testing, so we have to formulate a
7 new one.

8 We had a window as a single donor unit
9 but it is corrected during window period. This
10 passable unit was EIA2-negative -- EIA3-negative as
11 well -- RIBA2 was indeterminate but RIBA3 was
12 negative. The HCV RNA copy number is about 10^7 --
13 it's more than 10^7 copies per ml, and genotype is
14 1B. This is by sequence analysis.

15 So we sent this window period plasma
16 unit to other participants, to other testing
17 laboratories, and to ask them to quantitate for us.

18 First lab here, they gave us the result
19 in terms of genome equivalence per ml, whereas all
20 the rest of the laboratories gave us the results in
21 terms of copies per ml.

22 So other than just one lab which has a
23 very low value, most of them were around this range.
24 So the average value, it's about 5 X 10^7 copies per
25 ml. So we used this window period unit to formulate
26 a plasma pool standards.

1 These are the participants: Bayer,
2 Centeon, Chiron, NGI, Roche, and we also doing the
3 testing ourselves with their methods as you have
4 heard their presentations this morning. So we
5 formulate an HCV dilutional panel consisting of ten
6 members.
7 The HCV panel stock was diluted with a
8 defibrinated human plasma pool to formulate ten
9 panel members containing zero to 10^5 copies per ml
10 of HCV RNA. And we filled the first member 4,000
11 vials.
12 Each one of them is .75 ml per vial and
13 it's targeted to contain 1,000 copies per ml,
14 whereas the rest of the panel members is about 2,000
15 vials -- we filled 2,000 vials. Again, it's about
16 .75 ml per vial. Right now we store at -70 degrees
17 Celsius.
18 So we sent these panels consisting of
19 ten members to eight laboratories and asked them to
20 give us qualitative results as well as the
21 quantitative results. Now, number 2 and number 5
22 are just filled with the diluent, so these are
23 negative. And all laboratories, all test negative;
24 no false positive at all.
25 As you can see now, the laboratory A was
26 able to detect all the positive, you know, members;

1 the lowest one they can detect. And the B lab has
2 qualitative and quantitative assay methods. The
3 qualitative assay method is much more sensitive and
4 they can detect all. The quantitative only can
5 detect up to 500 copies per ml.
6 But as you can see here, most of -- in
7 fact all the labs can detect 500 copies per ml -- I
8 mean, this at the targeted level all of them were
9 able to detect 500 copies per ml -- except this
10 laboratory which is I. This is a branch DNA assay.
11 The cutoff is 20,000 copies per ml so they cannot
12 detect any of these panel members.
13 As you can see here, some of the
14 laboratories were able to detect 100 copies per ml;
15 quite a few laboratories. Now, some of them may not
16 be 100 percent hit here. One of the laboratories, 6
17 out of 8 positive, and then so forth. But again,
18 some of them can even go lower; below the 100 copies
19 per ml.
20 We haven't got all the results yet, but
21 we've got about almost all the data. Again, they
22 provide us the mean value. We haven't really got
23 the raw data and so forth to do the statistical
24 analysis.
25 These are the participating labs that
26 provide us the results. I just run through the

1 tests very quickly -- I mean, the results of all the
2 panel members, what are the levels of them.
3 This is panel member number one; the
4 targeted level is 1,000 copies per ml. And this is
5 the target level right here; solid line here. As
6 you can see, all the labs are pretty close. I mean,
7 the variation is about half a -- within half-log
8 difference. These are half-log scales.
9 Except one lab is a little lower. Then
10 this particular -- this is panel member number one.
11 We sent 150 vials to Dr. John Saldanha and to
12 participate in his collaborative studies. And I
13 guess soon we would know how much in terms of
14 international units for this member number one.
15 This is member number 3. The target
16 level is 100,000 copies per ml so it's quite high
17 level. As you can see all the labs were pretty
18 close. The variation is within half a log.
19 This member number 4 is 10,000 copies
20 per ml. Again, most close to the target level, 10^4,
21 except one lab is over half a log lower.
22 This is member number six. The targeted
23 level is 500 copies per ml. The two labs were a
24 little lower than the half-a-log; the difference.
25 So in the 100 copies per ml, this is for
26 member number 7, this is the targeted levels. You

1 can see there are two labs cannot provide us the
2 data. Now they have -- they can detect them
3 positive because qualitative assay is much more
4 sensitive, but they cannot provide us the
5 quantitative data. So that's close.
6 And then number seven, 50 copies. There
7 were four labs were still -- these are our
8 laboratories that -- all four labs were able to
9 provide us the data and close to the targeted level
10 which is 50 copies per ml.
11 And this is ten copies per ml, and only
12 two laboratories were able to give us the data.
13 And five copies per ml -- the lowest one
14 -- the target level five copies. And again, there
15 is only two laboratories can provide to us.
16 So in summary, an additional panel for
17 HCV is being developed and a difficult sensitivity
18 of diverse NAT can be compared, and it can be used
19 to the laboratories of NATs for HCV RNA. Current
20 proposed limit, 100 copies per ml for testing mini-
21 pools is achievable.
22 I would like to acknowledge those that
23 contributed to the work. Mr. Mason and Dr. Tan in
24 our laboratory. And Dr. Fashid, Dr. Yuwen, Dr.
25 Hsia, and Dr. Tabor. And they participated -- they
26 are the ones in our laboratory and Dr. Tabor's

1 laboratory collaborated this formulation and testing
2 of these panels -- the new proposed panels.
3 I also would like to acknowledge all the
4 participants -- all the participating laboratories
5 for their collaboration. Thank you.
6 CHAIRPERSON TABOR: Thank you. Now Dr.
7 Nubling will speak again; this time on
8 standardization.
9 DR. NUBLING: Okay, for me, quite easy
10 because I think the most important things have
11 already been said by the previous speakers. But
12 these, the topics remember reference preparations
13 and I think -- the topic of this section is
14 standardization and reference -- common reference
15 preparations are one, option to obtain
16 standardization; common requirements for routine
17 assays are a second option for obtaining
18 standardization.
19 Nevertheless I want to first come to the
20 first point. We've also, as I've already mentioned,
21 prepared so-called (unintelligible) HCV RNA. Like
22 Dr. Yu just told some minutes ago, we also use
23 window phase donations which was negative in all on
24 the HCV test but positive by PCR, and was quite
25 different a titer in the CBER preparation; namely
26 10^7 copies per ml.

1 This was diluted and several thousand
2 aliquots were lyophilized. During lyophilization we
3 had a loss of HCV RNA approximately in the range of
4 30 percent. By quantity PCR we have 10^5 copies
5 approximately, already still available in the
6 lyophilized ampoules, and this corresponds to
7 approximately 3 X 10^4 international units but the
8 exact unit shall be given after the evaluation of
9 the collaborative study organized by John Saldanha.
10 We used this concentration for the
11 reference preparation in order to have preparation
12 which is appropriate for spiking of plasma pools, as
13 well as give a suitable for preparing run controls.
14 And we will provide this preparation once
15 calibration is finished to all the blood donation
16 centers which require it, at least in Germany.
17 Nevertheless, we also encourage blood
18 donation centers to calibrate their own reference
19 materials. And this is just a table how we
20 recommend them to perform such assays: 24
21 replicates per dilution points. Here are two
22 dilution series.
23 One is a WHO center HCV RNA dilution
24 series -- different dilution factors, international
25 units per ml. And the second preparation is an in-

1 house HCV preparation of a blood donation center in
2 Germany.
3 And for both preparations dilutions were
4 performed and for each dilution point 24 replicates
5 were analyzed until the dilution range where
6 negative results were obtained.
7 By probit analysis of these data it is
8 possible for us -- this corresponds to the WHO
9 standard -- first to establish or to calculate the
10 95 percent cutoff of the assays in international
11 units; and the second possibility is to by potency
12 comparison of these two jobs to get the unit of the
13 in-house reference preparation.
14 So that's the system how we recommend it
15 and how it obviously, also works. The next point,
16 how standardization is obtained is by common
17 requirements for routine HCV NAT. As I told before,
18 we require a run control which should control the
19 complete NAT procedure starting from lyses ending
20 with detection, and should contain even HCV
21 particles.
22 And that's also the reason we are not
23 very happy with the amplicor tests as it is now
24 because there's only RNA which is used as a positive
25 control and also is the internal control of course -
26 - is only RNA.

1 The HCV RNA concentration in the run
2 control depends on the number of donations per pool,
3 and indicates for the regulations for erythrocytes
4 and thrombocytes should reflect the 5,000
5 international units; meaning a pool of, for example,
6 50 donations should have 100 international units as
7 a run control as a parallel assay for each run.
8 Also, negative controls are required for
9 detection of contamination, but I think that is not
10 the most important issue because most of the samples
11 are expected to be negative as well.
12 And the last point, standardization,
13 that we require also inhibition control for pools
14 consisting of more than 50 donations -- and then
15 this again. The background for this requirement,
16 experiments which are just a time ago but
17 nevertheless, I think are quite valid.
18 We spiked commercial size plasma pools
19 which were HCV PCR-negative with low amount of
20 viruses and with 10-fold higher amount of viruses,
21 and then we compared to extraction methods which was
22 first the extraction method with the amplicor HCV
23 kit 1.0 and the second extraction method was silica
24 columns.
25 And both eluents were then amplified and
26 detected using again the amplicoquet. And as you

1 can see here, dependent on the extraction method we
2 get false negative results due to inhibition. And
3 this inhibition is dependent on the RNA
4 concentration because with the 10-fold higher RNA
5 concentration it doesn't go to zero but it
6 decreases.
7 So I think inhibition control is an
8 important issue and is also, if it's required
9 generally a point of standardization for assays.
10 Inhibition control can consist either of synthetic
11 RNA which is co-amplified in the same assay, or
12 three percent of parallel HCV spiking control,
13 meaning that the same sample is tested spiked and
14 unspiked with low amount of virus.
15 In case of inhibition we recommend to
16 repeat the whole procedure and if it doesn't work,
17 if inhibition still occurs, to use a different
18 extraction method -- at least for these samples.
19 Okay, I promised to make it short. This
20 was it. Thanks.
21 CHAIRPERSON TABOR: Thank you. We have
22 time for five or ten minutes of discussion before
23 the break. If anyone in the audience has questions
24 please come up to the microphone and identify
25 yourself before the question. Yes?

1 MR. MARTINEZ: Hello. My name is Bill
2 Martinez. I'm from a community blood center in
3 Florida. And from Dr. Hewlett's presentation there
4 was a slide that described the ways that NAT testing
5 should be undertaken. And it described them as a
6 home brew kit or send them out.
7 Can the inference from that slide be
8 that all blood drawn in the United States should be
9 tested by NAT under one of those three options?
10 DR. HEWLETT: We do have an answer for
11 that, though. No, it isn't that those are the only
12 options, but those are the options we've been
13 presented with. So that's what I was addressing in
14 my slide.
15 MR. MARTINEZ: So the FDA is not going
16 to require that all blood drawn in the United States
17 be tested for the genome of HCV? Is that correct?
18 DR. HEWLETT: Are we requiring testing?
19 Nucleic acid testing?
20 MR. MARTINEZ: That's the question,
21 basically.
22 DR. HEWLETT: I think at this point
23 there are no formal requirements but we are
24 basically going along with the impetus from the
25 European community, as you've heard today. There's
26 a lot going on in Europe and that has, to a great

1 extent, influenced our policy in regard to nucleic
2 acid testing, and clearly if there's public health
3 benefit then -- I mean, that's what drives whether
4 it should be implemented or not.
5 CHAIRPERSON TABOR: This is a very
6 unusual regulatory situation in that it's been
7 driven entirely by the industry and by the pressures
8 from changes that are occurring very rapidly in
9 Europe, leading ultimately to the testing that will
10 be in place by next year.
11 It's not being required per se, by FDA,
12 but there's no question that market forces and the
13 wish for the safest blood supply possible will
14 almost certainly result in all plasma being tested
15 in this way in a very short time.
16 I think it's also going to be driven by
17 technological changes. We've heard today only about
18 mini-pool testing but I think all of us expect to
19 see technological changes that will permit
20 individual unit testing in the not-too-distant
21 future, and that will of course, change the whole
22 ballgame.
23 MR. MARTINEZ: Okay, so the FDA just
24 plans to go along with it?
25 CHAIRPERSON TABOR: Well, I would word
26 that differently. I mean, we're responding to

1 applications to evaluate and to validate and
2 standardize these assays, and by the wish of the
3 blood collecting community, to apply this new
4 measure of safety.

5 MR. MARTINEZ: One more thing. Is there
6 going to be a restriction on the number of INDs that
7 can be presented to FDA for the purposes of studying
8 this --

9 CHAIRPERSON TABOR: The only
10 restriction, in reality, is on the size of the FDA
11 staff, which is shrinking as we sit here.
12 Otherwise, there are no restrictions.

13 MR. MARTINEZ: Okay. The blood centers
14 that send their testing out, will they have to
15 participate in an IND?

16 DR. HEWLETT: I'm sorry?

17 MR. MARTINEZ: Are there obligations of
18 centers that send their tests out under -- defined
19 in terms of what their participation is likely to
20 be? Sorry about my questions.

21 DR. HEWLETT: Are you asking whether the
22 blood center has to submit an IND?

23 MR. MARTINEZ: Well, if a blood center
24 sends their tests out and what would they have to do
25 in order to help provide data under IND?

1 DR. HEWLETT: Well, they would be one of
2 the sites that is being tested by this service or by
3 --
4 MR. MARTINEZ: So they would be part of
5 an IND?
6 DR. HEWLETT: Yes.
7 MR. MARTINEZ: Okay. Thank you.
8 DR. KLEINMAN: Steve Kleinman. I'd like
9 to make a comment and then if there are members on
10 the panel that want to react to it. Today we've
11 heard about GAT testing and NAT testing. We heard
12 about master pools, maxi-pools, and mini-pools.
13 We've heard about genome equivalence per ml, copies
14 per ml, and international units per ml.
15 I think when we talk about
16 standardization I'd like to urge some
17 standardization of terminology because it's getting
18 -- I mean, I think people in this room can follow it
19 because we all have a special interest in it, but
20 when it gets brought out to the general community we
21 need to come up with definitions, and hopefully
22 those definitions and terminologies can be
23 international so we're all not having to question
24 whether the terminology we use is the same as the
25 terminology of somebody else.

1 I know international units and genome
2 equivalence is a different story, but I think it
3 would be nice and I think the studies are ongoing so
4 that we can -- I mean, even in listening to these
5 presentations it's difficult to kind of switch your
6 mind back and forth between one speaker presenting
7 genome equivalence per ml, another speaker
8 presenting a standard that's international units per
9 ml.
10 And all you have to do is lose your
11 attention for a few seconds and you're not sure
12 you've got the right thing down. So I think that's
13 important.
14 With regard to things like master pool,
15 primary pool, maxi-pool -- I think within the U.S.
16 we definitely need definitions. Everybody needs to
17 use those same definitions, otherwise we're not
18 going to know what each other are talking about.
19 So, any comments on that?
20 CHAIRPERSON TABOR: Dr. Saldanha.
21 DR. SALDANHA: Well, I can comment on
22 two of them. One is NAT and GAT. I think we
23 decided to call it nucleic acid amplification
24 technique rather than genome, because you don't
25 necessarily amplify a genome. You can do just any
26 bit of nucleic acid.

1 With units I think you made a very good
2 case for having international units and I think
3 that's the whole point of setting up an
4 international standard so that we're all talking
5 about the same thing.

6 It's an arbitrary unit but as long as
7 everything is calibrated against the international
8 standard then I think we're all working to the same
9 sensitivity. And I agree with you, it's very
10 confusing. Because I'm not sure about the
11 difference between copy numbers and genome
12 equivalence.

13 DR. HEWLETT: Yes, I'd just like to add
14 a few lines to that. I agree that definition of
15 international unit or the terms of reference in
16 terms of international units, copy numbers, and
17 genome equivalence does need some degree of
18 standardization.

19 But I think that's exactly what's going
20 on and we're already seeing that people are making
21 the effort to find some common ground, and I think
22 in a couple of months or maybe a year from now we'll
23 see a lot more in that area.

24 In regard to discussions about pools and
25 about defining primary pools and maxi-pools and so
26 on, I think we're just beginning to, again, just

1 beginning to see data long those lines, and as these
2 INDs mature I think we'll learn more about what
3 definitions are actually going to work in the field.
4 So I think at this point we're in the
5 situation of doing an interim assessment and I think
6 the issues are very pertinent but we'll learn more
7 as we go along.
8 DR. BIANCO: I'm Celso Bianco. I'm not
9 going to touch on that one. I understood from our
10 colleagues from Europe that there has been a certain
11 change, particularly by the Paul Ehrlich Institute.
12 Initially there was a definition of required
13 sensitivity for the assay that is being used for the
14 pool -- that was if I recall, first 400 units and
15 now 100 units.
16 But I understood from you of a
17 requirement that you would have to be able to pick
18 up 5,000 of something units in the pool. Is there a
19 change? Is there any standard that has been defined
20 for a sensitivity for a pick up of a unit in a pool?
21 DR. NUBLING: I'm sorry, that's a
22 misunderstanding, what you said. The 100
23 international units for the manufacturing pools are
24 defined for the plasma industry. And you cannot
25 define it in a different way because the pool sizes
26 are quite different and so on.

1 And the regulation in Germany concerning
2 erthyrocytes and thrombocytes defined 5,000
3 international units for the single donation, and it
4 depends on the technology which pool size is used or
5 is usable. And even if you don't have to use pools;
6 you can also -- some blood banks which make single
7 donation PCRs already -- small blood banks.

8 DR. BIANCO: So what you're saying is
9 that if the sensitivity of my PCR is 100 units, that
10 I would pool a maximum of 50 units in that pool?

11 DR. NUBLING: Correct.

12 DR. BIANCO: But is that -- so you are
13 defining the size of a pool?

14 DR. NUBLING: No, no. We are defining
15 the minimal sensitivity for the single donation, but
16 if we have a much higher sensitivity you can test by
17 pooling, and the pool size depends on the
18 sensitivity of the method.

19 CHAIRPERSON TABOR: We have time for one
20 more question.

21 DR. SIMMONDS: Peter Simmonds. There
22 are several standards and controls being discussed.
23 One of the difficulties with any of these standards
24 is that fact that they are derived from sero-
25 positive donations ultimately; and obviously we know

1 that these will concentrate differently when you
2 spin them.
3 Some tests use direct extraction, but
4 other ones are actually based on
5 ultracentrifugation, and I don't think that a sero-
6 positive donation or control is actually a fair
7 test of this concentration step.
8 So what I was really wondering was, are
9 there any plans to actually get some sero-negative
10 standards sorted out? Because, you know, we can't
11 really sensibly evaluate a test such as the new
12 Roche test which has this ultracentrifugation set
13 built into it.
14 DR. NUBLING: Yes, I agree totally with
15 you and it's not correct; not all standards are
16 sero-positive. For example, our standard is a window
17 phase in a donation --
18 DR. SIMMONDS: Sorry, sorry.
19 DR. NUBLING: -- which is negative in
20 all assays which we have used. And I had the same
21 thinking in the morning when the person from Roche,
22 the lady from Roche told the centrifugation step as
23 an enrichment step, but the target samples are
24 antibody-negative and the determination of detection
25 limit was performed with some of the materials which
26 are sero-positive. So it could be really a problem.

1 DR. SIMMONDS: I mean, was the new Roche
2 test 24,000g for half-an-hour, is that right?
3 DR. NUBLING: Yes.
4 DR. SIMMONDS: An hour, yes. There's
5 been actually a German paper in Vox this month
6 actually, showing that you can't concentrate virus
7 even spinning much harder than that if the donation
8 is sero-negative. So I'm not sure how much -- for
9 example, the Roche test has been tested, you know,
10 with sero-negative control.
11 DR. YU: I just want to comment, you
12 know, about ultracentrifugation. We did find when
13 in the presence of ten percent IgG -- in the
14 presence of antibody there is a tremendous
15 difference between the pelleting efficiency, you
16 know, with antibody and without.
17 CHAIRPERSON TABOR: Could you speak just
18 a little louder? I think people in the back are not
19 hearing.
20 DR. YU: I'm just, you know, with
21 experience that we have in the -- what Peter just
22 raised, the presence of antibody may have something
23 to do with the pelleting efficiency. And so, you
24 know, in our hands I know if it's ten percent IgG
25 the pelleting efficiency is so poor in the absence
26 of antibody.

1 But that is ten percent protein. And if
2 it's five percent protein like what is in the
3 plasma, I think you really need to evaluate in the
4 presence and absence of antibody. I think the
5 effect is not as great but it still needs to be
6 evaluated.
7 DR. PSALLIDOPOULOS: Are there enough
8 materials, you know, available to actually
9 distribute standards which are sero-negative? I
10 mean, Jens Bukh mentioned that there are chimpanzee
11 pre-sero conversions -- some of those samples from
12 different genotypes. I mean, are those suitable?
13 What sort of volume are they? What sort of volume
14 do you have?
15 DR. BUKH: Those chimpanzee pools are in
16 two different kinds. The ones are the ones that's
17 been tested for infectivity and they're primarily
18 going to be used for vaccine studies or studies
19 where infectivity is needed, so it's a cell culture.
20 And we have about 300 ml or 300 aliquots of such.
21 And then we have later plasma pheresis
22 units. Then we get slower to the sero conversion of
23 course, that's going to be made available. And we
24 will have 400 aliquots of each of these genotypes,
25 100 microliters each that were from the gatepost

1 inoculation. And those are available for PCR
2 testing, etc.
3 Now, of course they all have genome
4 titers of about 10^5 to 10^6, so potentially dilutions
5 could be made of them.
6 DR. CONRAD: I just want to say that
7 through the INDs that we've done and looked at,
8 we've found several hundred now and interdicted in
9 several hundred donations that are antibody-negative
10 and some of them are quite distal to the act of sero
11 conversion.
12 And we obviously saved the whole plasma
13 pheresis unit on those, either through Alpha,
14 Baxter, or ourselves. And those should probably
15 become available to you guys making standards,
16 because those are large, large volume amounts of
17 material that is antibody-negative.
18 DR. YU: For us, we also, you know, the
19 HCV stock -- the one I'm just talking about is the
20 window period units -- 1B and so forth. We do have
21 quite a few, quite a lot in volumes -- can I say
22 something? But anyway, in larger quantities. But
23 again, you know, in CBER member number 1 we do fill
24 4,000 vials. But our essential purpose is really
25 what we use as a standard for other -- for a lot of
26 these testing or other things.

1 CHAIRPERSON TABOR: I think we need to
2 move on. We're going to have a 15-minute break.
3 When we come back would the members of the panel --
4 that is, those who are participating in the panel
5 discussion -- please sit around the table prior to
6 Dr. Bianco's talk? Thank you.
7 (Whereupon, the foregoing matter went
8 off the record at 3:42 p.m. and went
9 back on the record at 4:00 p.m.)
10 CHAIRPERSON TABOR: The next speaker
11 will be Dr. Bianco from the New York Blood Center.
12 And would all members of the panel please sit at the
13 tables? Thank you.
14 DR. BIANCO: My role is to provide you
15 with some general lists of issues regarding the
16 implementation of nucleic acid testing by blood
17 centers. And I'll try to do that since I was the
18 only person in blood banking and transfusion
19 directly involved -- related here, except for some
20 of the presentations from the industry and Sue
21 Stramer -- I'll try to raise all these generic
22 issues.
23 First, Dr. Kleinman came with some
24 subversive words a few minutes ago, and I had
25 actually come with some -- to grapple with some of
26 these issues. And I see that GAT is an industry

1 favorite and NAT is FDA, and I realize also the
2 favorite of the European regulatory agencies. We'll
3 start calling it NAT, but for the purposes of this
4 presentation I'm going to call it GAT/NAT.
5 Where are we today? I think that we had
6 a superb review in this meeting of the status of the
7 technology. Obviously, the technology is here in
8 terms of the testing of each one of these samples --
9 be it the sample, the specimen derived from a pool
10 or derived from an individual blood donor sample.
11 Unfortunately, the logistics are not
12 here. There are a number of issues that we will
13 have to confront. We have the benefit of having a
14 task force that was put together under the umbrella
15 of the American Association of Blood Banks, that has
16 been trying to deal with some of these issues.
17 I'm listing here the names of the
18 members of this task force just to tell you that
19 there is representation from almost every sector of
20 the transfusion bloodbanking area.
21 There are representatives -- Dr.
22 McCullough is the chairman. There are
23 representatives from America's blood centers,
24 America's Red Cross, ABBA -- and it's been a very,
25 very effective committee. And this group kind of
26 kind of together -- there is a report, a short,

1 initial report that is coming out in Transfusion, I
2 believe next month, that lists the issues being
3 addressed by the task force.
4 And among those global issues are the
5 issues of the generic questions that we have. GAT
6 is being implemented, particular in Europe, for the
7 units that do not meet GAT. As plasma units they
8 are going to be subjected to viral inactivation
9 during their manufacture.
10 We are involved in collection and
11 distribution of blood products that are going to be
12 transfused into recipients of single units. They
13 are not going to be inactivated by any process.
14 They cannot be red cells at the present time or
15 platelets being activated.
16 So we would like to apply this
17 technology to the units that represent the real risk
18 to our population of patients. What viruses should
19 we test? Will every collecting facility have access
20 to GAT/NAT? And how are we going to deal with some
21 of the donor issues like autologous donors or
22 platelet pheresis donors?
23 Platelet pheresis are special. There
24 are no plasma products coming out of it; it's just
25 the platelets. Should they be tested?

1 Some of these issues I think that are
2 consensus are developing. Obviously, we are going
3 to test for both HCV and HIV, even at the risk --
4 according to the data that we saw today -- is the
5 one that will benefit the most. HIV is of such an
6 importance in the minds of our patient population
7 that I think that we cannot ignore it.
8 And there is also one of the
9 manufacturers that has a multiplex assay and by
10 default, that both viruses tested will be searching
11 for the nucleic acid of both viruses, not the
12 genome. And obviously, there of other of these type
13 of issues.
14 There are many of the regulatory issues
15 that are not exactly part of the recommendations
16 that have so far been issued by FDA by CBER; either
17 in written form or during our discussions. And we
18 would like as we develop those INDs, to have a
19 common sense among all of us of what are the best
20 procedures? What are the procedures that are in the
21 best interest of our donors and our recipients?
22 And we also have some concerns that
23 these are tests; they are not yet licensed. We are
24 in the process of IND; we are learning about them.
25 And we are concerned that some of the approaches
26 that we used today with regulated tests may be

1 applied in a rush to some of these tests that are
2 still experimental.
3 But the questions are there. Do we
4 retrieve and quarantine prior donations? How are
5 going to confirm? How are we going to notify? How
6 are we going to follow up these donors? And a lot
7 of it will be learned during the process of the IND.
8 A major issue with the whole blood
9 donation is a question of timing. The plasma
10 industry has actually the luxury in terms of safety,
11 of retaining those units, as we heard today, for a
12 period of months until they include these units in
13 the processing for the manufacture of the plasma
14 derivatives.
15 Our platelets have a short life -- shelf
16 life of five days. If we look at the time cycle of
17 everything that is happening, there is in a period
18 of 36 to 48 hours we'll have a donor sample that
19 will be subjected to the ELISA, and another donor
20 sample -- probably in one of those fancy tubes that
21 Dr. Stramer showed us -- that is going to be rushed
22 into an airplane and flown into some laboratory
23 somewhere.
24 When it gets to that lab we will have to
25 remove repeat reactives on the ELISA. Now we'll
26 have the results. So that the number of positive

1 pools that we have is reduced; otherwise the
2 frequency will be much higher.
3 And we would pool the negatives and then
4 subject those samples, those pools to be GAT/NAT.
5 And here it took us 36 to 48 hours. Even if we have
6 in-house testing and pooling this will be close to
7 the 36 hours.
8 Then we have a second cycle of 36 to 48
9 hours minimum; that is obviously if our pool here a
10 few hours later is negative we can release all the
11 negatives. But if we have to go to round 2 or round
12 3 to resolve which ones are the positive samples in
13 the pool, we will come to release the negatives for
14 transfusion at a minimum of 48 hours.
15 So timing in the best of all worlds,
16 GAT/NAT results will come out in 36 to 48 hours.
17 The GAT/NAT results may appear 48 to 96 hours before
18 we started the process. And our platelets expire in
19 120 hours. And obviously there are the issues of
20 contamination, sleet, rain, snow, and everything
21 that affects the Post Office.
22 One of the issues that is being
23 seriously considered among us is the possibility
24 that we will have to transfuse a portion of our
25 supply -- particularly the supply of platelets --
26 prior to the availability of the GAT/NAT test

1 results; and that we will then create a system
2 which, on a kind of a look-back but an immediate
3 look-back following that transfusion, we would
4 provide the physician and the recipient with the
5 molecular technology results and help them deal with
6 this fact.

7 But it will be a very serious balance.
8 It will be -- even if the frequency of those events
9 is rather small, as we heard the numbers today,
10 maybe a couple of hundred a year in the whole
11 country -- that certainly will not be an easy task.

12 And among the logistic issues there are
13 many. There's the transportation. We heard some of
14 the things about the needs for preservation of the
15 samples; the pooling hardware. And what we see as
16 one of the major obstacles at this point is
17 software: is software for sample management; is
18 software for pooling; is software for management of
19 test results.

20 You heard for instance, the Red Cross
21 that uses a single software for the lab, a single
22 type of barcodes; that the need for special handling
23 of those samples: the avoidance of duplicate
24 numbers, and the management of those test results
25 and the communications back to the laboratories.

1 If we size it up maybe we'll have a more
2 realistic view even, of what this represented in
3 terms of logistics. ABC centers and American Red
4 Cross centers, each one of them collect about half
5 of the -- about 45 percent each of the whole blood
6 supply, and hospitals collect the other ten percent
7 for those hospitals.
8 And the majority of them I suspect, are
9 going to ship their samples either to the Red Cross
10 or to centers among America's blood centers in order
11 to have them tested by GAT/NAT.
12 There are, for each one of these
13 systems, about 22,000 samples a day. ABC centers
14 are 70 centers. They have multiple software, they
15 have multiple communication systems, they have
16 different barcode systems, and they have overlapping
17 numbers. And those issues will have to be resolved
18 between now and early next year.
19 The American Red Cross I commented
20 already, but each one of these systems, depending on
21 the pool size and our ability to deal with these
22 test results and tests concomitantly, may have to
23 find among the 22,000 samples, 700 repeatedly
24 reactive samples -- about three percent or two
25 percent, depending on what they decide to pull out -
26 - before pooling every day.

1 And I don't think it makes a difference
2 if it is 50 or 700. You look in this one here full
3 with 22,000 samples, you probably will get to the
4 end of those tables and you'll go look for those
5 samples.
6 The number of pools actually, that is
7 going to be run, is relatively small. For a pool of
8 24 you see that ABC centers in total are going to
9 run less than 1,000 pools a day -- maybe a little
10 bit more with the repeats and resolution.
11 American Red Cross centers are going to
12 run less than 200 pools a day. So the staff for
13 that with the Gen-Probe may be one, two people, but
14 the staff that will be involved in the logistics and
15 the training and the software and the investment is
16 immense compared to the actual technology of test.
17 The initial plan -- and I'm giving you
18 as an advance and without authorization directly
19 from anybody -- but as it looks today we heard that
20 the American Red Cross has made an association that
21 seems to be very productive with Gen-Probe, and is
22 preparing, of what I heard, a very nice laboratory
23 in San Diego.
24 The 70 ABC centers made preliminary
25 arrangements, both with Gen-Probe, that it
26 apparently will set up four sites in four different

1 labs scattered around the country. And Roche, that
2 is planning to set up 11 labs. And we are starting
3 to work together with those manufacturers in a
4 collaborative manner, attempting to have as fast as
5 possible, our INDs in very good shape and so that we
6 can initiate those studies.
7 The likely, initial scenario is that
8 most or all donors are going to be tested,
9 ultimately, sometime next year. It's not easy or I
10 would not feel comfortable personally, testing
11 sample -- units that are going to be turned into
12 recovered plasma for further manufacture, and not
13 testing the red cells that are going to be
14 transfused to a recipient in a hospital. I don't
15 think it would be fair.
16 The size pools you heard today, American
17 Red Cross is 128, followed by an interrogation mark.
18 The plans from ABC, both with Roche and I don't know
19 yet if confirmed with Gen-Probe, but the plan with
20 Roche is 24.
21 The ARC plans to remove the reactive
22 samples. America's blood centers, there's an
23 intense search for the possibility -- in the
24 majority of these centers, about 80 to 85 percent of
25 the donors are repeat donors. and as you heard from
26 Dr. Kleinman this morning, the incidence of sero

1 conversion in repeat donors is rather small; about
2 four per 100,000 person-years for HCV.
3 So if we could segregate these samples
4 that come from first-time donors we would be running
5 a very low risk of finding a positive pool if we
6 tested it concomitantly with the ELISA test without
7 removing the reactives. But this is part 2 of the
8 plans, and this is directly linked to the pool size.
9 Well, we heard the orders from the
10 European regulatory authorities and we will try to
11 comply. We don't like April 1st so we chose March
12 31st.
13 (Laughter.)
14 And the European manufacturers of plasma
15 derivatives where we ship a lot of our recovered
16 plasma, required that we ship to them tested plasma
17 by PCR after March 31st. And we plan to implement
18 that testing in an IND format before that date.
19 However, there is a consensus that came
20 up very strongly at this task force, and
21 particularly after an impassioned speech by Dr.
22 Roger Dodd, that this is an experimental test and
23 that it's being performed under an IND and that we
24 should not behave as if we had just received a
25 licensed test in our laboratories, and implemented
26 with all the fancy bells and whistles without

1 actually knowing what the consequences are of what
2 we're implementing.
3 So the implementation should be well
4 planned and gradual, and in the first several months
5 until the systems are fully understood, we should
6 not rush in our opinion, to take action on results
7 of the initial pools.
8 For instance if a pool of 128 is
9 reactive or a pool of 24, of going back and
10 notifying recipients of all the prior units that
11 this donor now sero converted, we will be more
12 careful in the early days and we won't take action
13 against individual units or individual samples, or
14 related to individual samples from donors that prove
15 to be positive. But we will not try to rush into
16 action for things for which we have low degree of
17 certainty.
18 How can everybody that is in this room
19 help? In our view, CDC and NIH, that is our public
20 health system and the academic side, can help by
21 continuing to support research in these areas. We
22 need and we need a lot.
23 Yes, also by helping the public
24 understand the benefit -- or relatively limited
25 benefits -- of the GAT/NAT testing, through
26 particularly, epidemiologic studies. And the

1 somewhat of an illusion that exists in the mind of
2 the public that the implementation of GAT/NAT in
3 pools is going to close all the windows.
4 They will improve but it will not close
5 the windows. And again, to help educate blood
6 donors, blood recipients, physicians, and the
7 American public about GAT.
8 The FDA also can help; it can help a
9 lot. This meeting is an example. They also are
10 providing substantial support to the AABB task force
11 by validating a lot of our thinking and providing a
12 very open and supportive discussion.
13 Definition of standards. I asked a
14 question before about the 50 and one, and one -- but
15 the issue is that the initial regulatory actions
16 were very careful, and they referred to the
17 sensitivity of the single assay.
18 But now we have been confronted with a
19 pool, and we need support and guidance on particular
20 issues of sensitivity related to pool size. My
21 fantasy is very simple. I'd like Dr. Hewlett to
22 give me a tube and say, this is HCV-positive; pick
23 it up in your pool. If you don't pick it up your
24 test is bad. If you pick it up your test is good.
25 And I wouldn't care if it is genomes or
26 if it is copies or if it is units. And again, by

1 supporting us in the establishment of validations.
2 Can the industry help? Yes, if we continue behaving
3 as we have so far. That is, we are all looking at
4 it as a safety issue and as a scientific issues and
5 not necessarily as a competitive issue.
6 We have shared a substantial amount of
7 information. We are all supporting the inter-agency
8 task force and we certainly are supporting the
9 public health system. Thank you.
10 CHAIRPERSON TABOR: We are now scheduled
11 to have a panel discussion. The panel is rather
12 small at this point and I think maybe we should
13 combine this with continuing discussion from the
14 audience and questions for any of the speakers.
15 Maybe I'll -- while we're waiting to see
16 if there are any questions -- I see Harvey Alter in
17 the back; maybe we can get him to sit up here at the
18 table. Let me just ask the people who are here now
19 -- Dr. Seeff, Dr. Miriam Alter, Dr. Dodd, Dr. Bianco
20 -- do you have anything to add to the discussion?
21 Dr. Seeff.
22 DR. SEEFF: Well, it may be heretical to
23 extend what is being discussed here to other areas
24 and perhaps this is already in the process of being
25 done. I guess I'm looking at the panel and I may be

1 the only -- except for Harvey now -- clinician
2 seeing patients.
3 And the next step of course, is our
4 anxiety about getting standardization and licensing
5 of tests for therapeutic purposes. This is --
6 again, this is perhaps the wrong venue but I think
7 we're all aware of the fact that we're moving into a
8 new treatment era and for the first time, genotyping
9 and viral concentration may turn out to be critical
10 -- actually critical -- in making decisions.
11 Not only is this with respect to who
12 should be treated but for how long people should be
13 treated. And I couldn't urge more from the clinical
14 point of view, the need to have these tests
15 available, standardized, licensed so we can get
16 started and get moving for appropriate treatment.
17 CHAIRPERSON TABOR: Maybe -- I wonder if
18 some of the representatives of industry who spoke
19 this morning would comment on whether we're likely
20 to get a test for therapeutic purposes in the
21 foreseeable future? Do any of you have anything --
22 DR. MIRIAM ALTER: Why would that be
23 different?
24 DR. CONRAD: Speaking for industry, yes.
25 I think universally, industry is moving towards
26 developing tests in the therapeutic domain, both --

1 I think my colleagues at Amplicor -- Roche as well
2 as us and others are probably looking at it, or
3 doing it now.
4 DR. MIRIAM ALTER: I'd like to add a
5 comment to Dr. Seeff's in the same range, that I
6 realize we're focused today on protecting the blood
7 supply and making it as safe as possible.
8 But we can't bury our heads in the sand
9 and not realize that this has far-ranging
10 implications; both for reporting the test results
11 back to the donor as well as for the public health
12 campaign that's going to take place starting within
13 the year, which will broaden the amount of testing
14 for hepatitis C that's currently done in this
15 country.
16 And regardless of regulations, we know
17 that in practice PCR and it's being used widely to
18 test and diagnose patients with HCV infection, and I
19 think we need to recognize that as we go through
20 this process.
21 Because these tests, as Dr. Seeff said,
22 need to be made available in a standardized fashion,
23 and if the recommendations don't come from us that
24 patients are not getting a consistent message
25 because they're not being interpreted in the same
26 way from one place to another.

1 DR. DODD: I guess my comment would pick
2 up with on what Miriam and indirectly, Leonard said,
3 and also what Celso has said; and that is that I
4 think we've learned today that we've moved a very
5 long way down the track to implementing testing in a
6 routine fashion, and that I think that we can
7 probably reasonably well anticipate meeting the
8 needs of the plasma for further manufacture
9 component of our task within an appropriate
10 timeframe.

11 And I think I'd say I'm very excited
12 with the progress of the technologies made. And I
13 can remember hearing Dr. Kessler say, I want you
14 guys to tell me not if it can be done, but when it
15 can be done and how. And I think we are further
16 ahead than many of us thought when he uttered those
17 immortal words.

18 But what I think we do need to think
19 about very carefully, particularly as we involve
20 labile products, red cells and platelets in this
21 process -- and that's inevitable when we use
22 recovered plasma -- is the human side of this.

23 The issues of how we're going to manage
24 our donors, how we're going to deal with giving
25 effective information about what is still a research

1 test. I think we all have a lot of faith in what
2 nucleic acid amplification really means.
3 But we really don't have enough mass
4 experience to understand how often we're going to
5 get an uninterpretable or a non-repeatable or a
6 false positive. And I do think that we have to
7 recognize that, at least for the single donor
8 products, we are going to have some recipients on
9 the other end of the process.
10 And I actually would like to see another
11 workshop that deals with, if you will, the human
12 side of NAT testing rather than the genome side.
13 DR. SEEFF: I'm sorry, if I could just
14 extend what I said in fact, to again support what
15 Miriam has to say. We are starting look-back, and
16 this look-back is something that has real relevance
17 to this group.
18 I think everyone knows here that the VA
19 system, 172 hospitals, is starting its own look-
20 back. And on the basis of which I am aware, it may
21 be that something like 70 to 80 percent of everybody
22 who attends VA hospitals is going to be tested for
23 hepatitis C.
24 Once we come up with a positive result
25 for antibody -- we've all got algorithms, and the
26 algorithm then takes us to, are these people

1 viremic? Do we treat those who are not viremic?
2 And of course, most people will not do that.
3 We've got to have a test that can take
4 us to the next phase. We're all busy drawing all
5 these arrows all over the place, but if we don't
6 have any licensed tests then I don't think that they
7 have any meaning. This is extremely important.
8 CHAIRPERSON TABOR: Dr. Conrad.
9 DR. CONRAD: I just wanted say that
10 after hearing Dr. Bianco's talk I'm thoroughly
11 depressed. Thanks for pointing out the problems.
12 But I also wanted to maybe bolster my spirits and
13 others that, we've actually looked at three-and-a-
14 half million donations from 100,000 people.
15 And to say that -- you know, there are
16 logistical difficulties, there are enormous
17 logistical difficulties. But I've noticed a
18 difference from when we began this process several
19 years ago and now, and I think that all those
20 logistical difficulties are certainly not
21 insurmountable.
22 And I think that as all of us gathered
23 here should hear, that there is progress being made,
24 and that process is extraordinarily rapid. And so I
25 think that we can all acknowledge that there is
26 difficulty and there is a human side, and we have

1 notified donors and we have dealt with the
2 ramifications of counseling someone that you have a
3 virus and for 172 days didn't develop antibodies and
4 what you do with that.
5 And we're learning. And I think the
6 alternate to doing that and dealing with those
7 difficulties is to burying our head in the sand and
8 just not doing anything, and I think that's
9 unacceptable. We have to understand that there's
10 difficulties with the system but it's better than
11 the alternative, which is to do nothing.
12 CHAIRPERSON TABOR: Dr. Hollinger.
13 DR. HOLLINGER: Yes, you know, there's
14 also something very interesting about this whole
15 issue. We've heard of probably a number of patients
16 who might be out there who are in the window period
17 and are transmitting disease. And we don't see much
18 of this disease.
19 I know it sounds like heresy, almost
20 going back to the 1970s when we told the blood
21 bankers, you know, there is a disease out there,
22 non-A/non-B hepatitis that's occurring. Oh no, no,
23 there's no disease out there; we're not seeing it.
24 And we found a lot of it.
25 But there are some unique issues here
26 because everyone is looking for post-transfusion

1 hepatitis now. There's a much greater, I think,
2 view from the public -- at least from clinicians --
3 looking for this in people who have had disease.
4 So where is this disease in these
5 patients? Is there something unique? We know that
6 in these patients who are in the pre-antibody area
7 who are obviously positive -- at least from a
8 nucleic acid standpoint and presumably have
9 infectious virus present -- but is there something
10 unique about the disease that they're transmitting
11 to individuals?
12 Is it more likely these patients do not
13 become chronically infected? Maybe have a very mild
14 or acute disease that goes on. I didn't hear from
15 the industry -- particularly Centeon for example,
16 produced some data that suggested perhaps one out of
17 1,000 of their donors were sero converting.
18 But what I'd really like to know is what
19 happened to those that sero converted? I didn't
20 hear anything about the clinical aspects of them.
21 Did they develop acute hepatitis, did they develop
22 jaundice, or things of that nature, on any of these
23 groups that sero converted during following up of
24 these plasma donors?
25 So there are two issues: one is what's
26 happening to that group, but secondly, is there

1 something unique about transmission of disease in
2 the window period that may not be causing much in
3 the way of significant disease? Maybe somebody can
4 tell me if there are some patients that have been
5 discovered over the last four years or five years
6 from post-transfusion hepatitis C.
7 I've asked my colleagues, I've asked
8 many people in this room to, you know, give me five
9 patients that they know of -- or four patients or
10 one patient. And I'm sure it must be true but I'd
11 just like to hear that data.
12 DR. CONRAD: I can give you those
13 patients. I mean, they're not patients -- they were
14 donors -- but they must have gotten it acutely. I
15 mean, we presented the window period -- well, they
16 were PCR-negative, became PCR-positive; followed
17 them through sero conversion.
18 And many of those patients developed
19 elevated ALTs, and obviously we haven't followed
20 them to look for the -- I mean, we saw earlier
21 presentations where people went 20 years without
22 significant disease. So we've had, you know, 200
23 days.
24 But I mean, I can give you lots of
25 individuals who were HCV-negative by nucleic acid as
26 well as antibodies; became HCV-positive by antibody

1 -- by nucleic acids, then by antibody; and developed
2 elevated ALTs.
3 DR. MIRIAM ALTER: Blaine --
4 DR. HOLLINGER: That's not my question.
5 My question is not of the people who sero converted
6 from a plasma -- I mean, I understand those will
7 happen. I'm asking, the patients who received blood
8 from those individuals, the recipients, what has
9 happened to the recipients? I'm well aware that you
10 can get the disease and you go on and get
11 chronically infected. But I'm interested about the
12 others.
13 CHAIRPERSON TABOR: Dr. Harvey Alter.
14 DR. HARVEY ALTER: A lot of comments.
15 Overall, my general approach is sort of a Nike
16 approach to this thing. Just do it and get it
17 moving. And it's actually moving as fast as it
18 possibly can, I think.
19 But Blaine raised an interesting
20 question. I hate to agree with Blaine ever, but it
21 is interesting, and these cases have disappeared.
22 Since we've now followed 655 people -- it's a small
23 number but we've followed them intensively since
24 1992 and there hasn't been a single case of
25 hepatitis C. Miriam is seeing hepatitis C, acute
26 cases, disappear in the community.

1 In fact, I would like to delay testing a
2 little bit so we can get a few more acute cases to
3 study. It's just you just can't find them. So
4 that's one comment. But I think we have to do this,
5 but the bottom line is that this is an intensive,
6 expensive, complicated effort to narrow window which
7 is going to prevent a probable handful. It's
8 predicted a couple of hundred but it may really be a
9 handful of cases. We're so close to zero incidence
10 right now.
11 DR. DODD: Can I expand the question
12 just a little bit and ask the clinicians among you
13 whether you would treat these window period
14 individuals that are going to turn up as a result of
15 screening? Would you treat them for HepC?
16 DR. HARVEY ALTER: Well, the approach
17 we've used now -- and Leonard can probably answer
18 better -- but I think in people who have needle
19 sticks or if we knew of somebody with acute
20 hepatitis, would be not to treat them at the point
21 of potential exposure but to treat them as soon as
22 they became PCR-positive.
23 I think that's a -- there haven't been
24 good studies to prove that's efficacious but there
25 have been some hints of that, and if I were the one
26 infected I'd want to be treated very early.

1 DR. SEEFF: Well, there's no answer to
2 this. Well, there are some studies I guess, in
3 Europe where people with acute diseases were treated
4 and the sense was that there was a reduction in
5 progression to chronic liver disease. But there
6 really -- we don't have enough data.

7 I know that Miriam has been trying to
8 set up a study, a needle stick study, and others.
9 But I'd go along with what Harvey says; that if I
10 were exposed I would have myself followed and the
11 minute I became PCR-positive I would like to be
12 treated. I don't know how, but I would like to be
13 treated and I think the --

14 (Laughter.)

15 DR. MIRIAM ALTER: Which brings the
16 issue back to where we were originally in that,
17 although we're here to protect the blood supply, in
18 fact, post-transfusion HCV infection is an
19 extraordinarily rare event.

20 And as you two just brought up, this is
21 driving an issue that really has an impact -- has a
22 much broader impact in other settings. And that's
23 the diagnostic setting, not the blood safety
24 setting.

25 And it's because of this that perhaps
26 we're going to get licensed tests that can be used

1 to test the healthcare worker after the needle
2 stick, and to screen other people who have risk
3 factors, and to use in the clinical setting. In the
4 absence of an antigen, an IgM test and a variety of
5 other things that tells whether or not a person's
6 viremic.
7 CHAIRPERSON TABOR: Dr. Lelie.
8 DR. LELIE: Yes, I want to comment on
9 this discussion. I think there -- we talked about
10 standardization of blood screening tests, but
11 there's also a need for standardization of
12 diagnostic tests. And we tried to make that link by
13 of course, having our standards calibrated against
14 the WHO standard as the primary reference
15 preparation, but not only as the primary reference
16 reagent for blood screening tests but also
17 diagnostic tests.
18 And so the proficiency studies that are
19 planned, it's also industrial use own group, that
20 contacts of -- brings us in contact with the
21 manufacturers of the NAT kits. So that there is the
22 panels that are then approved of also by the
23 industry.
24 And so if we use this diagnostic test
25 also for confirmation I think -- and just an idea,
26 but I'd like to have a comment on this. Since we

1 are now collecting PPT samples that are in fact, PCR
2 (unintelligible), this opens the possibility to use
3 the same cubes to immediately, after doing RIBA or
4 Western Blot testing, also use the same test on the
5 NAT kits for confirmation.
6 And this would certainly improve the
7 quality of donor counseling I think, in this
8 country. Because I think there are a lot of donors
9 counseled, even though they are false-positive in a
10 sort of confirmatory test procedure that is applied
11 now.
12 And I think if those PCR kits can be
13 used for confirmation testing this would certainly
14 improve the quality of counseling donors in the
15 United States.
16 DR. MIRIAM ALTER: I'd like to just
17 follow up. I'm glad you brought that up because I'd
18 like to introduce a word of caution in the use of
19 the term "false positive". It means that the person
20 did not test positive for the virus.
21 It does not mean that the antibody was a
22 false positive. It does not mean that the person
23 was not infected with HCV in the past. And I think
24 that we have a responsibility to the individual
25 we're testing to tell them what -- the meaning of
26 their test results and not just that they weren't

1 virus positive on the day that we happened to test
2 them.
3 One, it is with this type of disease
4 this person could develop chronic hepatitis down the
5 line at some point, if in fact that antibody is
6 truly positive. And so I think that the RIBA or
7 other supplemental assay still plays a major role in
8 the counseling messages that you give an individual
9 and in their need for further medical evaluation.
10 Maybe not in protecting the blood supply but
11 certainly for the donor.
12 DR. KLEINMAN: Yes, Steve Kleinman. I'd
13 like to follow up on two of the points -- the first
14 one that Miriam just made -- and that is, I think
15 what Nico is saying is that amongst our antibody-
16 positive, PCR-negatives there are probably two
17 groups of people: those who have been exposed in
18 the past and cleared the infection, and those who
19 are truly false positive.
20 I mean, they have never been infected.
21 Our RIBA tests are not perfect. They use the same
22 antigens as our EIA tests; they just spread them out
23 in a different format. I'm not sure that
24 confirmatory is necessarily the right word for them.
25 They're supplementary for sure.

1 So I think that's probably only a small
2 number of individuals but we haven't really proven
3 whether that antibody-positive person by RIBA who's
4 PCR-negative and he comes back in and remains PCR-
5 negative, whether he's ever had past HCV infection.
6 That's an inference. I agree with you; we should
7 still counsel people.
8 The other things was, in Blaine's
9 comment about where are the transfusion associated
10 hepatitis cases. I think the numbers from modeling
11 would suggest that obviously those numbers are very
12 small. We're talking about 100 units a year or so
13 that are donated in the window phase, maybe made
14 into an average of 1.5 components each; therefore
15 exposing 150 recipients in the entire United States.
16 We know that 60 percent of those units
17 go to people who never survive their initial
18 hospitalization. So now we're down to 70 or so
19 recipients. We know that three-quarters of the
20 cases of non-A/non-B are anicteric. I mean, we're
21 really talking about 15 cases a year throughout the
22 United States.
23 Now, do they get reported back as post-
24 transfusion, non-A/non-B -- as post-transfusion
25 hepatitis? Well, I don't know what the reporting
26 rate is, you know, but it would have to be -- if

1 it's less than half -- I mean, very few of these
2 cases are going to be reported back to the blood
3 center and very few clinicians are going to see
4 them.
5 So I think it's consistent with the fact
6 that we don't see them. We wouldn't expect to see
7 them at this point, and it certainly supports the
8 point that it's a rare event and yet everybody has
9 sort of, I think agreed, at least tacitly if not
10 explicitly, that because we can narrow the window
11 it's something that we should do. But that's why we
12 don't see them.
13 DR. MIRIAM ALTER: Actually, I have a
14 question for both the agency as well as industry.
15 And that is, are the proposed studies that are going
16 to be done only going to address pool testing or are
17 they also going to address individual testing, such
18 that they would be useable in the clinical setting?
19 Diagnostic setting?
20 CHAIRPERSON TABOR: Will you respond to
21 that, Dr. Ticehurst?
22 DR. TICEHURST: Yes, more or less. I'm
23 John Ticehurst and I work at the Center for Devices
24 and Radiological Health in FDA, as well as Johns
25 Hopkins. And I'm here today largely as an observer
26 out of interest, but a lot of the issues that

1 particularly Drs. Alter and Seeff are bringing up
2 are what we deal with at CDRH.
3 This is sort of internal FDA stuff that
4 isn't very interesting but it's very relevant to the
5 discussion. Which is that for these particular
6 assays, the indications that pertain to the
7 diagnosis and management of individual patients is
8 going to be evaluated at CDRH.
9 That doesn't matter for anything except
10 that it's administratively handled separately and
11 the companies have to deal with it separately. And
12 to just make a comment: we're not really hearing
13 from the companies on this.
14 There's been very little of that and I
15 know from my Hopkins point of view and from talking
16 with colleagues and going to virology meetings, I'd
17 say what was stated before a little more strongly.
18 HCV RNA is a standard of medical care in this
19 country right now but it's an unstandardized
20 standard of medical care.
21 CHAIRPERSON TABOR: Miriam was saying
22 something sotto voce and maybe if you could say it
23 in a microphone?
24 DR. MIRIAM ALTER: You mean you can't
25 say it? Is there anyone from industry who'd like to
26 respond?

1 MR. WESOLOWSKI: I'm Alex Wesolowski.
2 I'm Senior Director of Regulatory Affairs at Roche
3 Molecular Systems. In response to Dr. Ticehurst's
4 comments, I do know a couple of members of industry
5 who had met with the Device Center -- Roche is one
6 of them -- discussing these very tests.
7 We're looking at submission of a
8 qualitative and a quantitative test for hepatitis C,
9 diagnostic testing as well as the work that we're
10 doing in blood screening. So they are on the way.
11 I know Dr. Ticehurst can't talk about specific
12 cases; however, since we're very close we're usually
13 a little more joyful about making these
14 announcements.
15 We're in the late stage of doing some
16 diagnostic, clinical studies and we expect that
17 these products will be available, approved by the
18 FDA relatively soon.
19 CHAIRPERSON TABOR: Dr. Bianco.
20 DR. BIANCO: Miriam, we have a deadline
21 and we want full attention from the agency, so the
22 diagnostics will have to wait a little bit.
23 (Laughter.)
24 CHAIRPERSON TABOR: Dr. Alter.
25 DR. HARVEY ALTER: I'm President of
26 Alter, Incorporated, and I am working on --

1 (Laughter.)

2 We are going to hopefully, do individual
3 -- since we have such a small donor population we're
4 hoping to do individual testing if I can get the
5 right cooperation from companies, just as a pilot to
6 see how this would work on line in getting platelets
7 out, etc. That's one thing.

8 The second thing is that not too far
9 behind, as I talked about before, is viral
10 inactivation of cellular products, which if given
11 the same impetus as this kind of testing, could move
12 just as rapidly.

13 And the data are now -- we just finished
14 the chimp data so I'm saying this with a bias
15 because I'm involved in it -- but we've just
16 finished the chimp data which show that this viral
17 inactivation will kill HBV and HCV and prevent
18 disease in the chimp model.

19 And there's already been a plethora of
20 in vitro data. So I don't know how that's going to
21 impact. You're going to have two powerful systems
22 for preventing a minuscule number of infections.
23 And will the system support both or will we have to
24 choose one from the other? I think the next year is
25 going to be very interesting.

1 CHAIRPERSON TABOR: The capacity of the
2 system to support these things is astonishing. If
3 you think back a few years when everyone was happy
4 that we were detecting hepatitis C for the first
5 time, now the -- basically the operating principle
6 is to have zero risk if at all possible.
7 DR. SEEFF: Do you have a means of doing
8 whole body viral inactivation of humans?
9 DR. HARVEY ALTER: Oh, yes, yes we do.
10 We're working on it. My goal is to go from zero
11 risk to zero meetings.
12 DR. BIANCO: But certainly, I think that
13 at least an approach I think that -- I find pooling
14 is a very interesting intermediate step because it's
15 giving everybody the opportunity to think, to
16 confront the issues, to confront the technological
17 issues in a reduced number of samples.
18 That is, we saw from 50,000 samples a
19 day we are going to reduce everything maybe to
20 1,000. And that is going to allow these systems to
21 move.
22 DR. HARVEY ALTER: Yes, I agree it
23 should be done that way, but we'd only be testing
24 one pool a day.

1 CHAIRPERSON TABOR: Are there any final
2 comments? I have a -- I wanted to show one or two
3 slides just in closing.
4 I think it's important to take a moment
5 to think about the fact that the concept of pooling
6 in a series of pools, starting with a group of
7 donors making one pool and taking -- say if these
8 were pools of 25 donors each -- taking several pools
9 of 25 donors each and making a tertiary pool and so
10 forth and then testing it, and then working back
11 from a positive test to identify the actual donor is
12 a real paradigm shift.
13 I mean, it's one of those concepts, one
14 of those simple concepts that changes the way we do
15 things. And this is certainly going -- is obviously
16 going to have that effect and it's basically the
17 availability of this concept that's allowed us to do
18 the kinds of things that have led to this meeting
19 today.
20 And this is just based on a slide of Dr.
21 Conrad's showing the cubicle concept that they use
22 and working back. And I think it's possible that
23 Dr. Conrad may even be the one who's responsible for
24 this concept, but it's really enabled us to do this
25 kind of testing.

1 And even if we're only eliminating a
2 small number transfusion-transmitted cases, we're
3 able to do something to improve the safety of blood
4 that is what the public wants today.
5 I'd like to say just a few words in
6 closing about the FDA's approach to nucleic acid
7 testing. I think it's fair to say, at least
8 unofficially, this is really just an interim measure
9 until we're able to test individual units.
10 But we do feel strongly that it should
11 be regulated as donor testing. We feel that it's
12 part of our responsibility to the donors and to the
13 concept of donor safety that donors should be
14 notified if they've been found to be positive.
15 And it's interesting that in the last
16 year-and-a-half or so that we've been discussing the
17 concepts of nucleic acid testing with industry that
18 this concept of nucleic acid testing of mini-pools
19 as donor testing seems to be fairly -- pretty much
20 accepted by everybody involved.
21 And I'd like to also point out that
22 there are really important public health benefits
23 from notifying the donors who would be identified
24 through nucleic acid testing in mini-pools.
25 First of all, we would be able not only
26 to prevent the donation -- the transfusion of the

1 donated materials at that time, but we would be able
2 to prevent repeated donations during the window
3 period that might affect other individuals.
4 Notification of these donors would allow
5 us to prevent transmission to close contacts, and in
6 addition it would allow donors to seek therapy
7 early.
8 Well, I think we've had a very long day
9 and I think the talks have been terrific and the
10 discussion has been stimulating. I think on that
11 note I'll close the meeting and I'll see many of you
12 at the Blood Products Advisory Committee tomorrow.
13 (Whereupon, the meeting was adjourned at
14 4:52 p.m.)
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